The Dependence of the Genesis of Induced Skin Tumors on the Fat Content of the Diet during Different Stages of Carcinogenesis

Albert Tannenbaum, M.D.

(From the Department of Cancer Research, Michael Reese Hospital, Chicago 16, Illinois)

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The genesis of induced skin tumors is facilitated by a diet rich in fat. Since the results in the preceding reports (7, 8) indicate that carcinogenesis proceeds through two distinguishable phases, (a) an initiatory, or preneoplastic, stage, which under favorable conditions leads to (b) a developmental or neoplastic stage that culminates in the appearance of a tumor, it becomes of interest to ascertain the stage or stages of carcinogenesis in which the facilitating effect of a high-fat diet occurs.

The results of other investigators, who have reported considerable increases in the incidence of induced skin tumors in animals consuming a high-fat diet, have been reviewed (6). In our experiments the increase in formation of skin neoplasms, though real, was of a decidedly lower magnitude. The present communication attempts to explain the reasons for this difference.

The experiments reported in this paper were performed according to the technic described in a previous work (7) on calorie restriction. An arbitrary separation of the phases of initiation and development is achieved by terminating the application of carcinogen just before the first tumor is expected. Under these conditions, the painting period roughly conforms to and contains what may be considered the initiatory phase, while the period following the painting covers the major portion, if not all, of the developmental phase. The effect of a fat-enriched diet on each of the two stages of carcinogenesis is determined by comparing the tumor incidence of mice receiving a high-fat diet during the limited painting period with the tumor incidence of mice receiving the high-fat diet only in the period following the application of the carcinogen.

METHODS

Pure-strain mice, obtained originally from the Roscoe B. Jackson Memorial Laboratory and inbred in our laboratory, were used. Each experimental series was divided into groups of 50 male mice equivalent as to age and weight. Many of the animals in one group of an experiment had litter mates in the other group or groups. The animals were housed 5 to a cage. Each was numbered and a separate record of its progress was kept.

The animals were inspected for tumors and weighed at 2 week intervals; the weights of those bearing carcinomas were not included in the group averages. Papillomas or carcinomas were distinguished by their gross appearance and by palpation; none of the papillomas regressed and eventually many were replaced by carcinomas (7). The tumor count and time of appearance refer to the first perceptible tumor, papilloma or carcinoma, that each mouse developed. Percentages of tumor formation were computed on the basis of the number of animals alive at the time the first tumor appeared in any group of an experiment (effective total) and also on the basis of an adjusted total described by Bryan and Shimkin (1), that accounts for the deaths of nontumor animals during the period in which tumors appear.

At postmortem, the tumors were sectioned and examined in the gross; preparations for histological study were made of many selected at random, and of all lesions about which doubt existed. The results of the histological studies indicate that the gross examinations were reliable.

Two diets were employed. The control diet (low-fat) consisted of 1.4 gm. Purina dog chow meal, 0.9 gm. skimmed-milk powder, and 1.9 gm. cornstarch per day for each animal. The high-fat diet was prepared by substituting 0.9 gm. of hydrogenated cottonseed oil† for the 1.9 gm. of starch. Thus, the two diets contained approximately equal quantities of protein, vitamins, and minerals, and differed only in their fat and carbohydrate content. The approximate compositions of the diets in grams per mouse per day were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>High-fat (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gm. Per cent</td>
<td>Gm. Per cent</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>0.62 15</td>
<td>0.62 19</td>
</tr>
<tr>
<td>Fat</td>
<td>0.08 2</td>
<td>0.98 31</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>2.92 70</td>
<td>1.22 38</td>
</tr>
<tr>
<td>Ash</td>
<td>0.16 4</td>
<td>0.16 5</td>
</tr>
</tbody>
</table>

† The hydrogenated cottonseed oil (Kremit) was generously furnished by Armour and Company.
A week’s supply of the weighed dietary constituents was mixed with sufficient water to form an easily molded mash, which was cut into equal blocks, permitted to dry over night, stored in a refrigerator, and fed daily. The actual average food consumption (reported under Results) of each experimental group was ascertained by weighing back each week the food left in the cages. All animals had free access to water.

For convenience the following letter combinations are employed to designate the dietary sequences used in these experiments: CC, control diet throughout the experiment; CF, control diet during the period of carcinogen application, high-fat diet in the succeeding period; FC, high-fat diet during the period of carcinogen application, control diet in the succeeding period; and FF, high-fat diet throughout the experiment.

