Urinary Bladder and Liver Cell Tumors Induced in Hamsters with o-Aminoazotoluene

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SUMMARY
Administration of o-aminoazotoluene at 0.1 per cent in the diet for 49 weeks to Syrian golden hamsters resulted in the occurrence of liver-cell and urinary bladder tumors in 45 per cent and 50 per cent of the animals. No definite sex difference could be established. In addition, three mammary adenocarcinomas out of fifteen females are believed to be related to the treatment. A similar relationship for other tumors seen could not be established with certainty.

INTRODUCTION
Research in carcinogenesis was significantly advanced when Yoshida in 1932 (16) reported that the azo dye o-aminoazotoluene gave rise to hepatomas when incorporated in the diet of rats. Subsequently, this compound has been studied extensively (9, 12), and most reports have been concerned with the induction of tumors in the liver of both mice and rats. However, a significant number of other studies have reported the induction of tumors in other organs, notably the urinary bladder. Yoshida (17) and Maruya (quoted by [15]) reported the induction of a few bladder papillomas in rats, and Yamasaki and Sato (15) reported a similar finding in rabbits which received o-aminoazotoluene (AAT) dissolved in olive oil or in water and injected into the bladder. In addition, proliferation and keratoses of the follicular epithelium of the thyroid in guinea pigs, rats, and rabbits were observed when AAT was administered either subcutaneously or orally (15, 17). Moreover, Maruya (quoted by [15]) found areas of squamous metaplasia of the bronchial epithelium in some cases. Nelson and Woodward (11) reported the occurrence of benign and malignant tumors of the urinary bladder and gall bladder in dogs fed with this compound. Andervont (1) reported the induction of local sarcomas and hemangiosarcomas in C strain mice given subcutaneous injections of AAT. The Syrian golden hamster has generally been proved to be sensitive to chemical carcinogens, although in some cases its reactivity varies from that seen in other rodents. Miller et al. (10) pointed out that the Syrian hamster differs from the rat in its response to azo dyes, being refractory to compounds exerting potent action in the rat. In a series of studies from this laboratory, it has been found that the hamster does differ markedly from the rat in its response to carcinogens, being refractory to some azo dyes (13), in confirmation to the work of Miller et al. (10), as well as to some other rat liver carcinogens such as ethionine and thioacetamide. In the instance of acetylaminofluorene (3) the hamster is particularly sensitive to the production of cholangiomatus tumors, whereas carbon tetrachloride (4) gives rise predominantly to liver cell carcinomas. The present study was undertaken to include AAT in this series.

MATERIALS AND METHODS
Male and female Syrian golden hamsters (Abrams Small Stock Breeders, Chicago, Ill.), 12–14 weeks old at the start of the experiment, were used. They were divided into two groups: Group 1.—Twenty-five male and fifteen female hamsters were fed powdered Rockland mouse diet mixed with 10 per cent corn oil (USP) for 2 weeks; thereafter, the carcinogen, o-aminoazotoluene (AAT), was added to the diet, dissoluted in corn oil, at a level of 0.1 per cent of the diet. This experimental diet was administered ad libitum continuously for 49 weeks and then replaced with Rockland mouse diet in pellets. AAT was obtained from Eastman Organic Chemicals Co. and purified in...
this laboratory by column chromatography on magnesia/Celite (2:1). The daily intake of food was measured throughout the experiment, and it was calculated that each animal consumed approximately an average of 60 gm. weekly, equivalent to a total of approximately 3 gm. of AAT during the 49 weeks of treatment.

**Group 1.**—Thirty-eight female and 33 male hamsters were fed powdered Rockland mouse diet with 10 per cent added corn oil for 50 weeks. Subsequently, they were fed Rockland mouse diet in pellets. In addition, another control group of 40 males and 63 females was carried out at the same time in conjunction with a parallel experiment. This group, fed Rockland mouse diet in pellets and otherwise untreated, has already been reported in a separate publication from this laboratory (2).

