The Effect of Rice Diets on the Formation of Induced and Spontaneous Hepatomas in Mice*

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By 1935, various investigators (9) principally Kinosita and his co-workers had established the hepato-carcinogenic activity of o-aminoazotoluene and N,N-dimethyl-p-aminoazobenzene. These azo dyes, administered in the diet or by subcutaneous injection, elicit changes—including neoplasms—in the livers of rats and mice and perhaps in the livers of other species. Subsequently it was established that in the rat the formation of hepatomas induced by o-aminoazotoluene or p-dimethylaminoazobenzene is strikingly dependent on the quality of the diet. For example, dried brewer's yeast (27, 5) or liver (18, 19) added in suitable amounts to a rice diet effectively inhibit, in comparison with an unsupplemented rice diet, the formation of liver cancer in rats being fed either of these azo dyes. The investigations of Sugiura and Kensler (26), of Rusch and associates (16, 22), and of others, employing diets consisting wholly or in part of purified food stuffs such as casein, crystalline B vitamins, etc., have led to the conclusion that diets containing high levels of protein and riboflavin protect against the toxic and carcinogenic action of p-dimethylaminoazobenzene in rats. The protective action of dried yeast or liver is generally attributed to their high content of protein and riboflavin.

The present studies with mice were initiated to determine whether in this species also the quality of the diet influences the formation of hepatomas, either induced by an azo dye or occurring spontaneously. Four experiments were performed: Hepatomas were induced by o-aminoazotoluene in three of the experiments. In the fourth, the spontaneous hepatoma was studied; that is, a mouse strain in which hepatomas form without exposure to a known carcinogen was employed, and no azo dye was administered. Dba strain mice were used in the experiments on induced hepatomas; spontaneous hepatomas are not usual in these animals even by 18 months of age. o-Aminoazotoluene was selected as the carcinogenic azo dye since mice have been shown to be more susceptible to the hepatoma inducing action of this compound than to that of p-dimethylaminoazobenzene, (2, 4). In the experiment with the spontaneous hepatomas, C3H male mice were used; these mice develop an appreciable incidence of spontaneous hepatomas by 13 months of age (1).

In each of the experiments two diets were compared: One consisted principally of rice, and the other was composed of a mixture of commercial foodstuffs with Purina fox chow as a base. Although the two diets differed in many particulars, principal attention is drawn to the differences in protein and riboflavin content because of the demonstrated significance of these dietary components in the formation of hepatomas in rats fed p-dimethylaminoazobenzene. The diets composed with Purina fox chow contained at least twice as much protein and 5 to 10 times as much riboflavin as the rice diets. From the extensive experience on the role of nutrition in the formation of liver cancer in rats fed p-dimethylaminoazobenzene, it was expected that hepatomas would appear more rapidly in the mice fed the rice diets.

Considerable work has been done in attempting to unearth the mechanisms involved in the dietary influence on the rate of hepatoma formation (22). It is probable that the rate of carcinogenesis in the liver, as in other tissues, is dependent, in part, on the concentration or amount of carcinogen to which the specific tissue is exposed. If so, the effects of diets on liver tumor formation may be dependent on the action of the diets in modifying the levels of the azo dye in the liver. Since the azo dyes are

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fat soluble, it appeared possible that the levels of liver carcinogen, (and, consequently, hepatoma formation) might be directly dependent on the concentration of lipids in the liver. For these reasons the effects of the experimental diets on the levels of o-aminoazotoluene and lipids in the liver were studied. Some of these data are presented because of their general interest even though they could not be correlated with the effects of the diets on hepatoma formation.

METHODS

All mice were of inbred strains raised in our laboratory. They were housed in groups of 5 in solid bottom cages with bedding of wood shavings, and were fed Purina fox chow checkers from weaning until transfer to the experimental diets.