### RESULTS

**Experiment 1.**—Two groups of mice, each consisting of 50 C57 black male mice, born within a span of 3 weeks, were transferred to their respective control and high-fat diets when they averaged 10 weeks of age. After 6 weeks they received the first of 26 semi-weekly applications of a 0.3 per cent solution of 3,4-benzpyrene in benzene. At each application 1 drop of the solution, containing about 0.05 mgm. of the carcinogen, was applied to the skin of the interscapular region by means of a dropping pipette.

No tumors were present at the time of the last application of the carcinogen (13th week after the first application). Two days later the following dietary changes were made: The group (S3) that had been fed the control diet was now transferred to the high-fat diet, while the group (S2) that had been on the high-fat diet was now given the control diet. The groups were designated as S3-CF and S2-FC respectively.

The mice of both groups consumed approximately 3.5 gm. per day when on the control diet and 2.8 gm. when on the high-fat diet. These quantities of food contain approximately the same amounts of essential dietary constituents (protein, vitamins, and minerals); the caloric intake is higher, and the mean weights of the mice 1 to 3 gm. greater, on the fat-enriched diet.

The results are given in Table I. At the end of the experiment, 49 weeks after the first application of the carcinogen, 18 mice of the S2-FC group had developed tumors, in comparison with 23 in the S3-CF group. A difference of 5 tumors (approximately 10 per cent) corresponds to the degree of augmentation obtained previously by the use of high-fat diets in 5 different experiments (6); the augmentation, though not of large magnitude, occurred consistently and with no exception, and therefore must be considered real. The mean times of appearance of the tumors were 33 ± 2.7 and 28 ± 2.0 weeks respectively. Thus a high-fat diet promotes a higher incidence of benzpyrene skin tumors when fed during the second, or developmental, stage of carcinogenesis than when fed during the initiatory stage (period of carcinogen application).

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet Pattern</th>
<th>Number of Mice Alive at End of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2-FC</td>
<td>high-low</td>
<td>28</td>
</tr>
<tr>
<td>S3-CF</td>
<td>low-high</td>
<td>21</td>
</tr>
</tbody>
</table>

### TABLE 1: DEPENDENCE OF THE INCIDENCE OF INDUCED SKIN TUMORS ON WHETHER A HIGH-FAT DIET IS FED DURING OR AFTER A LIMITED PERIOD OF CARCINOGEN APPLICATION

<table>
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<td>21</td>
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**Experiment 2.**—In this experiment 4 equivalent groups of 50 male dba mice, 10 weeks of age, were employed. Two of the 4 groups were placed on the control diet, while the other 2 were given the high-fat diet. After 4 weeks the first of 19 semi-weekly applications of a 0.3 per cent solution of 3,4-benzpyrene in benzene was applied to the skin of the interscapular region by means of a dropping pipette.

Two days after the last application of the carcinogen, and before tumors had developed in any of the mice, the following dietary changes were made: One of the groups (S10) being fed the control diet was continued on this ration while the other 3 were given the high-fat diet. After 4 weeks the first of 19 semi-weekly applications of a 0.3 per cent solution of 3,4-benzpyrene in benzene was applied to the skin of the interscapular region by means of a dropping pipette.

The mice of both groups consumed approximately 3.5 gm. per day when on the control diet and 2.8 gm. when on the high-fat diet. These quantities of food contain approximately the same amounts of essential dietary constituents (protein, vitamins, and minerals); the caloric intake is higher, and the mean weights of the mice 1 to 3 gm. greater, on the fat-enriched diet.
In the same order the mean induction times are 31 ± 1.8, 27 ± 2.2, 31 ± 2.6, and 30 ± 1.9 weeks.

The mice of the S12-FC group (high-fat diet during the period of carcinogen application only) did not develop any more tumors than did the control group (S10-CC), indicating that the high-fat diet had no augmenting effect during the initiatory phase. The other 2 groups, S11-FF (high-fat diet throughout experiment) and S13-CF (high-fat diet during the second period only), exhibited the augmenting effect of fat to approximately the same degree, suggesting that it is the developmental phase of carcinogenesis that is stimulated by the fat-enriched diet.

**DISCUSSION**

The effects of feeding a high-fat diet during varying periods of the process of carcinogenesis was first reported by Lavik and Baumann (3). They painted groups of mice with a 0.3 per cent solution of methylcholanthrene in dioxan for 2 months. The experimental groups differed only in that they were fed a high-fat diet during arbitrary periods of the experiment. It was found that the high-fat diet was most effective in causing an increase in tumor formation when it was fed throughout the experiment; when fed during only a limited period its effectiveness was of smaller magnitude. The authors conclude that "the highest incidence of tumors appeared when fat was given throughout the experiment, but measurable increases were also observed when fat was fed either during the first 2 months while the carcinogen was applied, or after the second month; e.g., after the application of hydrocarbon had ceased. The most effective [limited] period was 1½ to 3 months after the beginning of the application of hydrocarbon."

