

Renal Epithelial Neoplasms Induced in Male Wistar Rats by Oral Aflatoxin B₁¹

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SUMMARY

Purified aflatoxin B₁ was added to the diet of male Wistar rats, at concentrations of 1.0, 0.5, and 0.25 parts per million for 147 days. The animals were then maintained until death on a basal diet. Renal epithelial neoplasias, histologically similar to human kidney adenocarcinoma, were found in over one-half of the animals ingesting the highest level of aflatoxin B₁ and in about one-quarter of the rats given the two lower dosages. Malignant hepatocellular carcinomas were also encountered in many of the animals although the renal tumors were present as the only neoplasia in some of the rats. The gross and microscopic appearance of the kidney tumors is described.

INTRODUCTION

For a number of years we have been studying the morphologic, biologic, ultrastructural, and biochemical properties of hyperplastic liver nodules, induced in rat liver by a variety of chemical carcinogens (6-9, 13). In the course of similar experiments, using the carcinogen aflatoxin B₁ and male Wistar rats, an unexpectedly high incidence of renal papillary tumors was encountered. Although a rare renal carcinoma (12) and some kidney adenomas (15) have previously been reported by others, following the ingestion of aflatoxin by rats, to our knowledge no previous author has communicated the frequent occurrence of renal neoplasms induced by this carcinogen. The kidney tumors encountered in the present experiments were quite similar to some cases of human renal cell adenocarcinoma. In addition the aflatoxins, a generic name for an admixture of metabolites—some highly purified and characterized (1, 21)—produced by the fungus *Aspergillus flavus*, are either toxic or carcinogenic to a variety of species (2-5, 11-12, 14-19, 23). For these reasons our data are being communicated at this time.

¹This work was supported in part by a contract (PH-43 64 505) from the National Cancer Institute, by a research grant (G. M. 10269) from the National Institute of General Medical Sciences, and by a training grant (G. M.-135) from the National Institute of General Medical Sciences.

Received October 17, 1968; accepted November 23, 1968.

MATERIALS AND METHODS

Animals and Histology

Male, 150-200 gram, Wistar rats (Carworth farms) were used. The animals were housed, 2 to a cage, in screen-bottomed cages in an air-conditioned room, and they had access to both the diets and drinking water at all times. Animals were followed until death, at which time a thorough autopsy examination was performed and sections of *all* viscera, as well as any other lesions, were taken for histologic study. Tissue was fixed in Stieve's solution and the histologic technics were the same as described previously (6, 13).

Diets and Experimental Design

The aflatoxin B₁ used in these studies was a highly purified preparation generously supplied by Dr. G. N. Wogan of the Department of Nutrition and Food Science of the Massachusetts Institute of Technology. During the time the animals were ingesting the aflatoxin B₁, diets were made fresh weekly in lots of 2 kilograms for the experimental animals and four kilograms for the controls and were stored in the dark, at 4°C, in containers wrapped in aluminum foil. The circumference of their feeding dishes was similarly made impermeable to ambient light. For this time period, the rats were given fresh diets at least twice weekly.

There were 3 experimental groups and one control group of rats. For the former the aflatoxin B₁ was weighed out and solubilized in reagent grade acetone. This was then added (in a volume of 1 ml per kg of diet) to the corn oil used in preparation of the previously described basal diet (6, 7, 13). A similar volume of acetone devoid of any aflatoxin was added to the corn oil used in the preparation of basal diets for control rats. After addition of acetone (with or without aflatoxin B₁) the corn oil was vigorously stirred and then used for completion of the dietary mix. The 3 experimental groups of rats were given aflatoxin B₁ at levels of 1.0, 0.5, and 0.25 parts per million for 147 days. Thereafter they were maintained on the basal diet.

At the beginning of the experiment there were 16, 18, 17, and 26 rats in the 1.0, 0.5, 0.25 ppm aflatoxin B₁ and control groups respectively. However, of these 77 animals, a total of 8 (2, 0, 4, and 2 for the above groups respectively) were either lost or died of pneumonia during the first 100 days of the dietary regimens. Accordingly, the data in this paper are based upon the surviving 69 rats with 14, 18, 13, and 24 being in the

1.0, 0.5, and 0.25 ppm of aflatoxin B₁ and control groups respectively.

