The Cancer Producing Properties of Azo Compounds in Mice

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Industrial cancer in its various forms has stimulated the researches which have led to the discovery of the cancer producing properties of the various carcinogens. Recognition of the high incidence of cancer of the scrotum of chimney sweeps led eventually to isolation from coal tar of 3,4 benzpyrene, the compound responsible for the carcinogenesis. For many years it has been recognized that workers engaged in the manufacture of dyestuffs had a higher incidence of cancer of the bladder, "aniline cancer," than comparative groups of the general population. Yoshida (8) in 1932 first produced a liver cell carcinoma in rats following oral administration of an azo dye. Subsequently Japanese researches (3) have shown that a number of simple azo compounds have carcinogenic properties; i.e., 4'-amino-2,3'-azotoluene (o-aminoazotoluene) which gives liver cell tumors when fed to rats, 2,3'-azotoluene which gives malignant urinary bladder tumors when administered orally to rats, and p-dimethylaminazobenzene which produces hepatomas and cholangiomas in the liver of rats. Shear (7) obtained liver cell carcinomas in the M (leaded) and A strains of mice following sub cutem injections of approximately 70 mgm. of o-aminoazotoluene. It has also been shown (1), following the same technic, that this same compound induces lung tumors in mice of strains A and C, both susceptible strains.

These results are of extreme interest because of the use of certain azo compounds as food coloring matter, as healing agents in salves, and in specialized dyeing processes and the common usage of p-amino benzene sulfonamide (sulfanilamide) and related products in combating various types of infection.

Materials and Methods

The two inbred strains of mice used for a study of the effect of simple azo compounds on internal tumors were the C57 black strain and the dilute brown Dba strain, both from the Roscoe B. Jackson Memorial Laboratory. The C57 black strain has a relatively high incidence of internal tumors (4). Nonpethelial tumors occur in approximately 14 per cent of the animals and a high percentage of these are liver tumors, vascular in origin. The epithelial tumors arising in this strain are limited chiefly to the liver. The dilute brown strain, on the other hand, is a line with high incidence of mammary carcinoma with comparatively few internal tumors other than those involving the spleen and lymph nodes. Tumors of the connective tissue are extremely rare in both strains. Two sublines of the C57 black strain, VI and X, and subline IV of the Dba strain were used exclusively. Equal numbers of mice of both sexes were used in each experiment.

The four azo compounds used, o-aminoazotoluene, p-dimethylaminazobenzene (butter-yellow), 4'-hydroxy-2,3'-azotoluene, and 2,3'-azotoluene, were obtained from the Eastman Kodak Co. and used without further purification (Fig. 1). The compounds were dissolved in a good grade of commercial olive oil and injected subcutaneously into the right axillary region of mice 2 months of age. A total of 5 mgm. was injected in 3 doses during the first 2 months. At 4 months of age a 5 mgm. pellet was inoculated by sterile trocar, making a total dosage for each animal of 10 mgm. Mice were kept as virgins.

Results

There appeared in a C57 black animal 105 days of age, 45 days following initial injection of o-aminoazotoluene, a subcutaneous growth which proved to be a fibrosarcoma. Of 30 mice which lived beyond the time of appearance of the first tumor, 13 (43.3 per cent) developed fast growing fibrosarcomas. The first fibrosarcoma in the butter-yellow series appeared in a C57 black mouse 112 days of age. In all, 6 of 29 mice in this series developed fibrosarcomas. At 175 days and 322 days respectively subcutaneous growths appeared in the 4'-hydroxy-2,3'-azotoluene and in the 2,3'-azotoluene series. Eleven of 30 (36.6 per cent) C57 black mice in the former and 5 of 20 (25 per cent) C57 black mice in the latter series developed subcutaneous growths. A total of 35 subcutaneous growths, all fibrosarcomas, appeared in 109 injected mice of the C57 black stock (Table II). Sarcomas arising in each of the four azo series produced 100 per cent takes of

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fast growing tumors when transplanted to the same strain.

The mean tumor incidence of the o-aminoazotoluene group was significantly higher than the mean tumor incidence of the butter-yellow series \((\bar{X}_D = 4 \times \sigma_D)\) and also significantly higher than the 2,3'-azotoluene series \((\bar{X}_D = 3 \times \sigma_D)\).

The mean latent period of fibrosarcoma induction in the o-aminoazotoluene series was significantly shorter than the mean latent period of any of the other three azo compound series. The differences of the mean latent periods between this group and the butter-yellow, 4'-hydroxy-2,3'-azotoluene, and 2,3'-azotoluene groups are 15.2 ± 3.9, 16.0 ± 3.8, and 21.9 ± 3.0 weeks respectively. No significant differences of the mean latent periods exist among the other azo groups.

Only one fibrosarcoma appeared among 106 mice of the Dba strain similarly treated and this appeared at 476 days in the butter-yellow series.

