Relative Influences of Natural and Semipurified Diets on Tumor Formation in Mice*†

HERBERT SILVERSTONE, ROBERT D. SOLOMON,‡ AND ALBERT TANNENBAUM

(Department of Cancer Research,§ Medical Research Institute, Michael Reese Hospital, Chicago 16, Ill.)

For a number of years this laboratory has been engaged in examining the influence of nutrition on the formation of tumors in mice. Before 1945 these studies were conducted with rations composed of natural foodstuffs, the principal basic components being either wheat grain or Purina Fox Chow meal and milk powder. To these were added cornstarch and partially hydrogenated cottonseed oil, which were varied depending on whether the experiment was concerned with the influence of caloric intake or the proportion of dietary fat. Investigations dealing with the roles of proteins, vitamins, and minerals were initiated in 1945. For these purposes diets of more definitive composition were required, a condition that was met by the use of semipurified diets composed of casein, cornstarch, partially hydrogenated cottonseed oil, supplements of vitamins, and a complete salt mixture.

The first of such experiments dealt with the formation of skin tumors in strain DBA male mice. One hundred twenty-six of the animals lived until they were 14–16 months old; and, in 81 per cent, benign tumors of the liver were observed. Grossly and histologically, the neoplasms were of the same type that occur spontaneously in strains C3H and CBA mice (4, 8). The high incidence of hepatomas in DBA males that had consumed semipurified rations contrasted strikingly with the infrequent occurrence of this tumor in comparable mice of the same strain, sex, and age, but fed diets of natural foodstuffs. Our experience with females of this strain was of a parallel nature: Hepatomas had never been observed in DBA females living 2 years or longer on natural diets, whereas with semipurified rations hepatomas were found in approximately 10 per cent of the animals living past 20 months of age. To our knowledge, an appreciable relative frequency of spontaneously occurring liver tumors in strain DBA mice has not been reported previously. For some time Andervont has been interested in the spontaneous hepatoma of the mouse—the occurrence and how it is influenced by age, sex, and other factors. Recently (1), after comparing his results on hepatoma formation in C3H mice of both sexes with those of our laboratory, he was led to comment upon the probable importance of the nature of the diet.

The present studies were designed to confirm the apparent augmentation of hepatoma formation in mice ingesting semipurified rations and to determine whether there was any influence on the formation of other tumors in mice, particularly the carcinogen-induced skin tumor and the spontaneous mammary carcinoma.

MATERIALS AND METHODS

The relative effects of a "natural" diet and a "semipurified" diet were compared in each experiment. The natural rations were either Purina Laboratory Chow checkers, or a "chow mash" compounded of Purina Fox Chow meal, 38 per cent; skimmed milk powder, 33 per cent; cornstarch, 25 per cent; partially hydrogenated cottonseed oil, 3 per cent; and dried brewers yeast, 3 per cent. Purina Laboratory Chow and Fox Chow are commercial animal foods containing fish and meat meals, wheat germ, alfalfa meal, soybean oil meal, bread, corn grits, wheat, brewers yeast, skimmed milk, molasses, beet pulp, bone meal, iodized salt, supplements of riboflavin and animal protein factor, and vitamins A and D feeding oils. They are generally considered adequate for growth, reproduction, and lactation of the mouse.

Two semipurified rations were used: Diet SP-1, designed to simulate the chow checkers, consisted of vitamin-free casein, 24 per cent; gelatin, 2 per cent; cornstarch, 64 per cent; salts, 4 per cent; partially hydrogenated cottonseed oil, 5 per cent; rice bran concentrate, 1 per cent; and the following amounts of vitamins per gram of diet: thiamine hydrochloride, 4 µg.; riboflavin, 1.5 µg.; pyridoxine hydrochloride, 3.5 µg.; calcium pantothenate, 8.5 µg.; niacin, 11 µg.; choline chloride, 800 µg.; 5 U.S.P. units of A; 0.8 U.S.P. units of D; and 0.11 mg. of E. Diet SP-2, constructed to simulate the chow mash ration, was composed of vitamin-free casein, 20 per cent; gelatin, 2