All the animals were housed in plastic cages on wood shavings, in groups of four or five according to sex, and received tap water ad libitum. All of them were weighed and inspected every week until death. The animals were allowed to die spontaneously or were killed when in poor condition. Two animals, both females of the control group, were lost through cannibalism. All others were autopsyed, and histological study was performed on each of them. The organs were fixed in 10 per cent buffered formalin, and sections were routinely stained with hematoxylin and eosin. Van Gieson, P.A.S., Masson, Snook, and fat stains were used when necessary.

**RESULTS**

**Rate of growth and survival.**—The average weight of the animals of the first group was 110 gm. for the females and 117 gm. for the males at the beginning of the experiment. During the first 20 weeks of treatment, the weight of the females increased progressively, reaching an average of 125 gm. at the 20th week. In the same period, the males progressively decreased in weight, reaching an average of 95 gm. at the 20th week. Starting from the 20th week, both females and males increased in weight slightly. A sudden conspicuous increase in weight after the 45th week could almost always be related to the presence of liver lesions with ascites.

In the control group, the weight was constantly higher, reaching an average of 123 gm. and 127 gm., respectively, for the females and the males at the 20th week. The survival rate was similar for the two groups up to the 40th week, after which time tumors began to appear and a higher mortality was seen in the AAT-treated group (Table 1).

**Tumors of the urinary bladder.**—The first tumor of the urinary bladder was observed at the 45th week in the females and at the 55th week in the males. At that time nine females and sixteen males were still alive. As reported in Table 2, a total of five females and fifteen males each developed a single bladder tumor, 33 and 60 per cent of the initial number of females and males, respectively, or 50 per cent for both sexes together.

Most of the tumors were grossly recognized as papillomatous growths partially occupying the vesical lumen and causing a marked hydronephrosis. Histologically, two tumors, both in the females, were benign papillomas, and eighteen were papillary or transitional carcinomas, showing various degrees of differentiation (Fig. 3). Areas of squamous differentiation were not infrequently observed. In nine cases, adjacent to the characteristic papillary or transitional pattern, the neoplastic growth had areas of adenocarcinoma (Fig. 4), closely resembling the adenocarcinoma of the urinary bladder observed in the humans and related to the cloacal ancestry of the bladder (7). All the carcinomas were invasive (Figs. 5-6), but no metastases were found. In six of the twenty bladders with tumors, a calculus, ranging in size from 1 to 5.5 mm., was present. In two other cases, a small quantity of gravel was found.

During the systematic examination of the bladders, no noteworthy lesions were found in the ex-

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<th>GROUP</th>
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TABLE 1

**SURVIVAL RATE**
Experimental animals killed or dying before the 43d week (Fig. 1). Fields of epithelial hyperplasia of the bladder were observed in a male killed at the 43d week from the beginning of the experiment (Fig. 2). Findings of chronic cystitis were generally seen in the bladders in which a tumor was present.

Liver tumors.—In Table 2, the incidence of liver cell tumors is recorded. Three hepatomas were observed in two female hamsters, dying at the 59th and 73d week from the beginning of the experiment.

A higher percentage of tumors was observed in the males. Seventeen liver-cell tumors were recorded, nine of which were liver-cell carcinomas and ten of which were benign hepatomas. The first hepatoma in the males was seen at the 43d week, In addition, two cholangiocarcinomas were found in the male hamsters (Fig. 8). In the first of these two animals, dying at the 66th week from the beginning of the experiment, a liver-cell carcinoma was present in the left lobe, while a cholangiocarcinoma was present in the right lobe of the liver. Both tumors were largely necrotic and highly invasive and gave metastases to the lungs, mesenteric lymph nodes, peritoneum, and perirenal capsule. No cholangiocarcinomas were seen in the female hamsters.

