

THE HISTOLOGICAL CHANGES IN THE LIVER AND IN THE URINARY BLADDER IN MICE FED BENZIDINE WHOSE BLADDERS CONTAINED GLASS BEADS*¹

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INTRODUCTION

In general, the fact that benzidine reveals a powerful carcinogenic activity upon the human bladder has been accepted by the accumulation of the epidemiological reports^{4,5,8)}. The attempts to induce experimental bladder tumors with benzidine were successful in dogs (1950), but a long period more than seven years was required²⁾. Moreover, the induction of bladder tumors in mice or rats by oral administration of benzidine had not been accomplished.

One of the bladder carcinogens, 2-naphthylamine, had been definitely proved experimentally to be capable of producing bladder cancer in dogs, hamsters, monkeys, rabbits and rats but not in mice. However, it was reported that the major urinary metabolites of 2-naphthylamine among these experimental animals didn't show any differences³⁾. The result of this report indicated that bladder cancer induction in mice by oral feedings of 2-naphthylamine would be possible.

Yoshida and associates (1973) obtained bladder tumors in mice fed 2-naphthylamine for 42 weeks, whose bladders contained small glass beads, and suggested that it was useful to prepare the glass-bead-bearing mice for a test of chemical carcinogens⁷⁾.

Therefore, the experimental study was

carried out to induce bladder tumors in mice fed benzidine, in which the bladder contained a glass bead.

This paper is concerned with the result of this experiment.

MATERIALS AND METHOD

Animals: Female mice of the *d, d*-strain, bred and maintained in KITAYAMA Animal Breeders Inc., Kyoto, were used. At the start of the experiment, they weighed approximately 25 g. They were divided into three groups of fifty mice each. Glass beads of approximately 45 mg in weight were inserted by cystotomy into the bladders of group 1 and 2 totally 100 animals. Fifty mice containing no beads were used as control.

Group 1: was bearing the glass bead and received commercial cube diet (CE-2).

Group 2: was bearing the glass bead, and was fed 0.2 % benzidine.

Group 3: was not bead bearing and was fed 0.2 % benzidine.

Diet: 0.2 % benzidine (purchased from Nakarai Chemicals Ltd., Kyoto), which was added to commercial feed (CE-2, CLEA Japan Inc., Tokyo) was administered to the mice in group 2 and 3 freely throughout an average of 280 days.

Preparation of tissues: The bladder was carefully inflated with Bouin's fluid per urethram before removal. The bladders were bisected sagittally and examined for calculi, tumor and other changes. Sections of the urinary bladder, liver and other organs were stained with hematoxylin and eosin by the usual method.

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The animals were excluded if they died during the experiment before 140 days.

Probabilities of statistical significance were evaluated by the exact method for 2×2 tables¹⁹.

RESULTS

A total of 45 mice of the 100 initially subjected to the insertion of glass beads (45.0%) and 15 mice of 50 no-bead-bearing mice (30%) survived a minimum of 140 days and were suitable for evaluation. The incidence of hyperplasia and bladder tumors observed within each group was shown in Table 1.

The tumor was not found in the bladders of the 16 mice bearing the glass beads (group 1), and no tumor in 29 mice bearing the glass beads and exposed to benzidine (group 2) was observed. No bladder tumor in 15 mice exposed to benzidine (group 3) was found.

Hyperplasia of the epithelium were common in the glass bead bearing groups but rare in group 3 which bore no beads. The highest incidence of hyperplasia was provided by group 2, where 28 of the 29 animals examined had hyperplasia (96.6%). This incidence, however, was not significantly different from that of the control group (87.5%) ($p=0.2452$). While

in group 3, this incidence revealed 40.0%, 6 of the 15 mice, and showed a statistically significant difference between group 2 and 3 ($p=5.85 \times 10^{-5}$).

Of fifty mice surviving 140 days or more, all fifty mice exposed to benzidine showed evidence of histological changes in the liver (Table 2).

As shown in Table 1 and 2, nineteen hepatomas were observed in these animals, fed 0.2% benzidine-containing diet, but cholangioma was not. In addition, cirrhosis of the liver and bile duct proliferation were not found. The histological appearances except hepatoma revealed hyperplastic nodules with atypical area, hyperplastic nodules, diffuse atypical area and necrosis, abscess, infection, etc. Metastasis was not noted.