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‡ Present address: Terre Haute Medical Laboratory, Terre Haute, Ind.
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per cent; cornstarch, 67 per cent; salt; 5 per cent; partially hydrogenated cottonseed oil, 5 per cent; yeast extract, 1 per cent; and the following amounts of vitamins per gram of ration: thiamine hydrochloride, 15 ßg.; riboflavin, 7.5 ßg.; pyridoxine hydrochloride, 15 ßg.; calcium pantothenate, 45 ßg.; folic acid, 5 ßg.; biotin, 0.1 ßg.; niacin, 75 ßg.; inositol, 875 ßg.; p-aminobenzoic acid, 30 ßg.; choline chloride, 5.75 mg.; 5-iodoxine hydrochloride, 15 ßg.; calcium pantothenate, 45 ßg.; folic acid, 3 ßg.; biotin, 0.1 ßg.; niacin, 75 ßg.; inositol, 375 ßg.; thiamine hydrochloride, 15 ßg.; riboflavin, 7.5 ßg.; pyridoxine hydrochloride, 15 ßg.; calcium pantothenate, 45 ßg.; folic acid, 5 ßg.; biotin, 0.1 ßg.; niacin, 75 ßg.; inositol, 375 ßg.

The materials, preparation, storage, and feeding of the rations were similar to those previously described (7, 10).

Some features of the diets are compared in Table 1. The figures are averages based on manufacturers’ data and on direct analysis. The values for only four B vitamins are given, these being the ones for which the dietary requirements are relatively well defined. There also exist differences in kinds of protein, fat, carbohydrate, and minerals between the natural and semipurified rations. From what is now known, however, the actual differences in vitamin content and general composition are not of an order likely to affect the genesis of the tumors under study.

The mice were of strains DBA and C57, from colonies maintained in our laboratory by brother-to-sister mating. They were fed Purina Laboratory Chow checkers from weaning until they were transferred to the experimental rations and were housed in sets of five in cages with solid bottoms. The animals of each experiment were born within a span of a few weeks, and the two component groups were constructed by litter-mate distribution as far as was possible.

The routines of weighing and examining the animals for tumors and other deviations from normal appearance were those generally employed in our laboratory. The three tumor types studied were readily recognized grossly, their appearance and growth characteristics being valuable criteria. A considerable proportion of the lesions, including all that were questionable, were examined microscopically.

RESULTS

HEPATOMAS

The influence of the two diet types on the incidence of spontaneous benign hepatomas was examined in three separate experiments (Table 2).

Experiment 1.—Two groups of 30 DBA male mice, 15 weeks old and averaging 26 gm. in body weight, were employed. One group was continued on Purina Laboratory Chow checkers ad libitum. The other was transferred to semipurified ration SP-1; during the course of the experiment the average daily food intake of the individual mice of this group was 3.4 gm. Within 8 months the animals reached weight levels (Table 2, column 6) which, in general, were maintained. When the surviving animals were 67 weeks old, they were sacrificed and examined. No tumors were observed in the livers of those fed chow checkers, whereas 26 per cent of the mice fed ration SP-1 exhibited hepatomas (Table 2, column 8).

The formation of the spontaneously occurring hepatoma is remarkably sensitive to the influence of caloric intake and body weight, and attention might be drawn to the fact that the animals fed the semipurified diet weighed about 10 per cent more than those on the natural ration. Since the food consumption of the latter group was not determined, it is not known whether there was also a difference in caloric intake. Actually, there probably was no significant divergence in calories ingested, for, in our experience, when the two diets are consumed in amounts calculated to be equivalent, mice receiving the semipurified diets are 5–10 per cent heavier. Furthermore, a 10 per cent difference in body weight or caloric intake is not sufficient to produce the divergence in hepatoma incidence observed in the above study (11).

Subsequent experiments were designed, nevertheless, with the purpose of attempting to eliminate the possible effects of differences in caloric consumption and body weight. Apparently, the two diet types do not have the same nutritive value, possibly because of variations in digestibility, intestinal synthesis and destruction of vitamins, and other factors affecting the utilization of the ration. Thus, equivalence in all respects is impossible, and one must decide which is to be kept constant—caloric intake, body weight, or some other feature of the diet-animal relationship. In our opinion, body weight is the best single measure of nutrition in studies of this nature. Accordingly, in the following experiments the rations were fed in such amounts that the average body weights were about the same in both groups.