In eleven of the initial fifteen females and nine of the initial 25 males cholangiomatous lesions, similar to those induced by 2-acetylaminofluorene (3), were also seen. Fatty changes in the liver were seen in four animals killed or dying between the 5th and 33d week. Areas of hydropic degeneration and of regeneration were seen in most of the animals dead or killed after that period, very often near the cholangiomatous lesions; no cirrhotic changes were observed.

Tumors of the gall bladder and extra-hepatic ducts.—One carcinoma and one papilloma of the gall bladder were observed in female hamsters, dying at the 59th and at the 65th week from the beginning of the experiment. One papilloma of the gall bladder, one papilloma of the common bile duct, and one carcinoma of the ampulla of Vater were seen in the male hamsters, dying or killed at the 83d, 83d, and 67th week, respectively.

The carcinoma of the gall bladder observed in the female hamster involved the entire organ, with limited areas of initial invasion of the liver parenchyma (Figs. 9, 10).

The papillomas, both of the gall bladder and of the common bile duct (Fig. 11), had the same appearance as the liver tumors. They were pink-reddish nodules with a size ranging from 3 to 20 mm. in diameter. Histologically, the liver-cell carcinomas appeared as large, irregular masses without a defined border, and with a disordered pattern of invasiveness. No capsule surrounding the neoplastic growth was seen in any of these tumors. The neoplastic masses were composed of atypical and undifferentiated cells with numerous mitoses and several scattered giant multinucleated cells (Fig. 7). In several fields, a coarse trabecular arrangement was seen, some of the trabeculae having a central lumen that simulated the presence of ductules. Areas of necrosis were observed mostly in the central parts of the tumors. Areas of necrosis were also seen in the surrounding compressed parenchyma. In none of these cases were metastases observed. Areas of hydropic degeneration and of regeneration were seen in most of the animals dead or killed after that period, very often near the cholangiomatous lesions; no cirrhotic changes were observed.
preneural tumors of the colon, two hemangiomas of the liver, three of the spleen, and one of the brain. Varying amount of protein material was present in the pancreas, all of them in animals dying or killed between the 55th and 75th week from the beginning of the experiment. They were uni- or multilocular cysts, divided by thin septa and lined with flattened epithelial cells. Animal dying at the 65th week from the beginning of the treatment. X30, H. & E.

In addition, two females and three males developed polycystic lesions of the pancreas, all of them in animals dying or killed between the 55th and 75th week from the beginning of the experiment. They were uni- or multilocular cysts, divided by thin septa and lined with flattened epithelial cells. Varying amount of protein material was present in the lumen of the cysts (Fig. 16).

Control groups.—In control hamsters of group 2, fed powdered diet with 10 per cent corn oil for 50 weeks, two cholangiomas, one malignant myxoma of the cheek pouch, one polymorphous-cell sarcoma of the pleura, one angioma, and one adrenal cortical adenoma were seen in the females. One papilloma of the forestomach, one adrenal cortical adenoma, two malignant lymphomas, histiocytic type, one cholangioma, one angioma, and one carcinoma of the liver, combined liver and bile duct type, were observed in the males. In addition, scattered areas of cholangiofibrosis, proliferation of oval cells, and biliary microcysts of the liver were not rare findings.

The pathological observation of the previously reported untreated control group (2) showed the absence of urinary bladder tumors, liver-cell tumors, gall bladder tumors, and mammary tumors. As reported, a few other tumors were seen—namely, two papillomas of the forestomach and one angioma of the liver in the females; one papilloma of the forestomach, two malignant lymphomas, histiocytic type, one carcinoma of the salivary gland, and one plasmocytoma in the males.

DISCUSSION

The present results demonstrate that AAT is a powerful carcinogen in the Syrian golden hamster, giving rise to bladder carcinomas, liver-cell tumors, gall bladder papillomas and carcinomas, and mammary carcinomas in a high percentage of the exposed animals.