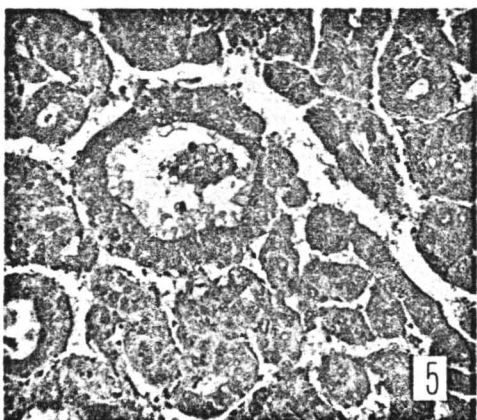
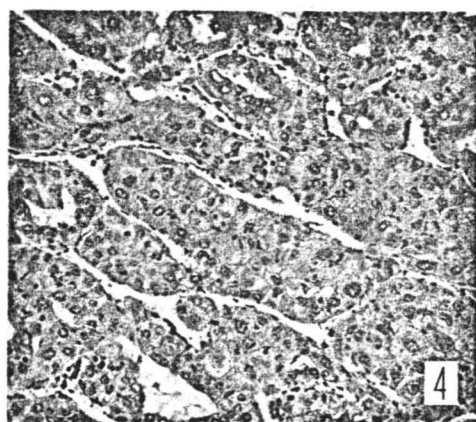
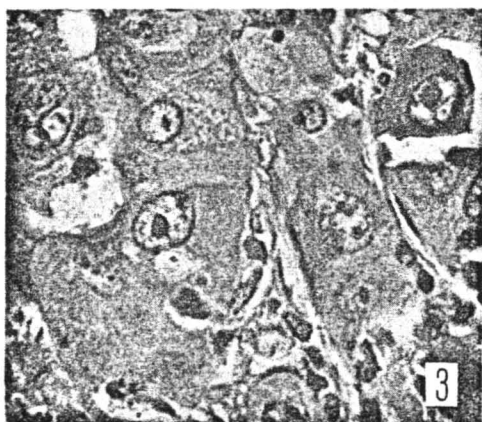
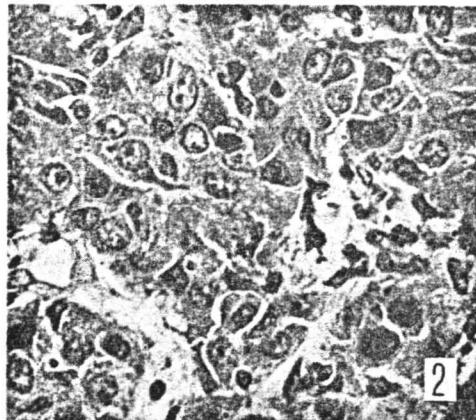
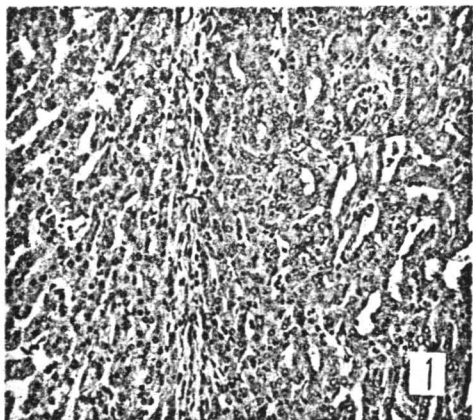
Histologically, the structure of the hepatoma varied. One type of tumor was encapsulated and showed cords of cells with poorly outlined cell margins and often very large with giant nuclei. The cytoplasm was stained faintly and was usually basophilic (Fig. 1, 2). The nuclei were large and vesicular with a single, large, prominent nucleolus (Fig. 3). The cells were arranged in cords like and resembling hepatic tissue (Fig. 4). Acinar structures were frequently encountered (Fig. 5).

Table 1. The effect of benzidine on the incidence of hepatoma and urinary bladder tumor.

Groups	Number of mice End/Start	Average survival days	Number of mice with bladder lesions		Number of mice with hepatoma (%)
			Hyperplasia (%)	Tumor	
1. Glass bead	18/50	358.6	14/16 (87.5%)	0	0/18
2. 0.2% BN+glass bead	32/50	276.2	28/29 (96.6%)	0	11/32 (34.4%)
3. 0.2% BN	18/50	247.6	6/15 (40.0%)	0	8/18 (44.4%)

Table 2. Incidence of the hepatic lesions.

	Group 1	Group 2	Group 3	Total (%)
Hepatoma	0	11	8	19 (38)
Hyperplastic nodules with atypical area	0	4	3	7 (14)
Diffuse atypical area	0	7	4	11 (22)
Hyperplastic nodules	0	7	3	10 (20)
Others	0	3	0	3 (6)



- Fig. 1. Encapsulated tumor
- Fig. 2. Irregular cells
- Fig. 3. Vesicular nuclei with prominent nucleoli
- Fig. 4. Cords of cells resembling hepatic parenchymal cells
- Fig. 5. Acinar formation in hepatoma

DISCUSSION

The fact that benzidine, a compound which is known to cause bladder cancer in man, induced bladder cancer in dogs after a latent period of more than 7 years demonstrated its carcinogenic properties²⁹. However, this result showed how inconvenient dogs are for the testing of carcinogenic activity of substances which might present a hazard to man. Therefore, an attempt to induce bladder cancer in other animals by benzidine was made. Reports from many investigators had proved that benzidine was carcinogenic for rats in several nonurogenous organs, liver, alimentary tract, auditory canal etc.^{5,6}, but tumors of the bladder had not been produced experimentally. Clayson showed that the implantation of a small glass bead into the lumen of the bladder increased the mitotic rate in the mouse bladder epithelium and it is necessary to increase the mitotic rate in order to induce tumors²⁹. Recently, the fact that 2-naphthylamine induced bladder cancer in mice containing glass beads in the bladder was revealed by Yoshida et al.⁷ However, the result of this experiment performed under the same conditions revealed failure to obtain bladder tumors with benzidine. Hyperplastic changes in the bladders were observed in glass bead bearing mice but the incidence was not significantly different from that of the control group.

The result described in this paper suggested that oral administration of benzidine caused tumors of the liver in female d, d strain mice. The failure to induce bladder tumors in mice may be explained by the hypothesis that they succumbed to hepatomas as Clayson described²⁹. This hypothesis was supported by his observation that a limited amount of chemical which did not lead to hepatomas followed by the implantation into the lumen of the bladder of a paraffin wax pellet was effective in

inducing bladder tumors.

The possibility that hepatoma or other liver damages influencing the induction of bladder tumors should be pursued in the next investigation of this carcinogen.

SUMMARY

Administration of benzidine followed by the implantation of a glass bead into the lumen of the bladder did not lead to tumor of the urinary bladder. There was no bladder tumor induction in mice given the chemical alone or in mice implanted with a glass bead alone. Hepatoma was observed in 19 out of 68 animals fed benzidine. Benzidine seems to be hepatocarcinogenic to female d, d strain mice but not carcinogenic to the bladder even under the above condition.

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