Inhibition of Azo Dye Carcinogenesis by Thorotrast and Iron Oxide

J. D. SPAIN† AND C. C. CLAYTON

(Department of Biochemistry, Medical College of Virginia, Richmond, Va.)

Evidence that the reticulo-endothelial system (R.E.S.) may be involved in the carcinogenic process has been presented by Stern and Willheim (22) and by Jaffe (11). This system has also been implicated in the metabolism of certain fat-soluble materials such as Vitamin A (3, 10, 17) and cholesterol (3, 23) and the water-soluble vitamin, riboflavin (2, 9, 10). Since riboflavin is a factor in the carcinogenicity of the azo dyes (5, 6, 20) and since the lipide-soluble azo dyes may be taken up by the reticulo-endothelial cells of the liver (Kupffer cells), it was of interest to determine the effect of altered R.E.S. activity upon the incidence of liver tumors produced by carcinogenic azo dyes. Kinosito has reported that R.E. blockade "retards" the development of liver tumors (13) and, conversely, that it "facilitates" the development of the neoplasms (14) by the azo dyes.

MATERIALS AND METHODS

Young adult male rats (Holtzman Rat Co., Madison, Wis.), weighing approximately 200 gm., were kept in wire-bottomed cages in groups of five to eight, with food and water supplied ad libitum. Food consumption was measured at intervals during the dye-feeding period. The diet was similar to that used previously (19, 20) and had the following composition: vitamin-free casein, 12 per cent; Wesson salts, 4 per cent; corn oil, 5 per cent; and Cerelose (glucose monohydrate), to 100 per cent. Vitamins were added so that each kilogram of diet contained 2 mg. of riboflavin, 2.5 mg. of pyridoxine, 3 mg. of thiamine monohydrochloride, 7.5 mg. of calcium pantothenate, and 1 gm. of choline chloride. Halibut liver oil was given by dropper every 2-3 weeks. The azo dyes were incorporated into the diet as an acetone solution, mixed, and the acetone was allowed to evaporate before the addition of the corn oil. When 4-dimethylaminoazobenzene (DAB) was used, it was added to the diet at a level of 0.06 per cent and fed for 16-20 weeks. 3'-Methyl-4-dimethylaminoazobenzene (3'-Me-DAB) was fed at a level of 0.064 per cent of the diet for 8 weeks. In all tumor experiments the animals were maintained for an additional 8 weeks on the dye-free diet, after which interval they were killed for examination for liver tumors.1

In one series the effect of the removal of the spleen upon azo dye carcinogenicity was determined. The animals were kept 1 week postoperatively on rat chow and then placed on the experimental diet. In other studies, Thorotrast2 or 4 per cent Fe3O43 suspended in dextran was administered to influence the reticulo-endothelial system. The colloids were injected into the marginal tail vein, in the amounts and frequency indicated below. Certain groups also received only the colloid without the azo dye, since Thorotrast itself has been implicated in tumor formation (8).

Analyses of the livers were also made at various times for total and bound azo dye (21) and for riboflavin (1, 4) to determine the effect of the azo dye and Thorotrast on these components.

RESULTS

Tumor studies.—Experiment 1.—In this preliminary experiment the amount of Thorotrast was 1 ml. of the suspension/kg body weight at the start of the experiment and ½ ml/kg body weight every 14 days thereafter during the 16-week dye

1 We wish to thank Dr. G. Z. Williams for the microscopic examination of livers.

2 Colloidal thorium dioxide (25 per cent) in dextran, Heyden Chemical Corp., New York.


4 This experiment was done at the Biochemistry Department at the University of Wisconsin with Dr. C. A. Baumann and appeared as a portion of a Ph.D. thesis (C.C.C.), 1949.
feeding period. A group not receiving the dye re-
ceived a comparable amount of Thorotrast. There
was only a slight inhibiting effect of the adminis-
tered colloid upon the incidence of tumors (Table
1, Groups 1-4). Sixty per cent of the untreated ani-
mals and 47 per cent of those that received Tho-
rotrost developed tumors. However, of the non-
tumorous survivors, the livers of the animals re-
ceiving Thorotrast were normal, while half of
those not getting the colloid were cirrhotic.
Splenectomy appeared to have no effect on the in-
cidence of hepatic neoplasia or upon the appear-
ance of the nontumorous livers, compared with the
controls. In this experiment one of the eight sur-
viving rats receiving Thorotrast, but no dye, had
mild liver cirrhosis.

Experiment 2.—The Thorotrast and the iron
oxide suspension were administered every 14 days.
The initial injection was 0.4 ml., and all subse-
quent injections were 0.2 ml., irrespective of body
weight. This amount is approximately double that
given in the first experiment. The carcinogen,
DAB, was fed for 20 weeks. Both of these colloids
markedly reduced the incidence of liver tumors;
e.g., from 53 per cent of the controls having tu-

### TABLE 1

**EFFECT OF ALTERED RETICULO-ENDOTHELIAL FUNCTION UPON THE INCIDENCE OF LIVER TUMORS INDUCED BY FEEDING AZO DYES**

<table>
<thead>
<tr>
<th>Exper.</th>
<th>Group</th>
<th>Dye</th>
<th>Time fed (weeks)</th>
<th>R.E.S. treatment</th>
<th>Survival*</th>
<th>Tumors† (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>.06 per cent DAB‡</td>
<td>16</td>
<td>None</td>
<td>15/16</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>.06 per cent DAB</td>
<td>16</td>
<td>Splenectomy</td>
<td>11/18</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>.06 per cent DAB</td>
<td>16</td>
<td>Thorotrast</td>
<td>15/16</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>None</td>
<td>16</td>
<td>Thorotrast</td>
<td>8/10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>.06 per cent DAB</td>
<td>20</td>
<td>None</td>
<td>17/30</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>.06 per cent DAB</td>
<td>20</td>
<td>Thorotrast</td>
<td>8/15</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>.06 per cent DAB</td>
<td>20</td>
<td>FeO₂</td>
<td>12/15</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>None</td>
<td>20</td>
<td>Thorotrast</td>
<td>9/10</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>.064 per cent S'-Me-DAB§</td>
<td>8</td>
<td>None</td>
<td>16/30</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>.064 per cent S'-Me-DAB</td>
<td>8</td>
<td>Thorotrast</td>
<td>17/30</td>
<td>35</td>
</tr>
</tbody>
</table>