The experimental diets, with the exception of the Purina fox chow checkers, were prepared by mixing the weighted ingredients with sufficient water to make an easily moulded mash; this was spread in pans, cut into blocks of appropriate size, and stored in a refrigerator at 40°F. The diets were prepared once each week and fed daily. For convenience, the diets composed principally of rice are designated as the R or rice diets, and those composed of Purina fox chow with or without other foodstuffs are indicated as C or chow diets. Water was available to the mice at all times.

During the course of the experiment the animals were weighed and inspected at biweekly intervals. All animals were examined post mortem. Hepatomas were recognized both grossly and by microscopic sections. Other details regarding the mice, the diets, and the administration of carcinogen are given in the descriptions of the individual experiments.

At various times during the experiment, mice of the several groups were sacrificed and their livers examined for morphologic changes and analyzed for lipids and o-aminooazotoluene. Occasionally, brain, kidney, abdominal depot fat, urine, and blood were analyzed. In animals which were injected subcutaneously, the residue of the injected oil and o-aminooazotoluene was found in cysts, in various stages of organization and adhering to the underside of the skin; in several series these "injection sites" together with the adjacent skin were analyzed to determine the rate of disappearance of subcutaneously administered dye. The analytical procedures were similar to those previously reported (23). For tissues other than blood, these procedures consisted essentially of the following steps: (a) Simultaneous extraction of the lipids and azo dye by means of hot acetone, ethyl ether, and petroleum ether, followed by gravimetric determination of the total lipids. (b) Separation of the o-aminooazotoluene from the lipids by chromatographic adsorption; diazotization and coupling of the isolated dye with p-naphthol, and colorimetric quantitation of the resulting bis-azo dye.

The procedure for extraction of the dye from the tissues failed completely when applied to blood, which was consequently extracted by a modification of the method of Miller and Baumann (14). Fifteen to 1.0 cc. of blood drawn from the vena cava into a heparinized syringe, was mixed with 2 cc. of 95 per cent ethanol and 8 cc. of 1N NaOH; this was extracted 3 or 4 times with 5 to 10 cc. volumes of petroleum ether; the o-aminooazotoluene present in the combined petroleum ether extracts was then determined by the colorimetric procedure indicated above. Recovery of known amounts (between 1 and 20 micrograms) of o-aminooazotoluene added to the various tissues ranged from about 85 per cent for liver to about 95 per cent for urine, blood, and depot fat. The sensitivity of the method was not limited by the amount of tissue analyzed but only by the amount of o-aminooazotoluene in the sample: Less than 0.5 microgram of the dye per analytical sample could not be determined satisfactorily.

EXPERIMENTAL

HEPATOMA FORMATION

Experiment 1.—Thirty dba male mice, 10 weeks of age, were divided into 2 equal groups. Group 1C was fed a diet consisting of 25 per cent Purina fox chow meal, 25 per cent skimmed milk powder, 3.1 per cent Kremax2 (partially hydrogenated cottonseed oil), and 46.9 per cent cornstarch; this ration contained 15 per cent protein and 5 mgm. riboflavin per gm. of food. Group 1R was fed a ration of 91 per cent white rice flour, 1.5 per cent dried brewer’s yeast, 1.5 per cent cod liver oil, 3 per cent Kremax, and 3 per cent salts (30), and contained 7 per cent protein and 1 mgm. riboflavin per gm. of food. The 1R diet was designed to simulate the brown rice plus carrot diet which, in rats fed p-dimethylaminoazobenzene or o-aminooazotoluene, promotes a high rate of hepatoma formation.

One month after institution of the diets, each mouse was injected subcutaneously in the interscapular area with 10 mgm. of o-aminooazotoluene6 dissolved in 0.2 cc. olive oil; 9 such injections were given at 1 month intervals. Eleven months after

6Furnished generously by Armour and Co., Chicago.

7o-Aminooazotoluene was obtained from Eastman Kodak Co.
the first injection of the dye, the surviving mice—13 in group 1C and 14 in group 1R—were sacrificed and examined for hepatomas. The observed incidences of hepatomas and the average weights of the mice during the course of the experiment are shown in Table I.