Bladder tumors have been previously reported from studies with this compound in the rat, in the rabbit, and in the dog (11, 15–17). The incidence of bladder tumors in our hamsters fed AAT was much higher than that previously observed in other species: 50 per cent of tumor-bearing animals out of the initial number of animals and 80 per cent out of the survivors at the time of the first tumor. Thus, a total of twenty tumors was recorded, only two of them being benign papillomas, all others being carcinomas. Male hamsters seem to be more susceptible than females to the induction of this type of tumor, but this difference needs further confirmation in a larger group of animals.

It has been generally assumed in many studies with aromatic amines known to be bladder carcinogens in the human that the dog was the species of choice for such studies. The present finding indicates that the hamsters may be equally susceptible and may facilitate the testing for many materials of interest as environmental carcinogens in man.

Fig. 1.—Normal urinary bladder. Animal dying at the 30th week from the beginning of the treatment. X25, H. & E.

Fig. 2.—Hyperplasia of the transitional epithelium of the urinary bladder. Animal killed at the 49th week from the beginning of the treatment. X90, H. & E.

Fig. 3.—Papillary carcinoma of the urinary bladder with broad base, with several areas in which glandular structures are clearly visible. Animal dying at the 62d week from the beginning of the treatment. X100, H. & E.

Fig. 4.—Papillary carcinoma of the urinary bladder, invasion of the muscular wall. Squamous and undifferentiated epithelial cells. Animal dying at the 69th week from the beginning of the treatment. X100, H. & E.
ments is enhancing the incidence of a usual tumor, whereas carbon tetrachloride is having a some-
which different action in giving rise to liver-cell tu-
mors that are very rare in the control animals (6, 
these, although the incidence was not high, cholan-
tumors in the rat (9, 1~), results in the occurrence 
hamsters (10, 13) ; on the other hand, carbon tetra-
benzene, do not give rise to tumors on feeding to 
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carcinogen notable for the diversity of its action in 
this study may also be attributed to the action of 
oral administration of urethan (5) .

However, in addition, there was a pronounced en-

ACKNOWLEDGMENTS

REFERENCES
Fig. 5.—Papillary carcinoma of the urinary bladder. Areas of invasion at the basis of the tumor. Animal dying at the 69th week from the beginning of the treatment. ×100, H. & E.

Fig. 6.—Adenocarcinoma of the urinary bladder. Animal killed at the 81st week from the beginning of the treatment. ×250, H. & E.

Fig. 7.—Liver-cell carcinoma. Numerous mitoses and one giant cell are visible. Animal killed at the 69th week from the beginning of the treatment. ×250, H. & E.

Fig. 8.—Cholangiocarcinoma. Animal dying at the 70th week from the beginning of the treatment. ×115, H. & E.
accidental induction of neoplasms is enhancing the incidence of a usual tumor, whereas carbon tetrachloride is having a somewhat different action in giving rise to liver-cell tumors in the hamster (4). Only of many cholangiomas and cholangiocarcinomas in the rat (9, 13), results in the occurrence of papillomas and carcinomas of the gall bladder and of the ampulla.

The hamster has generally been proved to be remarkably sensitive to chemical carcinogens, although the incidence was not high, cholangiocarcinomas were recorded. It might therefore be asked whether the acetylaminofluorene in these experiments from studies with chemical carcinogens generally had the expected course of events. We should like to record that experiments with chemical carcinogens generated effect in the rat, such as p-dimethylaminoazobenzene and 3'-methyl-4-dimethylaminoazobenzene. Well known for their pronounced carcinogenic effect in the rat, as it does in the mouse. In the rat, it induces tumors in the liver with the production of many eholangiomas and cholangiocarcinomas, and of the ampulla. The authors wish to thank Mrs. Sushila Conger for technical assistance and Mr. Manuel Suciro for histological preparations.

References

FIG. 9.—Adenocarcinoma of the gall bladder. Animal dying at the 59th week from the beginning of the treatment. ×30, H. & E.

