Effect of Limited Food Intake on Survival of Mice Bearing Spontaneous Mammary Carcinoma and on the Incidence of Lung Metastases*†

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It has long been known that the growth of neoplasms in animals can be retarded by the reduction of food intake. This applies to spontaneous and deliberately induced tumors as well as to those developing from transplants. Both simple underfeeding and restriction of carbohydrate only (caloric restriction) are effective (11). The influence of food restriction on the longevity of the tumor-bearing host, however, has received little attention. Sugiyama and Benedict demonstrated that underfeeding, instituted after surgical removal of spontaneous mammary tumors, delayed recurrence of the neoplasms and extended the life of the mice (8). Flory and co-workers investigated the effect of underfeeding on mice which had been inoculated with leukemic cells 2 days previously (8). With two strains of transplantable leukemia survival time was increased, whereas with two other strains no influence was observed. Neither study answers the question whether underfeeding or caloric restriction—without surgery or other treatment—alters the life span of animals with established, growing tumors. This was a principal objective of the present study with mice bearing spontaneous mammary carcinoma. Furthermore, since mice of the strain utilized often develop more than one breast tumor and exhibit metastases to the lungs, it was possible to garner information relevant to the influence of underfeeding on these events.

MATERIALS AND METHODS

Employing the following general conditions, three experiments were performed consecutively over a period of several years. A large group of Swiss female mice, born within a 2-week span, were housed in sets of five per cage; they were offered Purina Laboratory Chow, an adequate commercial diet, and water ad libitum. At 2-week intervals the mice were weighed and inspected for neoplasms. The animals in which tumors had emerged were paired according to their body weights and the location and dimensions of the neoplasms; those which could not be paired were discarded—resulting in rejection of approximately half the original stock. The selected animals were housed individually and entered into the experiment. One animal of each pair was assigned to the "full-fed" group, and fed a daily ration of 15 Calories; the other, placed in the "restricted" group, received 7.4 Calories.

In Experiments 1 and 2, both full-fed and restricted mice were fed a diet of the following composition: Purina Fox Chow meal, 50 per cent; skimmed milk powder, 25 per cent; cornstarch, 20 per cent; brewer's yeast, 3 per cent; and partially hydrogenated cottonseed oil, 2 per cent. Full-fed mice were given and consumed 3.6 gm., underfed mice 2.0 gm. daily. In Experiment 3, the full-fed rations of 3.6 gm. consisted of Purina meal, 1.3 gm.; milk powder, 0.7 gm.; brewers yeast, 0.1 gm.; and cornstarch, 1.6 gm. The restricted ration contained the same amounts of meal, milk powder, and brewers yeast (i.e., the same amounts of protein, fat, minerals, and vitamins), but the cornstarch was reduced to 0.1 gm. to make a total of 2.1 gm. daily. Thus, the restricted animals of the first two experiments were "underfed," those of the third experiment were "calorie-restricted" (11).

The animals were weighed and inspected at 2-week intervals, and their general condition, including the appearance of new mammary carcinomas, was noted. All neoplasms were measured when the mice were placed in the experiment and at death. In Experiment 2 the growth rates of the tumors were evaluated by semiweekly measurements; in Experiment 3 all mammary tumors of a mouse, and its carcass (exclusive of tumors), were weighed.

The first two experiments were designed to determine mainly the influence of reduced food intake on the life span of the tumor-bearing animal, and, hence, all mice were permitted to survive until death; supplementary observations were made on the occurrence of additional mammary carcinomas and the presence of grossly visible metastases to the lungs. On the other hand, the primary objective of the third experiment was evaluation of the effect of caloric restriction on the occurrence of metastases and additional mammary tumors; consequently, when one animal of a pair succumbed, its mate was sacrificed.

The mammary tumors and lungs of each mouse with lung nodules were examined histologically. In every instance the lung tumors were found to be secondary to the mammary neoplasms. Sections of breast tumors of numerous animals without metastatic lesions also were examined.