None of the mice fed the rice diet had visible hepatomas, while 9 (70 per cent) of the mice fed the chow ration had hepatomas ranging from 3 to 10 mm. in diameter. In this experiment, the rice diet did not augment the rate of formation of hepatomas, but actually had an inhibitory effect.

<table>
<thead>
<tr>
<th>TABLE I: THE EFFECTS OF RICE DIETS AND CHOW DIETS ON THE FORMATION OF INDUCED AND SPONTANEOUS HEPATOMAS IN MICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exper. number Hepatomas produced by Group number and diet Average weight of mice Months after beginning of experiment Mice with hepatomas over total mice sacrificed in indicated interval Final incidence of hepatomas</td>
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</tr>
<tr>
<td>1. 9 subcutaneous injections at 1 month intervals of 10 mgm. o-aminoazotoluene 1C-chow 1R-rice</td>
</tr>
<tr>
<td>2. 7 subcutaneous injections at 1 month intervals of 10 mgm. o-aminoazotoluene 2C-chow 2R-rice</td>
</tr>
<tr>
<td>3. Feeding of 0.05% o-aminoazotoluene for 11 alternate weeks over a period of 23 wks. 3C-chow 3R-rice</td>
</tr>
<tr>
<td>4. Spontaneously occurring in C3H male mice. 4C-chow 4R-rice</td>
</tr>
</tbody>
</table>

In both groups, the mice ate approximately 4 gm. of food daily and the rice diet supported body growth and "general health" as well as the chow diet.

**Experiment 2.**—Two groups of dba female mice, 3 months of age were employed: Group 2C (45 mice) was continued on the Purina fox chow checkers while group 2R (55 mice) was transferred to a diet of 97.75 per cent ground brown rice, 2 per cent olive oil, and 0.25 per cent cod liver oil. The fox chow checkers contained approximately 24 per cent protein and 5 μgm. of riboflavin per gm. of food; the brown rice ration contained only 8 per cent protein and 0.5 μgm. riboflavin per gm. of food.

Two weeks after institution of experimental diets each mouse was injected subcutaneously in the interscapular area with 0.2 cc. of olive oil containing 10 mgm. of o-aminoazotoluene. Seven such injections were given at approximately 1 month intervals. During the experiment some of the mice died or were sacrificed because of palpable mammary tumors or lymphomas (which occur spontaneously in dba mice); in addition, mice were sacrificed during the course of the experiment for analytical purposes and for study of morphologic changes in the livers. The experiment was terminated 52 weeks after the first injection of o-aminoazotoluene. The results are shown in Table I.

As in Experiment 1, the relatively inadequate rice diet did not result in an augmented rate of formation of tumors. Although the total incidences are nearly equal, among the mice surviving to 46 weeks of age slightly fewer of the group 2R mice had hepatomas—31 per cent compared to 50 per cent in group 2C. The difference, however, is not statistically significant and is considered suggestive only in connection with the results of Experiment 1.

The mice fed the brown rice ration did not grow as well as those fed the fox chow checkers. In addition the death rate in group 2R (rice diet) was appreciably higher than that in group 2C (chow diet); this was most noticeable during the first 26 weeks of the experiment when several of the 2R mice either died or were killed because of severe diarrhea or extensive dermatitis. The augmented death rate and depressed growth probably were due to the combination of diet and treatment with azo dye since the diet alone did not have these effects. This was determined in small groups of dba female mice fed the same diets for the same period of time but untreated with o-aminoazotoluene; a similar inference is indicated by the data of Experiment 4.