RESULTS

Renal Papillary Tumors

Renal tumors developed in 57%, 28%, and 23% of the animals on the 1.0, 0.5, and 0.25 ppm aflatoxin B₁ regimen respectively (Table 1). These were diagnosed at mean autopsy times of 603, 696, and 783 days from the start of the experiment for the above 3 respective groups (Table 2). No neoplasms of any type were encountered in control rats. Of the 16 kidney tumors in the 3 experimental groups, 5 occurred in rats with no other neoplastic lesion. The incidence, and the times of diagnosis, of the renal tumors are summarized in Tables 1 and 2.

Gross Appearance of Renal Tumor

Approximately half of the renal tumors were bilateral (7/16). The smaller neoplasms (0.2–0.4 cm) appeared most often as solid, ovoid to circular, sometimes multifocal, and generally intramedullary in location. The tumors were somewhat dry, fairly homogeneous, well demarcated, but nonencapsulated, yellow to grey-tan lesions readily identified with the naked eye. Larger tumors ranged to a maximum of 10 and 12 cm in greatest dimension. The larger tumors were all, at least in part, localized by an apparent capsule. However, the bigger neoplasms were cystic and, when sectioned, hemorrhagic and necrotic loci, as well as foci distended with serous fluid, were evident. Often the peripheral portions of these large tumors were of a dull grey hue although other loci had a golden yellow to yellow-tan color. The consistence of these areas was variably friable to firm to rubbery. On 2 occasions,

Table 1

Dietary level of aflatoxin B ₁ in ppm of diet	Rats in group available for study ^a	Rats in group developing renal neoplasms	Percent rats in group with renal neoplasms	Rats in group with malignant hepatoma	Percent in group with malignant hepatoma	Rats with renal tumor but no hepatoma	Percent rats with renal tumor not having hepatoma
1.0	14	8	57	12	86	2	25
0.5 ^b	18	5	28	13	72	2	40
0.25 ^c	13	3	23	8	62	1	33
Basal diet only throughout	24	0	0	0	0		

Incidence of renal epithelial and malignant hepatic tumors in male Wistar rats ingesting aflatoxin B₁ for 147 days. Rats were maintained on basal diet following their dietary exposure to aflatoxin B₁ for 147 days.

^aA total of 8 rats in the 3 experimental and the control groups (2, 0, 4, and 2 respectively) died during the first 100 days and had pneumonia at autopsy.

^bOne rat in this group, with no other primary tumor, had an epidermoid carcinoma of the lung when he died 364 days after starting the experiment; another animal in this group had a benign subcutaneous fibroma—as well as a malignant hepatoma—when he died 761 days after the start of the experiment.

^cOne rat in this group with no other primary tumor had a benign subcutaneous fibroma when he died of pneumonia 271 days after starting the experiment; another animal in this group, who died 861 days after starting the experiment, had an undifferentiated 5 cm soft tissue sarcoma—in addition to having both a malignant hepatoma and renal cell tumor.

Table 2

Dietary level of aflatoxin B ₁ in ppm of diet	Rats in group surviving initial 100 days	Days from initiation of experiment to diagnosis of malignant hepatoma	Days from initiation of experiment to diagnosis of renal epithelial neoplasia
1.0	14	463, 468, 481, 502, 577, 635, 637, 653, 683, 718, 802 [Mean: 611]	468, 481, 538, 577, 637, 653, 683, 790 [Mean: 603]
0.50	18	520, 594, 606, 613, 633, 640, 670, 718, 753, 754, 761, 763, 856 [Mean: 622]	594, 640, 684, 704, 856 [Mean: 696]
0.25	13	503, 668, 670, 715, 761, 850, 861, 906 [Mean: 742]	670, 761, 919 [Mean: 783]

Chronology of autopsy diagnosis of malignant hepatic and renal epithelial tumors in male Wistar rats ingesting aflatoxin B₁ for 147 days. Control rats [24] had no malignant tumors. Rats were maintained on basal diet following their dietary exposure to aflatoxin B₁ for 147 days.

tumor focally permeated into the adjacent peritoneal and retroperitoneal area so that kidney, adrenal, perirenal fat and fascia, and spleen (1 instance) formed a confluent mass, in the midst of which was a still partially encapsulated renal tumor which could not be dissected free of adjacent structures.

Microscopic Appearance of Renal Tumors

The renal neoplasms, both in any one tumor and in lesions from different animals, have a fairly uniform cytologic and histologic appearance. The major components were thin papillae, with sparse stroma, that supported high to low columnar cells whose cytoplasm was generally eosinophilic and granular (Figs. 1–5). Less frequently, loci of acinar and tubular formation were present (Figs. 2, 4). The lumens of the latter often contained hemorrhagic debris. The lumens also encased amorphous, eosinophilic, granular material (Figs. 2, 3, 5). Indeed in some instances deposits of the latter type were so large as to give under low magnification the impression of a lymphatic channel dilated with abundant proteinaceous debris. At higher magnification, however, one could ascertain that such aciniform spaces were lined by compressed epithelial cells.