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RESULTS

Chronic restriction of food intake had a beneficial effect on the life span\(^1\) of mice bearing mammary carcinoma (Table 1). This is shown in both Experiments 1 and 2, in which the average survival time of the restricted animals was 2–3 weeks longer than that of their full-fed mates. In all experimental mammary carcinomas or grossly visible metastases to the lungs (Table 2). Moreover, their metastatic lesions were less numerous and smaller in size. These salutary effects occurred in all three experiments. They were more critically evaluated in Experiment 3, in which differences in life span were eliminated by sacrificing the mate when an animal died. In this study, the proportion of calorie-restricted mice with additional mammary neoplasms or metastases was significantly less, statistically, than the corresponding values for the full-fed controls.

The lower frequency of additional mammary tumors and of metastases was not the basis of the prolonged life span of the restricted mice. The

### Table 1

<table>
<thead>
<tr>
<th>Exp.</th>
<th>No. pairs</th>
<th>Survival time* of full-fed mice (days)</th>
<th>Increase in survival time† of restricted mice (days)</th>
<th>Proportion of pairs in which the restricted mouse outlived its full-fed control (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>85 ± 4.8 (87–145)</td>
<td>+23.8 ± 7.93 (−79 to +162)</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>74 ± 4.5 (69–176)</td>
<td>+16.2 ± 6.61 (−101 to +190)</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>92 ± 4.4 (85–140)</td>
<td><strong>†</strong></td>
<td>69</td>
</tr>
</tbody>
</table>

* Interval from detection of the tumor until death of the animal: mean ± standard error of the mean and, in parentheses, range of individual values.
† Survival time of restricted mouse minus that of its respective full-fed control: mean ± standard error of the mean and, in parentheses, range of differences.
† These data were not obtained in Experiment 3—when an animal died, its mate was sacrificed.

### Table 2

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Group</th>
<th>No. mice</th>
<th>Proportion of mice bearing additional mammary tumors (per cent)</th>
<th>Incidence of mice with visible lung metastases (per cent)</th>
<th>Tumor growth*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Full-fed</td>
<td>40</td>
<td>50</td>
<td>35</td>
<td>Diameters (mm.)†</td>
</tr>
<tr>
<td></td>
<td>Underfed</td>
<td>40</td>
<td>15</td>
<td>20</td>
<td>26.0 ± 1.29 (14–36)</td>
</tr>
<tr>
<td>2</td>
<td>Full-fed</td>
<td>53</td>
<td>51</td>
<td>36</td>
<td>Growth rates (mm/day)‡</td>
</tr>
<tr>
<td></td>
<td>Underfed</td>
<td>53</td>
<td>42</td>
<td>27</td>
<td>0.35 ± 0.05 (0.10–0.77)</td>
</tr>
<tr>
<td>3</td>
<td>Full-fed</td>
<td>70</td>
<td>51</td>
<td>23</td>
<td>Weights (gm.)‡</td>
</tr>
<tr>
<td></td>
<td>Calorie-restricted</td>
<td>70</td>
<td>33</td>
<td>6</td>
<td>10.2 ± 0.57 (1–25)</td>
</tr>
</tbody>
</table>

* These are means ± standard errors of the means and, in parentheses, the ranges of various measures of tumor growth. In Experiments 1 and 2 the values refer only to those animals with a single tumor; similar figures were obtained when only initial tumors of all animals were considered. The differences between the full-fed and restricted values are statistically significant.
† Final diameter (average of major and minor axes) of the tumor.
‡ Rate of increase of diameter.
§ Total weight of all mammary neoplasms born by the individual animal.

three experiments, furthermore, the mouse on the limited regimen outlived its control in approximately two-thirds of the pairs. These findings are statistically significant.

Fewer of the restricted mice developed additional mammary carcinomas or grossly visible metastases to the lungs (Table 2). Moreover, their metastatic lesions were less numerous and smaller in size. These salutary effects occurred in all three experiments. They were more critically evaluated in Experiment 3, in which differences in life span were eliminated by sacrificing the mate when an animal died. In this study, the proportion of calorie-restricted mice with additional mammary neoplasms or metastases was significantly less, statistically, than the corresponding values for the full-fed controls.