**Experiment 3.**—In the two preceding studies, the o-aminoazotoluene was administered by periodic injection. In the present experiment the dye was
incorporated in the diets. Each of the two groups consisted of 50 dba female mice, 14 to 16 weeks old when the experimental diets were instituted. Group 3C was fed a ration of 29 per cent Purina fox chow meal, 29 per cent skimmed milk powder, 3 per cent dried brewer's yeast, 37 per cent cornstarch, and 2 per cent olive oil; the protein content was 19 per cent and the riboflavin 10 μgm. per gm. of food. Group 3R was fed a diet of 97.75 per cent ground brown rice, 2 per cent olive oil and 0.25 per cent cod liver oil, containing 8 per cent protein and 0.5 μgm. of riboflavin per gm.

The animals were fed these diets without added o-aminoazotoluene for 4 weeks, after which the dye was added to the ration (in the olive oil) at the level of 0.05 per cent of the diet. The dye was incorporated in the diet for 1 week, omitted the following week, returned the next week, etc. This alternate incorporation and omission of the azo dye was continued for 21 weeks (dyed fed during 11 alternate weeks) after which the same diets, free of o-aminoazotoluene, were fed until the end of the experiment 24 weeks later. This procedure of intermittent feeding was adopted because continued feeding of the dye at the concentration employed was found, in a scout experiment, to be sufficiently toxic to cause the early death of most of the animals.

The mice of group 3C (chow diet) consumed approximately 3 gm. of food daily when fed the dye-free rations, and about 2 gm. when fed the ration containing the dye. Because of considerable scattering of food, the intake of the group 3R mice (rice diet) could not be satisfactorily estimated but seemed to be less than that of the mice of group 3C.

A few of the mice fed the chow diet developed mammary or lymphatic tumors, and were sacrificed; none died of causes attributable to the toxicity of the dietary regimen. On the other hand, during the period in which the o-aminoazotoluene was being fed, some of the mice receiving the rice diet developed severe dermatitis and died or were sacrificed, and others died showing no gross morphologic changes. The experiment was terminated 45 weeks after the first feeding of the azo dye. The weights of the mice during the experiment and the incidences of hepatomas are shown in Table I.

No hepatomas were observed in the mice dying before 36 weeks; at this time 35 of the mice fed the chow diet (3C), and only 24 of those fed the rice diet (3R), were alive. The incidences of hepatomas in the mice alive at the 36th week of the experiment (and sacrificed by the 45th week, when the experiment was ended) were 46 per cent in group 3C and 42 per cent in group 3R. As in Experiments 1 and 2, the rice diet did not augment the formation of hepatomas.

Experiment 4.—C3H male mice spontaneously develop hepatomas which begin to appear in appreciable number when the mice reach 11 to 12 months of age (1). Each of the two groups in the present experiment was composed of 20 C3H males, 5 to 7 weeks old. The diets employed were the same as those of Experiment 2: Group 4C was fed Purina fox chow checkers ad libitum, and group 4R was fed the rice diet. Throughout the experiment, the mice of both groups consumed an average of 3.8 to 4.2 gm. of food daily. The general condition of the mice was excellent, only 1 mouse dying (in 4C) previous to the termination of the experiment when the mice were 13 to 14 months of age. The results are given in Table I.

Again, as in the other 3 experiments, the mice fed the rice diet did not develop more hepatomas than those fed the chow diet. Statistically, there is no difference between the incidence of 5 per cent in the group fed the rice diet, 4R, and the incidence of 18 per cent in the group fed the chow diet, 4C. The brown rice ration supported growth and health as well as Purina fox chow checkers despite its significantly lower protein and riboflavin content.

LIVER LIPIDS

During the period in which o-aminoazotoluene was being administered in both Experiments 2 and 3, the total liver lipids of the mice sacrificed for analysis did not vary significantly with the diet. The average total hepatic lipids of the 4 groups ranged from 3.6 to 5.7 per cent of the wet weight of the livers. In the groups fed the chow diets and given the azo dye by subcutaneous injection (group 2C) or by feeding (group 3C), the total liver lipids of individual mice ranged from 3.4 to 5.0 per cent and averaged 4.0 per cent. In the mice of group 2R (rice diet, dye administered by injection) the liver lipids of individual mice ranged from 4.4 to 7.6 per cent, averaging 5.7 per cent; and in group 3R (rice diet, dye fed in the diet) the values ranged from 2.5 to 4.6 per cent, averaging 3.6 per cent.