By far the most frequent cell seen in these tumors was columnar in type (Figs. 1–5). The nucleus was generally central or subcentral and most often contained a single large nucleolus (Figs. 3–5). Mitotic figures (both normal and abnormal) were present, but generally one had to survey many fields to locate them. Giant cells, generally with 3 or 4 nuclei and dense eosinophilic cytoplasm, were also infrequent. The luminal margin of the neoplastic cells often had a “frayed” appearance which suggested (Figs. 3–5) “brush border” differentiation. Intraluminal eosinophilic debris was seen in association with such cells. On occasion the tumor was almost arrayed in sheets, and focal calcification resembling psammoma bodies was seen in such cord-like areas. In yet other loci the generally sparse stroma was more abundant (Fig. 4). In such areas, epithelial cells irregularly permeated the stroma, which was often loose and myxomatous (Fig. 4). In other scattered areas, the tumor cells lacked affinity for eosin so that the cytoplasm was quite clear (Fig. 5). Such cells most frequently contained central, uniform nuclei. Areas such as the latter (Fig. 5) were remarkably similar to foci seen in human renal cell adenocarcinoma. These clear cells also formed papillae, tubules, and acini, and such lumens contained both amorphous debris and cholesterol-like clefts. In 2 instances the sparse residual, non-tumorous renal parenchyma resembled the pattern associated with chronic pyelonephritis.

Small loci of scattered tubular hyperplasia were seen in some kidneys which had no evidence of neoplasia. The diminutive nests of hyperplastic cells in these instances had a cytoplasm with a marked basophilic propensity.

In 2 other kidneys, devoid of evidence of primary malignant disease, small medullary adenomas, completely circumscribed by benign renal parenchyma, were found. These small adenomas (Fig. 6) had a similar cytology and histology to the larger renal epithelial neoplasms described above. No direct evidence, however, was noted that would definitively suggest that the larger tumors arose from such adenomas.

Distal metastases were *not* found although in 2 animals the kidney tumor did extend locally into adjacent soft tissue and viscera. In these extensions, as in the primary tumor, the neoplastic cells were generally quite uniform.

Liver Lesions

Malignant hepatomas developed in 86%, 72%, and 62% of the rats on the 1.0, 0.5, and 0.25 ppm aflatoxin B₁ regimens respectively. Neither the incidence nor the time of appearance of these hepatocarcinomas seemed as rigorously dose-dependent as were the renal tumors (Tables 1 and 2). The gross and microscopic appearance of these malignant liver tumors resembled those described by others (2, 5, 12, 14–17, 19, 23). Local, and distal intraabdominal and intrathoracic, metastases of these hepatocarcinomas were found. In some rats on the aflatoxin diets dying of pneumonia during the first 100 days of the experiment as well as in many experimental animals dying at later intervals, hyperplastic liver nodules were found. Such lesions have been described in detail by others as occurring in the livers of rats fed diets containing aflatoxin (15, 19, 23).

Extrarenal and Extrahepatic Tumors

A squamous cell carcinoma of the lung was the only malignant lesion found in one rat in the 0.5 ppm aflatoxin B₁ group which died 364 days after starting the experiment. This tumor invaded the major vessels and both cardiac atria.

A fairly anuclear benign subcutaneous fibroma was found in one rat which died of severe pneumonia 271 days after beginning the 0.25 ppm aflatoxin B₁ regimen. A similar fibroma was found in another animal on the 0.5 ppm regimen for 761 days before death. This latter rat also had a malignant hepatoma. Although no such fibromas were found in control rats, we have occasionally noted identical lesions in control Wistar rats used for other experiments in this laboratory.

One rat in the 0.25 ppm aflatoxin B₁ group had a soft, rubbery, moist, pink, ovoid 5-cm submandibular anaplastic undifferentiated sarcoma, as well as a malignant hepatoma and renal tumor, when he died on Day 861 of the experiment.

DISCUSSION

The finding of renal tumors in rats ingesting aflatoxin B₁ for 147 days immediately raises the question as to whether such kidney tumors are primary or represent metastases from a hepatocarcinoma. The most convincing evidence that the renal lesions were true primary tumors is the fact that five of 16 were found in rats who had no other malignant disease. In addition, the renal tumors appeared to be more rigorously dose-dependent, both in relation to incidence and to time of diagnosis, than were the hepatocarcinomas. Furthermore, the histology and cytology of the kidney tumors differed markedly from that of the malignant hepatomas.