Our results tend to confirm the work of Lavik and Baumann, since in both investigations the high-fat diets were more effective when fed after the period of carcinogen application than when fed during carcinogen application. However, there are some differences in the results obtained by the two laboratories. In our experiment: (a) feeding the fat-enriched diet only after the application of the carcinogen had been terminated was as effective as feeding it throughout the experiment; (b) no augmenting effect was obtained when the high-fat diet was fed only during the period of carcinogen application; and (c) the fat-enriched diet fed throughout the experiment did not cause a large increase of tumors.

In order to facilitate comparison of the experiments performed by the two laboratories, the results are listed in the following table:

<table>
<thead>
<tr>
<th>Table II: Stage in Which a Fat-Enriched Diet Is Fed as a Factor in Facilitating the Formation of Skin Tumors Induced by a Limited Period of Carcinogen Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
</tr>
<tr>
<td>Low-fat control.</td>
</tr>
<tr>
<td>High-fat throughout experiment.</td>
</tr>
<tr>
<td>High-fat during application of carcinogen only.</td>
</tr>
<tr>
<td>High-fat after application of carcinogen only.</td>
</tr>
</tbody>
</table>

It is probable that these differences are real, and that they are not due to any fundamental error on the part of either laboratory, but to the variables and conditions of the experiments. The most important difference between these two experiments is the degree of oiliness of the skin produced by the contact with a fat-enriched diet; i.e., the fur of Lavik and Baumann's mice was probably oily, while the fur of our mice was not.

It is known (4, 9) that fat applied locally to the skin of mice in the region where the carcinogen is applied increases tumor formation. The mechanism of this effect is as yet unknown, but it may be due to the solvent action of the fat fixing the carcinogen to the painted area, thus permitting less to be lost mechanically (through desquamation, movement, or rubbing) or to easier passage of the carcinogen through the epidermis.

Watson and Mellanby (9), in pioneer experiments, showed that tarred mice maintained on a diet containing a high proportion of butter-fat developed many

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2 Present author's addition.
more tumors than did controls on a low-fat diet. The coats of the animals on the high-fat diet assumed a characteristic oiliness. Such a high-fat diet produced both an oiliness of the skin and an increase of tumor formation comparable to that seen in groups of animals maintained on the control diet, but whose skins were treated with either mouse fat or olive oil preceding each application of the carcinogenic tar. In a recent publication (4) Lavik and Baumann have also come to the conclusion that much of the effect of the high-fat diets they employed was exerted locally through oiliness of the skin, arising from local contact with the fatty ration.

In contrast, it is important to stress that the coats of the animals in our experiment showed no visible greasiness, even though the fat content of the high-fat diet was higher than in those used by Watson and Mellanby and by Lavik and Baumann. This is probably due to our method of preparing the diet.

The small but consistent increase in tumor formation on a high-fat diet reported by us previously (6) and in this communication, did not occur through the medium of oily skins. Gross examination revealed no significant differences in the skins of the control and fat-fed groups, and examination of sections stained for fat confirmed this impression. It should be emphasized again that the mice of the S12-FC group (fed the high-fat ration during the period of carcinogen application) developed no more tumors than the mice consuming the control diet (S10-CC). This suggests that the increase in tumor incidence in the S11-FF group is not due primarily to an increase of fat on the skin during the period of carcinogen application.

It is likely that the increase in tumor formation in groups S11-FF and S13-CF was due to fat absorbed from the gastrointestinal tract and carried to the skin, where it acted in some unknown way. This effect probably exerts its influence on the transition of the pre-neoplastic to the neoplastic stage.

Thus, fat in high concentration in the diet may have two separate effects: (a) If, through physical contact, it produces an oiliness of the skin in animals that are being subjected to carcinogen application, it facilitates the absorption of the carcinogen and thus produces a considerable increase in tumor formation; (b) the dietary fat is absorbed through the gastrointestinal tract producing systematically a small but definite cocarcinogenic effect on tumor formation. This concept is an example of the general hypothesis stated in an earlier paper (6), dealing with the effects of a high-fat diet on spontaneous mammary tumors, primary lung tumors, and induced skin and subcutaneous tumors. It was stated “that the effects reported may be the resultant of two properties of fat: (a) ‘solvent action’ on the carcinogen; and (b) ‘cocarcinogenic action’ on the developing tumor cell.”