In contrast, in Experiment 4 (in which o-aminoazotoluene was not used) the mice fed the brown rice diet developed mildly fatty livers—containing from 9.0 to 13.1 per cent fat, while those fed Purina fox chow checkers had livers with fat contents from 4.0 to 5.6 per cent.
Before investigating the effects of the diets on the accumulation of o-aminoazotoluene in the livers of mice, a few studies were conducted on the distribution and persistence of the dye in the animal body following subcutaneous injection. Only a few representative experiments are given here.

**Persistence in tissues following subcutaneous injection.**—Twelve C3H male mice were injected subcutaneously with 10 mgm. of o-aminoazotoluene; 1, 2, 4, and 6 days later they were sacrificed in groups of 3 mice. The analytical results are given in Table II.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 day (mgm.)</th>
<th>2 days (mgm.)</th>
<th>4 days (mgm.)</th>
<th>6 days (mgm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (per g.)</td>
<td>7.2-8.4 (7.8)</td>
<td>0.4-2.0 (1.0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood (per cc.)</td>
<td>7.5-12.2 (10.1)</td>
<td>10.0-14.5 (11.7)</td>
<td>7.1-9.7 (8.5)</td>
<td>6.9-10.2 (8.1)</td>
</tr>
<tr>
<td>Urine (per cc.)</td>
<td>16.4-22.0 (18.7)</td>
<td>2.1-8.0 (5.7)</td>
<td>0.8-2.0 (1.4)</td>
<td>0-0.8 (0.3)</td>
</tr>
<tr>
<td>Remaining at site</td>
<td>4500-6100 (4900)</td>
<td>1000-3600 (2600)</td>
<td>300-1200 (850)</td>
<td>89-122 (104)</td>
</tr>
</tbody>
</table>

In the many experiments of this kind, appreciable amounts of the dye were found in the livers 20 to 27 hours after the subcutaneous injection of o-aminoazotoluene. By 48 hours, the hepatic azo dye was much lower and by 72 hours after injection the levels found were in a range at the limit of sensitivity of the method (about 0.5 mgm. of dye). This decrease of o-aminoazotoluene concentration with time was observed in other tissues such as brain, kidney, and depot fat, but did not obtain for blood.

It is likely that the kidney is a principal route of excretion of not only the metabolic split products (24) but also of the unchanged azo dye. Thus the decreasing concentration of urinary o-aminoazotoluene paralleled the disappearance of the azo dye from the tissues and from the injection site.

Relatively rapid disappearance of o-aminoazotoluene from the site of injection was consistently observed. During the first 4 days following injection the dye decreased at a rate of about 50 per cent per day. The rate of removal then slowed down so that as late as 1 month following injection of 10 mgm. of o-aminoazotoluene 80 to 150 μgm. of o-aminoazotoluene remained at the site of injection.

In a number of animals of Experiment 2, sacrificed 1 month after the 7th and final injection of o-aminoazotoluene, from 80 to 250 μgm. of dye was found in the combined injection sites of the individual animals.

In contrast to the other tissues studied, the blood continued to show relatively high levels of o-aminoazotoluene for at least 6 days after administration of the dye. As with p-aminoazobenzene in blood (17), all the o-aminoazotoluene was found in the cells, and none in the plasma. If the method employed for determination of the dye in liver was applied to blood of animals treated with o-aminoazotoluene—i.e., extraction of the blood with hot acetone and ethyl ether and petroleum ether—none of the dye known to be present was recovered.