* Survival = Number alive at conclusion of experiment (8 additional weeks added to time in column 4) over
number at start.
† Per cent tumors = per cent of survivors with liver tumors.
‡ DAB = 4-dimethylaminoazobenzene.
§ S'-Me-DAB = 3'-methyl-4-dimethylaminoazobenzene.

### TABLE 2

**CHANGES IN LIVER TOTAL AZO DYE AND RIBOFLAVIN AS AFFECTED BY THOROTRAST AND TIME**

<table>
<thead>
<tr>
<th>Time</th>
<th>Regimen</th>
<th>Total azo dye (unit/gm.)</th>
<th>Riboflavin (mg/gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>S'-Me-DAB*</td>
<td>1480</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>S'-Me-DAB+Thorotrast</td>
<td>1690</td>
<td>19.2</td>
</tr>
<tr>
<td>3 weeks</td>
<td>S'-Me-DAB</td>
<td>1730</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td>S'-Me-DAB+Thorotrast</td>
<td>1895</td>
<td>19.3</td>
</tr>
<tr>
<td>4 weeks</td>
<td>S'-Me-DAB</td>
<td>1260</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>S'-Me-DAB+Thorotrast</td>
<td>1580</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>33.2</td>
<td>39.4</td>
</tr>
<tr>
<td>6 weeks</td>
<td>S'-Me-DAB</td>
<td>1410</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>S'-Me-DAB+Thorotrast</td>
<td>1185</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>27.8</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>Thorotrast</td>
<td>24.7</td>
<td>24.7</td>
</tr>
</tbody>
</table>

* S'-Me-DAB = 3'-methyl-4-dimethylaminoazobenzene.

Food consumption was comparable among
groups of all series, except the splenectomized ani-
mals in Experiment 1, which ate somewhat less.

**Analytical studies.**—Thorotrast was adminis-
tered weekly, with 0.4 ml. given the first week and
0.2 ml. the subsequent weeks. Animals were killed
for analyses after 2, 3, 4, and 6 weeks on the regi-
men. All experiments had four to five rats per
group, except at the 6-week period, when nine to
ten animals were used. Thorotrast had little effect
upon the concentration of total azo dye or of ribo-
favin in the liver (Table 2). Although the effect of
the colloid upon the average liver riboflavin concentration was minimal, it is of interest to note that the concentration of this vitamin in all the series was slightly less when Thorotrast was administered. About 60–65 per cent of the total azo dye was protein-bound, and this percentage was not altered by the injection of Thorotrast.

DISCUSSION

Small amounts of colloid injected intravenously will stimulate the reticulo-endothelial system, while large amounts may impair certain functions of these cells (11). Although no tests were performed on R.E. function in these experiments, the decreased incidence of liver tumors resulting from the feeding of the azo dyes in conjunction with R.E.S. treatment by intravenous colloid would seem to indicate a role for the Kupffer cells in this carcinogenic process. This decreased tumor incidence is of greater interest when considered with the effect of the Thorotrast upon riboflavin and liver azo dye. The colloid tended to lower liver riboflavin concentration slightly, which in itself is a tendency of the carcinogen, and from this evidence one would be inclined to think it should promote the development of neoplasia by the dye (5, 6). In a similar manner, Thorotrast did not lower the liver dye concentration, which is the usual situation when the incidence of tumors is decreased by a lower dietary level of carcinogen, (16), weaker carcinogens (16), added riboflavin (15), or high levels of dietary copper salts (12).

The attributed carcinogenic action of Thorotrast was not apparent in these experiments, although one animal not fed the azo dye did have a cirrhotic liver after receiving this agent. There was no additive or co-carcinogenic effect induced by the Thorotrast; in fact, the effect was more like that of other carcinogens such as methylcholanthrene (18) or nitrogen mustards (7) in inhibiting azo dye liver tumor formation. However, the smaller number of tumors when the apparently noncarcinogenic iron oxide was used as the colloid would indicate that the inhibitory effect was due to the R.E. function per se. The effectiveness of the iron oxide would also rule out the irradiation effect of Thorotrast which could possibly inhibit tumor development.

The fact that the liver azo dye was as high in the colloid-treated animals as in the noninjected would indicate that the colloid did not inhibit the uptake of dye by the liver. It may, however, affect the dye distribution in the liver among different cell types and thus lower concentration in certain cells which are normally the type more predisposed to the development of the neoplasm.

SUMMARY

The development of liver tumors in rats by the feeding of 4-dimethylaminoazobenzene or 3'-methyl-4-dimethylaminoazobenzene was inhibited by the intravenous administration of Thorotrast or colloidal iron oxide. The inhibition occurred, even though the colloids had little effect upon riboflavin or total azo dye concentration in the liver.

REFERENCES


Inhibition of Azo Dye Carcinogenesis by Thorotrast and Iron Oxide

J. D. Spain and C. C. Clayton


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/18/2/155

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.