Experiment 2.—Two groups of 50 DBA male mice were utilized. When the animals were 15 weeks of age one group was transferred to chow mash and the other to semipurified diet SP-2. The mice on the chow mash consumed an average of 3.8 gm/mouse daily. In order to maintain the two groups at equal mean body weights, the animals fed SP-2 were restricted to an average daily ration of 3.4 gm., about 10 per cent less than that given the mice on the natural diet. Throughout the experiment, the differences between the average body weights of the two groups did not exceed...
1 gm. Forty-three of the mice fed chow mash and 45 of those fed SP-2 lived until they were 65 weeks of age. At this time 21 animals in each group were sacrificed (Table 2, Experiment 2a). Only one animal in the group fed chow mash had a hepatoma, and this was 2 mm. in diameter. In contrast, hepatomas were found in six of the mice that had been ingesting the semipurified diet. The remaining mice were allowed to live on (designated as Experiment 2b); the survivors were killed at 92 weeks of age. Hepatomas were found in four of the 22 animals on the chow mash and in thirteen of the 24 fed SP-2. When the results for the two age periods were combined, the animals given the natural diet had a relative frequency of hepatomas of only 12 per cent—significantly lower than the relative frequency of 42 per cent among the mice on the semipurified diet. Not only was there a decided difference in the number of mice with tumors, but, in addition, none of the former animals had more than one hepatoma, while one-third of the tumor-bearing animals on the SP-2 ration had multiple hepatomas.

Experiment 3.—To determine whether a similar influence occurs in another strain of mouse and whether the effect could be obtained if the diet differences were instituted at a later age, 34-week-old CSH males, averaging 39 gm. in body weight, were tested. The mice of one group were given and consumed 3.4 gm. of chow mash daily. The second group was fed ration SP-2 and was kept at a mean body weight approximately equal to that of the first group by giving them 3.2 gm. of food daily. The mice of both groups lost an average of about 2 gm. and then maintained these weights recrystallized rutin and 3 mg. of ascorbic acid, and (c) SP-2 with dried aqueous extract of liver (Wilson Laboratories 1:20 Liver Powder-Liver Concentrate, N.F.) equal to 5 per cent of the diet replacing the same amount of cornstarch. The diets were fed and consumed at a level of 3.4 gm/mouse daily. At the age of 67 weeks, when the surviving mice were sacrificed, the proportions with hepatomas were identical in the three groups (Table 3). Obviously, the supplements utilized did not inhibit the formation of tumors, and the experiment offers no clue to the factor responsible for the differential action of the two diet types.

### Table 2

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Mouse strain</th>
<th>Diet</th>
<th>Age in weeks at start of experiment</th>
<th>Examination for hepatomas</th>
<th>Body weights* (gm.)</th>
<th>No. animals†</th>
<th>Relative frequency of mice with hepatomas* (per cent)</th>
<th>Size of hepatomas (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DBA</td>
<td>Chow checkers</td>
<td>15</td>
<td>67</td>
<td>29.4 (26-36)</td>
<td>26</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>2a</td>
<td>DBA</td>
<td>SP-1</td>
<td>15</td>
<td>65</td>
<td>32.8 (28-41)</td>
<td>27</td>
<td>26</td>
<td>4.4 (2-14)</td>
</tr>
<tr>
<td>2b</td>
<td>DBA</td>
<td>SP-2</td>
<td>15</td>
<td>92</td>
<td>38.5 (32-46)</td>
<td>21</td>
<td>29</td>
<td>8.3 (3-18)</td>
</tr>
<tr>
<td>3</td>
<td>CSH</td>
<td>SP-2</td>
<td>34</td>
<td>57</td>
<td>36.0 (30-44)</td>
<td>22</td>
<td>18</td>
<td>4.0 (2-7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chow mash</td>
<td></td>
<td></td>
<td>36.5 (30-45)</td>
<td>24</td>
<td>54</td>
<td>10.1 (4-24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SP-2</td>
<td>37.5</td>
<td>50</td>
<td>38.7 (30-46)</td>
<td>47</td>
<td>28</td>
<td>7.3 (3-15)</td>
</tr>
</tbody>
</table>

* Body weights during last 3 months of life: group means and, in brackets, range of individual values.
† Number of animals surviving to the age indicated in column 5, except in 2b, where a number of animals had died with hepatomas between 76 and 94 weeks of age.
‡ Diameters of hepatomas: group means and, in brackets, range of individual values.

**Induced Skin Tumors**

The relative influence of natural and semipurified diets on the formation of carcinogen-induced skin tumors was investigated in the following two experiments (Table 4).
Experiment 5.—Two groups of 88 strain DBA male mice, 15 weeks old and averaging 26 gm. in body weight, were utilized. One was continued on the stock diet of Purina Laboratory Chow checkers, the other was transferred to semipurified ration SP-1. Two weeks later the mice of both

<table>
<thead>
<tr>
<th>Supplement*</th>
<th>Body weight†</th>
<th>Incidence of hepatomas§</th>
<th>Diameter of hepatomas (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>33.7 (28-41)</td>
<td>40</td>
<td>7.6 (3-16)</td>
</tr>
<tr>
<td>10 mg. rutin plus 3 mg. ascorbic acid</td>
<td>32.6 (25-42)</td>
<td>45</td>
<td>8.5 (3-15)</td>
</tr>
<tr>
<td>5 per cent liver powder (Wilson 1:20)</td>
<td>32.0 (26-40)</td>
<td>44</td>
<td>5.9 (3-12)</td>
</tr>
</tbody>
</table>

* The basic ration in all groups was the semipurified diet, SP-4.
† General body weight 10-14 months of age; group means and, in brackets, range of individual values.
‡ Number of mice alive when experiment was terminated. Mice were 67 weeks old.
§ Group means and, in brackets, range of individual values.