In order to extract the dye it was necessary to treat the blood with dilute NaOH, as described under Methods. It was also found that blood laked in water and dialyzed against running tap water for 24 hours lost none of the dye. On the other hand, o-aminoazotoluene added to blood (laked or whole), of animals which had not been exposed to o-aminoazotoluene, could be extracted quantitatively with acetone and ether. These observations suggest that there is formed, in vivo, some linkage, salt or otherwise, between o-aminoazotoluene and a blood cell component. It also may be inferred that the procedure of extracting with hot acetone and ether permits determination of the o-aminoazotoluene in tissues such as liver, kidneys, etc., with assurance that the residual blood in these tissues is not an interfering factor.

**Distribution in tissues following subcutaneous injection.**—In the following tabulation, the analytical data on two mice, one sacrificed 24 hours and the other 48 hours after injection of 10 mgm. of o-aminoazotoluene is given (Table III).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 day (μgm.)</th>
<th>2 days (μgm.)</th>
<th>4 days (μgm.)</th>
<th>6 days (μgm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (per g.)</td>
<td>4500-6100</td>
<td>1000-3600</td>
<td>300-1200</td>
<td>89-122</td>
</tr>
<tr>
<td>Blood (per cc.)</td>
<td>2600-2900</td>
<td>850-900</td>
<td>500-550</td>
<td>150-170</td>
</tr>
<tr>
<td>Urine (per cc.)</td>
<td>16.4-22.0</td>
<td>2.1-8.0</td>
<td>0.8-2.0</td>
<td>0-0.8</td>
</tr>
<tr>
<td>Remaining at site</td>
<td>4500-6100</td>
<td>1000-3600</td>
<td>300-1200</td>
<td>89-122</td>
</tr>
</tbody>
</table>

At least in these tissues, the dye appeared to be distributed in proportion to the tissue fat rather than to the total tissue. That even when based on the fat content, the concentration of o-aminoazotoluene in the kidneys was consistently high, may
Liver
Per gram of tissue 11
Per gram of tissue fat 300

Brain
Kidney
Depot fat
Liver
Per gram of tissue 23
Per gram of tissue fat 292

Kidney
Depot fat
Liver
Per gram of tissue 21
Per gram of tissue fat 400

Brain
Kidney
Depot fat
Liver
Per gram of tissue 204
Per gram of tissue fat 250

Brain
Kidney
Depot fat
Liver
Per gram of tissue 2.2
Per gram of tissue fat 55

Kidney
Depot fat
Liver
Per gram of tissue 3.9
Per gram of tissue fat 49

Brain
Kidney
Depot fat
Liver
Per gram of tissue 3.2
Per gram of tissue fat 76

Brain
Kidney
Depot fat
Liver
Per gram of tissue 28
Per gram of tissue fat 35

be due to the fact that the dye is excreted in the urine. On the other hand, possibly because of a smaller blood supply (6), the fat deposits almost always were found to contain less of the dye in relation to the tissue fat.

Effects of the diets on levels of azo dye in the livers.—In Experiment 2, in which the o-aminazoazotoluene was administered by subcutaneous injection, 6 mice of each group were sacrificed at 24 hours and 6 mice at 48 hours after injection. The range of values for the individual animals, and the average levels of o-aminazoazotoluene are given in Table IV.

<table>
<thead>
<tr>
<th>Group</th>
<th>Per gram of tissue</th>
<th>24 hours after injection</th>
<th>48 hours after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C-chow diet</td>
<td>1.8-10.3</td>
<td>1.5-5.2</td>
<td>1.5-5.2</td>
</tr>
<tr>
<td>2R-rice diet</td>
<td>5.7-11.0</td>
<td>2.0-4.7</td>
<td>2.0-4.7</td>
</tr>
</tbody>
</table>

It is apparent that the mice fed the two rations did not differ with respect to the levels of o-aminazoazotoluene in the livers 24 or 48 hours after injection. Whether differences existed at times later than 48 hours could not be ascertained because the levels observed were too low to be determined with satisfactory precision. The removal of the azo dye from the injection site, the distribution of dye in the tissues other than liver, and excretion in the urine, did not depend upon the diet: the differences between individual mice fed the same diet were so great that the average differences observed between the experimental groups had no significance.