FIG. 10.—Enlargement of Fig. 9. Tubular formations are included in an abundant fibrous connective stroma. The epithelial cells are rather well differentiated with hyperchromatic nuclei, several of them with mitotic figures. ×115, H. & E.

FIG. 11.—Papillomatosis of the common bile duct. Animal killed at the 83d week from the beginning of the treatment. ×250, H. & E.

FIG. 12.—Papillomatosis of the gall bladder. Animal dying at the 65th week from the beginning of the treatment. ×90, H. & E.
The hamster has generally been proved to be remarkably sensitive to chemical carcinogens, although its reactivity varies considerably from that of many species, including the induction of bladder tumours in the mouse and in the rat. Thus, certain azo dyes, such as p-dimethylaminoazobenzene and 3'-methyl-4-dimethylaminoazobenzene, are capable of producing hepatomas when administered to the rat, such as p-dimethylaminoazobenzene. The authors wish to thank Mrs. Sushila Conger for technical assistance and Mr. Manuel Suciro for histological preparations.

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Fig. 13.—Enlargement of Fig. 12. Areas suggesting an initial invasion of liver parenchyma. ×250, H. & E.

Fig. 14.—Adenocarcinoma of the ampulla. Invasion of the duodenal mucosa. Animal killed at the 67th week from the beginning of the treatment. ×250, H. & E.

Fig. 15.—Hemangioma of the brain. Animal killed at the 55th week from the beginning of the treatment. ×100, H. & E.

Fig. 16.—Poly cystic lesions of the pancreas. Animal dying at the 73rd week from the beginning of the treatment. ×100, H. & E.
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talk on April 13, 2017. © 1961 American Association for Cancer Research.
The hamster has generally been proved to be remarkably sensitive to chemical carcinogens, although its reactivity varies considerably from that seen in the mouse and in the rat. Thus, certain azo dyes, well known for their pronounced carcinogenic effect in the rat, such as p-dimethylaminoazo benzene and 3'-methyl-4-dimethylaminoazobenzene, do not give rise to tumors on feeding to hamsters (10, 13); on the other hand, carbon tetrachloride gives rise to many hepatomas in the hamster (4), as it does in the mouse. In the rat, it induces pronounced cirrhosis, but no tumors of the liver have been observed. Acetylamino fluorene, a carcinogen notable for the diversity of its action in many species, including the induction of bladder tumors in the rat (9, 12), results in the occurrence only of many cholangiomas and cholangiocarcinomas in the hamster (4).

In untreated hamsters used in these studies certain tumors occurred with some frequency; among these, although the incidence was not high, cholangiomas were recorded. It might therefore be asked whether the acetylamino fluorene in these experiments is enhancing the incidence of a usual tumor, whereas carbon tetrachloride is having a somewhat different action in giving rise to liver-cell tumors that are very rare in the control animals (6, 7). It might appear as though AAT is having both actions, which would be the expected course of events from studies with chemical carcinogens generally.

In our experiment, the major effect of AAT on the liver was the production of liver-cell tumors. However, in addition, there was a pronounced enhancement of cholangiomas with the production of two cholangiocarcinomas and, more noteworthy, of papillomas and carcinomas of the gall bladder and of the ampulla.

The three mammary carcinomas observed in this study may also be attributed to the action of this carcinogen, since this tumor has never been observed in our untreated controls and just one reference to a single case is reported in the literature (Habermann, quoted by [5]). Recent investigations from this laboratory have revealed that mammary carcinomas can be induced by parenteral administration of 20-methylcholanthrene and oral administration of urethan (2).

Thus, in the present study at least four tumors were recorded that have not been observed or are extremely rare in our untreated controls—namely, urinary bladder tumors, liver-cell tumors, mammary carcinomas, and gall bladder tumors. The other tumors seen at different sites cannot be considered to be specifically related to the carcinogen used, particularly when compared with the incidence observed in the controls.

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