Balanced against the apparent validity of these findings are a few points that caution reservation. Among the animals on the semipurified ration, as many as one-third of the tumors degenerated into ulcers; early in their development they appeared grossly to be neoplastic and proved so on histologic study. Moreover, in half the 22 animals without tumors, 3- to 12-mm. ulcers were present in the interscapular area (the site of tumor development); clinical history and microscopic examination indicated that these were not neoplastic. Perhaps these denuded areas precluded the induction of tumors and thus were a source of bias in the tabulated results. Ulcerating degeneration of neoplasms and occurrence of non-neoplastic ulcers were entirely absent among the mice on the natural diet.

Experiment 6.—An attempt was made, in this study, to avoid the conditions of the preceding experiment which might have interfered with obtaining a valid result. Diets possibly more comparable were utilized, and differential action upon and injury to the skin were avoided. One hundred DBA male mice, 10 weeks old and 24 gm. in average body weight, were placed on the chow mash diet and given sixteen semiweekly applications of a 0.2 per cent solution of benzpyrene in acetone. Ten days after the final treatment with carcinogen, they were divided by a randomization procedure into two equivalent groups of 50 animals. One continued to receive chow mash, the other was fed semipurified ration SP-2. Thus, the animals ingested identical diets during the period of treatment with carcinogen, and any differential action of the two diet types operated only during the interval of tumor appearance—the so-called developmental stage (9).
The mice of the group given chow mash voluntarily consumed an average of 3.8 gm/mouse daily. Those fed SP-2 were restricted to an average daily ration of 3.5 gm. At no time during the course of the experiment did the mean body weights of the two groups differ by more than 1 gm.

The investigation proceeded smoothly, the general appearance and condition of the skins being excellent except for the development of tumors. These began to appear in both groups 21 weeks after the initial application of benzpyrene. The experiment was terminated at the 53d week.

There were only small differences between the two groups in both the incidence and the mean time of appearance of skin tumors (Table 4), but these were in the same direction as was observed in Experiment 5: a slightly lower relative frequency and a small retardation in time of appearance of tumors in the animals ingesting the semi-purified diet.

**Spontaneous Mammary Carcinoma**

*Experiment 7.*—The relative effects of natural and semi-purified diets on the development of this tumor type were investigated in only one experiment. 150 DBA strain females, 11 weeks old and 21 gm. in average body weight, were divided into two equivalent groups. One group was fed chow mash and the other, diet SP-2. The former voluntarily ingested an average of 3.1 gm/mouse/day; the mice given the semi-purified diet were restricted to an average daily intake of 2.9 gm. The groups maintained equivalent mean body weights which ranged between 32 and 33 gm. during the second year of the experiment. The resulting incidence of spontaneous mammary carcinoma and the mean age at which they appeared are given in Table 5. There was a small enhancement of tumor formation in the mice on the semi-purified diet, but the differences are not statistically significant. In both groups grossly visible lung metastases were found in about one-fourth of the animals with mammary tumors, and the average duration of life following appearance of a mammary tumor was about 11 weeks.

**Leukemia**

In our colony of DBA mice, leukemia develops in approximately 7 per cent of the animals before they are 1 year of age. The disease is grossly characterized by a striking enlargement of one but generally more of the following: thymus, spleen, liver, kidneys, and deep and superficial lymph nodes. The white blood cell counts are markedly elevated. In the six experiments in which strain DBA mice were employed, the nature of the diet had no consistent influence on the relative frequency of these neoplasms.

<table>
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<th>TABLE 5</th>
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<tr>
<td><strong>RELATIVE INFLUENCE OF NATURAL AND SEMIPURIFIED DIETS IN THE FORMATION OF SPONTANEOUS MAMMARY CARCINOMA IN STRAIN DBA FEMALE MICE (EXPERIMENT 7)</strong></td>
</tr>
<tr>
<td>(EXPERIMENT 7)</td>
</tr>
<tr>
<td><strong>Diet</strong></td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Chow mash</td>
</tr>
<tr>
<td>SP-2</td>
</tr>
</tbody>
</table>

* The diets were instituted when the mice were 11 weeks old.
† Initially there were 75 mice in each group. These were adjusted for deaths of nontumor animals during the experiment (8).