When o-aminazoazotoluene was fed at a level of 0.05 per cent of the ration as in Experiment 3, the amounts of azo dye found in the livers of individual mice—sacrificed at various times during the day and during the experiment—were about 1 /gm. or less. In order to obtain reasonably accurate data on the distribution and levels of the dye in such animals it would have been necessary to employ pooled samples of the tissues from 3 or more mice. Nevertheless, such data as were obtained indicated that the dye concentrations in the livers of the mice in the two groups did not differ in a sufficiently consistent way to show any dependence on the diet.

MORPHOLOGY OF THE HEPATOMAS

The hepatomas, both the induced and the spontaneous, observed in these experiments, generally bulged above the hepatic surface as pink to grey, but sometimes yellow or red-brown, globular masses covered by the intact hepatic capsule. Often the induced hepatomas were multiple.

Sections stained with hematoxylin and eosin revealed that the tumors were sharply delineated, often compressing the adjacent hepatic tissue. Both the induced and spontaneous hepatomas were composed of cells which resembled those of the hepatic parenchyma. Although the cells generally were arranged in liver-like cords, there was a lack of true lobular pattern. Often the cells of the tumor would exhibit far fewer degenerative changes, such as fatty infiltration, than those of the liver, but in some instances the reverse was found.

These tumors have been studied by various investigators (3, 10, 25) and the question has arisen as to whether they should be regarded as extreme examples of active non-architectural regeneration or as tumors. Certainly there seems to be no good clinical or morphologic reasons to consider them malignant. A careful evaluation of the studies of others, and our own, suggests that the mouse hepatomas—either those induced by o-aminazoazotoluene or those occurring spontaneously (as in the C3H strain)—should be considered benign tumors.

DISCUSSION

In the four experiments concerned with hepatoma formation in mice, the animals fed rice diets developed either the same or a lower incidence of hepatomas than the mice fed the more adequate chow diets. In none of the experiments did the mice fed the rice diets develop a higher percentage of hepatomas. This experience is contrary to that which might be expected since such rice diets, in comparison with more adequate rations, effect a marked acceleration of liver tumor formation in rats fed p-dimethylaminoazobenzene.

In the three experiments with induced hepatomas, the unexpected failure of the rice diet to accelerate hepatoma formation in mice exposed to o-aminazoazotoluene may be due to (a) the difference in carcinogen—o-aminazoazotoluene was employed as the carcinogen in these studies while most of the
definitive dietary work with rats has been done with p-dimethylaminoazobenzene, or (b) the difference in species—the mouse responding differently from the rat.

The nature and extent of the modifying effect of diet on tumor formation is dependent on the particular carcinogenic agent utilized. Liver extract or increased dietary protein are reported to have no demonstrable effect on the formation of hepatomas induced by 2-acetylaminofluorene fed to rats (8); and riboflavin, hydrogenated coconut oil, or rice bran in the diet have a smaller and less consistent effect on tumor formation when m'- or o'-methyl-p-dimethylaminobenzene is employed to induce tumors than occurs with p-dimethylaminobenzene (7). In rats receiving o-aminoazotoluene, however, tumor formation is definitely modified by the dietary regimen. For example, rats fed rice containing the dye for about 8 months developed a high incidence of hepatic tumors, while no liver tumors were found in rats fed wheat containing the same amount of the dye for as long as one year (5, 29); 8 of 24 rats fed rice (and 0.02 to 0.1 per cent o-aminoazotoluene) for 1 year developed hepatomas, in contrast to only 1 of 38 rats fed the rice diet with 10 per cent liver powder and the same amounts of dye (18). Similarly, Maisin (11) failed to observe any hepatomas in 100 rats fed o-aminoazotoluene in a diet of wheat, wheat germ, and beef for 700 days, in sharp contrast to the results obtained by workers feeding the dye in a rice diet. Thus it is probable that hepatomas would form more rapidly in rats fed o-aminoazotoluene in a rice diet than in rats given the dye in a ration of fox chow alone or with added milk powder and cornstarch.