It is of interest that a higher incidence of renal tumors has not been reported earlier as occurring in rats ingesting aflatoxin. One group of investigators (15) did report on the occur-

rence of kidney adenomas in rats exposed to aflatoxin. However, more recent reports from that laboratory (14, 16, 17, 19, 23) have not mentioned any significant renal tumors. These investigators, however, have recently reported a tubular lesion in animals exposed to aflatoxin (18). This tubular lesion was *not* neoplastic, and, in addition, it appeared related to the experimental diet and *not* to the aflatoxin. A single rat exposed to aflatoxin, reported by another group (12), had a kidney adenocarcinoma.

It should be borne in mind that the strain of rats, basal diet, and aflatoxin dosages used in our studies differs from that used by others. However, in recent unpublished investigations in which a strain of inbred rats similar to those used by others were the experimental animals (19, 23), and in which the basal diet and aflatoxin dosage were identical to that in the present report, no renal tumors were diagnosed. This would suggest that the Wistar strain is probably more susceptible to the induction of renal tumors than are other strains of rats. Since aflatoxin appears to be mainly excreted in the feces (22), the incidence of renal tumors is quite possibly unrelated to kidney concentration of metabolites of aflatoxin B₁. Potential mechanisms of strain susceptibility to tumor formation in a specific organ could become testable when more data are available relating to the molecular events occurring during the process of oncogenesis induced by the aflatoxins. Some studies of the chemical sequelae of aflatoxin administration have been reported (10, 20). However, it is not immediately evident if the data from these experiments can be related to the observed renal tumors. The present finding of this possible strain difference in a single species might serve as a "model" for testing future proposed hypotheses relating to intermediary reactions thought to be prerequisite for aflatoxin to manifest its oncogenic potential.

Finally, since aflatoxins can contaminate food ingested by humans, it is of interest that the presently reported tumors resembled both grossly and microscopically clinical cases of renal cell carcinoma.

ADDENDUM

Since this paper was accepted for publication, it has been brought to our attention that approximately 30% of the rats, ingesting one diet containing peanut meal possibly contaminated with aflatoxin, developed renal adenomas (W. D. Salmon and P. M. Newberne, Occurrence of Hepatomas in Rats Fed Diets Containing Peanut Oil as a Major Source of Protein. *Cancer Res.*, 23: 571-575, 1963). More recently, renal adenocarcinomas, or carcinomas of the renal pelvis, have been reported in approximately 9% of male rats fed diets containing known amounts of aflatoxin B₁ as a contaminant (W. H. Butler and J. M. Barnes, Carcinogenic Action of Groundnut Meal Containing Aflatoxin in Rats. *Fd. Cosmet. Toxicol.*, 6: 135-141, 1968).

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Figs. 1–6. All photomicrographs were obtained from hematoxylin and eosin-stained, paraffin-block sections of tissue fixed in Stieve's solution.

Fig. 1. Low power photomicrograph of renal tumor in rat who was on 1.0 ppm aflatoxin B₁ regimen for 481 days. The overall pattern is that of a papillary tumor with scant stroma. However, occasional loci of tubular and acinar formation are seen. Hemorrhagic debris is focally seen. × 67.

Fig. 2. Renal tumor tissue from rat on 0.25 ppm aflatoxin B₁ regimen for 919 days. Note the striking uniformity of cell structure within the section. Nuclei show little irregularities. × 140.

Fig. 3. Renal tumor tissue from rat on 1.0 ppm aflatoxin B₁ regimen for 463 days. Note the amorphous, granular, eosinophilic debris within the luminae. Cells adjacent to the lumens often appear to have "brush borders." Nuclei generally have a single prominent nucleolus. × 430.

Fig. 4. Another area of the same tumor seen in Fig. 3. Stroma is more abundant here and appears invaded by epithelial cells. The "brush border" appearance of some cells is again noted. × 270.

Fig. 5. Renal tumor from rat on 1.0 ppm aflatoxin B₁ regimen for 577 days. In this field the tumor resembles a clear cell adenocarcinoma of human kidney. × 270.

Fig. 6. Single renal lesion found in rat on 0.5 ppm aflatoxin B₁ regimen for 676 days. This is *not* considered a malignant neoplasm. However, its cytology, histology, and medullary locus do resemble some of the other tumors found in the kidneys of these rats. Possibly an adenoma such as this was the origin of the large neoplasms. × 67.