**DISCUSSION**

The results of the present experiments confirm our earlier observations that, among mice ingesting semi-purified diets, the incidence of spontaneous benign tumors of the liver is considerably greater than among animals consuming diets composed mainly of natural foodstuffs. The genesis of skin tumors induced by methylcholanthrene or benzpyrene is certainly not affected in the same way. Actually, there may be some relative inhibition of these neoplasms by a semi-purified diet, but the rather small differences observed in the present study do not firmly establish a differential effect between the two diet types. In this connection, Boutwell, Brush, and Rusch found about the same incidence of carcinogen-induced skin cancer in mice on a natural diet of wheat, milk powder, and salt, as in mice on a semi-purified diet (2). The formation of spontaneous mammary carcinoma may be slightly enhanced by a semi-purified diet, although again the differences observed were not of appreciable magnitude. Fenton, Cowgill, and Stone reported, for strain A females on semi-purified diets, a small acceleration in time of appearance of mammary carcinoma as compared to that.
in animals fed Purina Laboratory Chow checkers (6). The incidental observations on the occurrence of spontaneous leukemia indicate that the genesis of this tumor type is probably independent of whether the diet is constructed of natural or semipurified components.

That a particular experimental procedure has diverse influences, depending on the tumor type studied, is not an unusual finding. With regard to the effects of semipurified as compared to natural diets, the genesis of the spontaneous hepataoma was strikingly enhanced; that of the spontaneous mammary carcinoma possibly enhanced; that of the carcinogen-induced skin tumor possibly inhibited; and that of the spontaneous leukemia unaffected. Here again, as with variations in dietary protein and caloric intake (10-12), the hepatic tumor responded more readily than neoplasms of other tissues. This is further evidence for the view that the liver may be more sensitive than other tissues to the nutritional alterations influencing tumor formation.

Engel and Copeland have recently reported another instance of the differential influence of the two diet types (5). In a study of various tumors induced in rats by the feeding of the carcinogen 2-acetylaminofluorene, they found that the rate of appearance and incidence of mammary carcinoma was considerably greater in animals ingesting semipurified diets than in those fed various stock diets; a smaller enhancing effect was noted for liver and ear duct tumors, but apparently the production of all three types of neoplasms was modified in the same direction. That all three kinds of tumors responded similarly may be less the result of effects upon the tissues of the host or the carcinogetic process than a consequence of diverse influences, depending on the tumor type studied, is not an unusual finding. With regard to the effects of semipurified as compared to natural diets, the genesis of the spontaneous hepataoma was strikingly enhanced; that of the spontaneous mammary carcinoma possibly enhanced; that of the carcinogen-induced skin tumor possibly inhibited; and that of the spontaneous leukemia unaffected. Here again, as with variations in dietary protein and caloric intake (10-12), the hepatic tumor responded more readily than neoplasms of other tissues. This is further evidence for the view that the liver may be more sensitive than other tissues to the nutritional alterations influencing tumor formation.

Returning to our results with the spontaneous hepataoma, it seems that their increased frequency in mice fed semipurified diets cannot be attributed to a relative deficiency of any of the known factors necessary for growth and reproduction; these were amply supplied in diets such as SP-2 and SP-3 containing 5 per cent aqueous extract of mammalian liver. Our present experience does not support any statement as to mechanism beyond this purely negative conclusion. Collateral observations and analytical determinations have shown that the livers of mice on semipurified diets are larger and paler and contain more fat than those of controls fed natural diets. These and other findings relating to body structure and function will be reported elsewhere. It may be stated here, however, that they do not elucidate the factors responsible for the differential development of certain tumors in mice consuming natural and semipurified diets. The problem awaits further exploration.

SUMMARY

The present studies concerned the relative influences of natural and semipurified diets on the genesis of tumors in mice. The natural diets were either a commercial food alone or mixed with milk powder, cornstarch, fat, and brewers yeast. The semipurified rations consisted mainly of casein, cornstarch, partially hydrogenated cottonseed oil, a salt mixture, and supplements of vitamins.

The genesis of the spontaneous benign hepataoma in strains DBA and CSH mice was notably enhanced in animals fed the semipurified rations. This effect was not a consequence of obvious differences in caloric intake or body weight, or in the proportions of protein, fat, vitamins, and minerals in the diets. In experiments with the spontaneous mammary carcinoma and the carcinogen-induced skin tumor only small effects were obtained.

ACKNOWLEDGMENTS

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