The lower frequency of additional mammary tumors and of metastases was not the basis of the prolonged life span of the restricted mice. The
differences in survival for only those pairs in which both animals bore single neoplasms and no metastases were essentially the same as those given in Table 1 for all animals.

The last column of Table 2 illustrates the influence of limited food intake on the growth of mammary carcinoma. The tumors of the restricted mice, compared to those of the controls, averaged 16 per cent smaller in final diameter (Experiment 1), grew at approximately two-thirds the rate (Experiment 2), and attained less than half the weight (Experiment 3). Furthermore, in over 80 per cent of the pairs these measurements were smaller in the restricted animal.

As should be expected the restricted mice lost more weight than the full-fed. For example, in Experiment 3 the mean initial body weights for both groups was 33 gm.; at death the carcass weight of the full-fed animals averaged 24 gm., the calorie-restricted animals 19 gm.

DISCUSSION

Underfeeding or caloric restriction of mice bearing mammary carcinoma of spontaneous origin increased their life span, decreased the rate of growth of the tumors, hindered the formation of additional neoplasms of the mammae, and decreased the frequency of lung metastases.

Initial criteria and statistical evaluation.—Before proceeding with a discussion of the results, the initial conditions, the criteria used for pairing, and some statistical considerations are reviewed. The initial conditions were those present when the neoplasms were first detected, and the animals paired and entered into the study: the factors of selection were the diameters and sites of the tumors, and body weights and ages of the mice. Construction of the two groups of a study by pairing the animals according to these four criteria—rather than through random distribution—was not only rational but also resulted in increased precision of the comparisons (7).

The actual initial conditions may be summarized as follows: diameters of the carcinomas (average of major and minor axes) ranged from 3 to 15 mm. (mean, 8 mm.); the body weights varied from 26 to 43 gm. (mean, 33 gm.); the tumors were in many sites, all related to the anatomic location of the mammae; and the mice were paired at from 30 to 70 weeks of age.

In selecting the two members of a pair, virtual equality of age of the animals and of location of the tumors was achieved. Equating them with respect to body weight and dimensions of the tumors presented more difficulties. In fact, relatively lenient limits were employed: the diameters of tumors accepted for a pair differed by as much as 5 mm., although in 80 per cent of the pairs the difference was less than 1.5 mm. Similarly, body weights differed occasionally by as much as 10 gm.; in about 80 per cent of the pairs the difference was less than 3 gm. For both measurements the means of the differences were negligible. Analysis of our material demonstrated that the divergence in initial body weight or in initial tumor diameter of a pair was not a factor in the effect of food restriction on survival time, growth of the tumor, occurrence of additional mammary neoplasms, and frequency of metastases.

Statistical evaluation of the results was complicated by the fact that, in the main, the data did not follow normal distributions. It was therefore necessary to transform the data in order to permit application of the usual tests of significance (2). In Experiments 1 and 2, for example, the survival times of the mice, as well as the differences between the two members of each pair, were markedly skew in distribution. However, the logarithms of the ratios of the survival times of the restricted mice to those of their full-fed mate were normally distributed with mean values of 0.089 ± 0.039 and 0.072 ± 0.029 for Experiments 1 and 2, respectively. Both means are statistically greater than zero and indicate that, on the average, the underfed mice lived 20 per cent longer—from the time they were entered into the study—than the full-fed controls.

Growth of tumors.—The present results agree with the generally accepted fact that underfeeding or caloric restriction hinders the growth of tumors (11). This is shown not only in Experiment 3, where the underfed and full-fed mice had equivalent survival times, but even in Experiments 1 and 2, where the tumors of the underfed mice had about 20 per cent more time for growing. In all three studies the findings related to growth proper, thus differing from some reported experiments with implanted tumors where the results were compounded from effects on establishment and on growth.