On the other hand, the diversity of the effects in mice and rats may be due to species differences in general, or more specifically to the difference in the nature of the types of liver tumors produced in the two species by the azo dyes. In the mouse the hepatomas, induced by o-aminoazotoluene or occurring spontaneously, are benign tumors the cells of which fundamentally resemble the normal parenchymal cells of the liver. On the other hand, in the rat the hepatomas induced by p-dimethylaminobenzene and other azo dyes are varied in type—bile-duct carcinoma and cystadenoma, liver cell carcinoma, and occasionally benign hepatoma—but the majority are malignant tumors (20, 21). It may be that the carcinogenic process involved in the production of benign hepatoma in the mouse is affected by dietary changes in an entirely different way than the carcinogenic process involved in the production of the malignant tumors in the rat.

The original object in performing analyses for hepatic lipids and o-aminoazotoluene was based on the anticipation of a striking dietary effect on hepatoma formation. Since the differences in hepatoma formation were not in the direction expected, and not of great magnitude, the information gained from such analyses is rather limited. No differences were observed between animals on the two types of diet with respect to the levels of o-aminoazotoluene in the liver, the distribution of the dye in the tissues, or the rate of removal of the dye from the site of injection. Nor were there any consistent differences in total liver lipids. These results were obtained in Experiments 2 and 3 in which the differences in incidences of hepatomas also were small. Consequently, the present experiments do not give any information on whether dietary effects on the formation of hepatomas induced in mice by azo dyes are mediated through an effect on hepatic lipids or on levels of carcinogen present in the liver.

That the incidence of spontaneously occurring hepatomas in C3H male mice is not augmented, but possibly depressed by the rice diet (Experiment 4), is in agreement with experiments indicating that a low protein (9 per cent casein) diet, sufficient to support growth and body health, retards the formation of spontaneous hepatomas in mice as compared with otherwise similar diets containing 18 or 45 per cent casein (28). It does not seem likely at present that the increased level of hepatic lipids observed in the mice fed the brown rice diet was of any significance in the possibly depressed hepatoma formation.

In the rat, the rate of formation of hepatic tumors induced by various carcinogens is known to be dependent on diet. The dependency on diet, however, appears to be modified by the nature of the carcinogen (7, 8). There is suggestive evidence that diets control hepatoma formation in rats through an effect on the levels of hepatic riboflavin (13), or on the levels of the carcinogen or its derivatives in the liver (12, 15, 23), although they may be only associated changes rather than causative factors. The experiments reported in this paper indicate that the species of animal (or perhaps the type of tumor induced) may be another factor, controlling the degree and even the direction of the change in hepatoma formation produced by dietary means.

**SUMMARY**

Mice fed diets composed essentially of rice developed hepatomas at the same or a slower rate
than mice fed a more adequate commercial diet containing higher amounts of protein, riboflavin, and other essential components. This was observed in two experiments in which hepatomas were induced by injection of o-aminoazotoluene, in one experiment in which hepatomas were induced by the feeding of o-aminoazotoluene, and in another experiment in which the spontaneously occurring hepatoma of the C3H male mouse was employed.

This observation, that a rice diet did not accelerate the formation of hepatomas in mice, is in contrast to the well-established fact that comparable rice diets do accelerate the formation of hepatomas, induced by p-dimethylaminoazobenzene in rats. It is suggested that this difference between mouse and rat may be a species difference—particularly related to the fact that the hepatomas induced in the mouse are benign while those induced in the rat are, in the main, malignant.

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The Effect of Rice Diets on the Formation of Induced and Spontaneous Hepatomas in Mice

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