On the basis of the facts and hypothesis discussed above the differences between our results and those of Lavik and Baumann are to be expected. Since the coats of Lavik and Baumann’s mice were oily, the results of these investigators can be explained as the joint effect of both solution of the carcinogen in the greasy skin during the pre-neoplastic stage, and a cocarcinogenic action in the subsequent neoplastic stage. In contrast, our results were obtained with mice whose fur did not become greasy from contact with the diet, and are due only, or mainly, to the cocarcinogenic action of fat in the neoplastic stage; in other words, to nutritional variation and not to a change in oiliness of the coat. It may be helpful to emphasize the necessity of distinguishing between the results that may be obtained under these two conditions.

In addition to giving a better understanding of the effects of fat solvents and high-fat diets on epidermal carcinogenesis, the experiments substantiate the importance of differentiating the stages of carcinogenesis. In a previous publication (7) it was shown that 3 entirely different means, wound healing, croton resin, and caloric restriction, exert a considerable effect on the incidence of neoplasms when applied during the developmental phase, while they exert little or no effect when applied during the initiation phase. To these factors one may now add the augmenting effect of a high-fat diet eaten during the developmental phase of carcinogenesis.

It is probable that at the present time there are not enough facts to explain the promoting effect of a high-fat diet. In both this and an earlier communication we have expressed our general views. Lavik and Baumann (4) have suggested that “much of the systemic or cocarcinogenic activity of dietary fat, if not all, is exerted through the medium of a voluntarily increased intake of calories on diets high in fat.” We cannot agree with this conception, since high-fat diets have no effect on the incidence of lung tumors (6); this should be augmented if the effects of such a diet were due to increased caloric intake. In addition, there are data (unpublished) suggesting that tumor formation is accelerated by fat-enriched diets of caloric levels equal to or even less than those of low-fat control diets.

From a practical and clinical viewpoint it becomes important to differentiate between: (a) the results caused by external application of lipids and organic solvents during exposure to carcinogenic agents, and (b) the results of ingestion of a high-fat diet. Fats, oils, and solvents may have a decided augmenting effect on the production of industrial skin cancers or tumors produced elsewhere by agents absorbed through the skin. On the other hand, the ingestion of high-fat diets may produce its augmenting effect independently of oily agents on the skin surface.

In a review of insurance statistics on the relationship
of body weight to cancer (5) it was shown that persons
of average weight or less are not so likely to develop
the disease as are those who are overweight. If the
results on the relationship of nutrition to cancer in-
cidence in mice can be carried over to man it follows
that a calorie-restricted and low-fat diet may aid in the
prevention of human cancer, or at least delay its onset.
Besides, as indicated in this communication, these re-
strictions should produce beneficial results even if begun
after carcinogenic stimuli have been acting for a con-
siderable period of time.

SUMMARY

A fat-enriched diet promotes the production of skin
tumors induced by carcinogenic hydrocarbons. Since
the carcinogenic process can be divided into two dis-
tinguishable stages, initiation and development, it
seemed of interest to determine whether the facilitat-
ing effect occurs in one or both of these stages. The
carcinogen was applied for a limited period only, and
the high-fat diet was fed to different groups either dur-
ing the period of application, in the period following
application, or throughout both periods. The ingestion
of the high-fat diet during the period following applica-
tion of the carcinogen exerted a small but definite
facilitating influence on the formation of skin tumors,
equal to that obtained when the high-fat diet was fed
throughout the experiment; no such effect was observed
if the high-fat diet was fed only during the limited
period of carcinogen application. This suggested that
the facilitating effect of the high-fat diet operated
principally in the neoplastic or developmental stage
of carcinogenesis. In contrast, facilitating effects of
greater magnitude have been reported in animals
whose skins have become oily from contact with a fat-
enriched ration. This effect, due to carcinogen applica-
tion in the presence of an oily skin, which affects solu-

bility and absorption of the carcinogen, must not be
considered a primary effect of a fat-enriched diet. Our
high-fat diet was prepared and fed in such a way that
the fur of the animals was not oily. Therefore it
must be emphasized that one can distinguish between
the tumor-promoting effect due to oily skins, in the
initiatory stage, and the tumor-promoting effect that
is produced systemically by ingested fat, in the devel-
opmental phase. The experimental and clinical impor-
tance of such differentiation is discussed.

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