No tumors involving the urinary bladder or digestive tract were observed and the incidence of other tumors, chiefly lymphoblastoma in the C57 black strain and lymphoblastoma and breast tumors in the Dba strain were not significantly different from the spontaneous incidences given for these strains.

**Liver Lesions**

Thirteen hepatomas were found in the experimental series, 9 of these in the Dba strain in which internal tumors exclusive of lymphoblastoma are comparatively rare and 4 in the C57 black strain which has a relatively high incidence of liver tumors (Table II). The majority of these were observed in the o-aminoazotoluene series.

There were no visible macroscopic changes in the livers of mice which died or were sacrificed within 2 months following initial injections. Histologically there was a distinct swelling of liver cells with a hyperchromatic appearance of nuclei. There also appeared in localized regions large dark staining hepatic cells, some with extremely large nuclei, others binucleate. Following these changes there appeared regions of local fatty degenerative infiltration in advanced condition macroscopically visible in the form of a definite nodule (Figs. 4 and 5). In later necropsies there were evident regions of definite necrosis with apparent regeneration around these areas of hepatic tissue in the form of hepatic adenomas.

\(\bar{X}_D = \text{Difference of means; } \sigma_D = \text{Standard error of the difference.}\)

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**DESCRIPTION OF FIGURES 2 TO 6**

Fig. 2.—Fibrosarcoma in C57 black mouse following subcutaneous injection of o-aminoazotoluene. \(\times 300.\)

Fig. 3.—Fibrosarcoma in C57 black mouse following subcutaneous injection of butter-yellow. \(\times 300.\)

Fig. 4.—Nodule of liver in Dba mouse following subcutaneous injection of o-aminoazotoluene. \(\times 200.\)

Fig. 5.—Same as Fig. 4. \(\times 160.\)

Fig. 6.—Hepatoma in Dba mouse following subcutaneous injection of o-aminoazotoluene. \(\times 200.\)
All liver cancers observed were trabecular hepatomas. There was found only slight adenomatosis of the bile ducts and this condition was by no means general. Two hepatomas were associated with reticulo-endotheliomas involving the spleen, mesenteric-intestinal lymph nodes, and liver.

Unlike the changes occurring in the liver of rats there was little or no proliferation of fibrous connective tissue nor was there extensive proliferation of butter-yellow (3). Failure to obtain subcutaneous tumors in mice may have been due to selection of resistant strains.

In the above experimental series of mice o-aminoazotoluene was more effective in inducing both fibrosarcomas and hepatomas than the other three azo compounds used. Substitution of OH for NH$_2$ in the azotoluene molecule ($4'$-hydroxy-$2,3'$-azotoluene) did not significantly change the incidence of induction of

<table>
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<tr>
<th>Azo compound</th>
<th>Strain of mice</th>
<th>Number</th>
<th>No. of fibrosarcomas</th>
<th>Per cent fibrosarcomas</th>
<th>Mean age at appearance of tumor</th>
<th>Mean age at necropsy</th>
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<td>6</td>
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<td>Hepatoma</td>
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<td>Hepatoma, reticulo-endothelioma</td>
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bile duct epithelium toward cholangioma formation. Likewise tumor nodules were not multiple; not more than 3 nodules were found in any one liver.

**DISCUSSION**

Although the azo compounds have been used extensively both in feeding and sub cutem injection in rats by the Japanese (3) and to a lesser extent in mice (1, 2, 7), subcutaneous growths have not been commonly encountered. Schmidt (6) observed an infiltrating sarcoma on the neck of a mouse fed a Sudan dye. This appeared rather late in life and may have been spontaneous. A single sarcoma, partly fibrosarcomatous and partly reticulosarcomatous, was produced late in life following subcutaneous injections in the rat of sarcomas but there did result a statistically significant lengthening of the latent period. If the amino or hydroxy group is removed from the molecule there results a statistically significant decrease in sarcomas induced as well as a significant lengthening of the latent period.

Perlmann and Staehler (5) were of the opinion that substances were carcinogenic if they had aminoazobenzene in their structure and that the carcinogenic effect would be lost if H of NH$_2$ were substituted for other groups. However, the N-methylated compound, butter-yellow, used in this study did induce sarcomas but the carcinogenic effect as measured by tumor incidence and latent period apparently was significantly decreased by such substitution.
SUMMARY

1. Thirty-five fibrosarcomas have been induced in 109 mice of the inbred C57 black strain of mice following injections sub cutem of 10 mgm. of four related simple azo compounds.

2. In these experiments with mice, o-aminoazotoluene (4'-amino-2,3'-azotoluene) was a more potent carcinogen in inducing both sarcomas and hepatomas than p-dimethylaminoazobenzene, 4'-hydroxy-2,3'-azotoluene, and 2,3'-azotoluene.

3. Only one sarcoma appeared in 106 mice of the Dba strain similarly treated, this in the p-dimethylaminoazobenzene series.

4. The chief liver reaction in the mice used in this experiment was the formation of trabecular hepatoma with only a slight adenomatosis of bile ducts and little or no proliferation of connective tissue.

REFERENCES


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