Multiple mammary carcinomas.—Early studies by Sugiura and Benedict (8) suggest that, in mice from which initial mammary carcinomas had been excised, the development of new neoplasms was significantly inhibited by underfeeding. Morris and Robertson reported that additional mammary tumors formed much less frequently in tumor-bearing mice placed on a riboflavin-deficient diet (5). Inasmuch as riboflavin deficiency leads to reduced food intake and loss of body weight, their results are probably related to those of the present investigation in which underfeeding
or caloric restriction alone hindered the appearance of additional mammary tumors.

These findings are added proof that the genesis of neoplasms can be inhibited by caloric restriction instituted shortly before tumors are expected to appear. To this extent they support the view that restricted food intake exerts its main inhibitory effect on tumor genesis during the developmental, rather than the initiatory, stage of carcinogenesis (10).

Metastases.—To our knowledge, a decrease in frequency of metastases as a result of limited food consumption has not been previously demonstrated. Metastases may be considered to be the consequence of a series of events: invasion of blood or lymph vessels by the primary neoplasm, formation of emboli, survival and establishment of the emboli in the tissue of lodgement, and, finally, growth. Caloric restriction or underfeeding might very well act on all these stages. Little is known about the influence of nutrition on invasion and embolism, but its action on the establishment and growth of a metastatic embolus may be analogous to that on implants—particularly autotransplants. Underfeeding nearly always retards the establishment, and sometimes the growth, of transplants. Perhaps, as suggested by Rous (6), it limits “the host’s ability to form a connective tissue scaffolding and vascularization necessary for their (the implants’) support.” The same idea, invoked for the action of food restriction on the establishment and growth of metastases, may explain their decreased frequency in the restricted mice.

Longevity.—Probably, the prolonged survival of the tumor-bearing underfed mouse was a result of two primary influences: (a) on the animal; (b) on the neoplasm.

It has been demonstrated conclusively that caloric restriction prolongs life (1, 4, 9). This beneficial effect on the animal presumably occurs even though a tumor is present and growing. However, the neoplasm, too, plays a part in determining the life span of the host: Generally, the more slowly a tumor grows, the longer is the survival time. In Experiment 2, the co-efficient of linear correlation between growth rates of the carcinomas and longevity was —0.66 for the full-fed controls and —0.54 for the underfed mice. If a cause-and-effect relationship is assumed, these values can be broadly interpreted as implying that a little more than half the factors that controlled survival arose from the rapidity of tumor growth. Probably, any procedure which retards the growth of the neoplasm—and in itself has no untoward effect on the host—is likely to prolong the life span. Moderate underfeeding or caloric restriction is in this category. In accordance with this viewpoint, the difference in life span between members of a pair was compared to the difference in growth rate of their tumors: there was a statistically significant linear correlation (—0.40), denoting that, to some extent, the greater the inhibition of tumor growth through underfeeding, the greater the increase in survival time.

The longer average life span of the restricted animals was not attributable to the reduced incidence of additional neoplasms and metastases. Furthermore, there was no association between which animal of a pair died first and which developed metastases or multiple mammary tumors. However, these events did curtail the life of some individual animals. Actually, in about 10 per cent of the full-fed mice and a few of the underfed, death was due to virtually complete replacement of the lungs by metastatic nodules. However, these were mainly animals of longer-than-average survival time, and the over-all mean values for the differences between the full-fed and restricted mice were not modified by excluding them from the computations.

Conclusions.—In this study the salutary action of underfeeding or caloric restriction on longevity and the frequency of metastases has been demonstrated for a single tumor type—one that grows slowly, permits a relatively long survival, and metastasizes with only moderate frequency and almost exclusively to the lungs. It is not at all probable that effects of the same degree would be observed with rapidly growing neoplasms and with those that metastasize extensively. Nevertheless, since caloric restriction has been shown to repress strikingly the genesis and growth of so many different kinds of neoplasms, it is not unlikely that the beneficial influence upon longevity and metastases also has generality. We believe that, with many neoplasms, the early institution of caloric restriction might result in retarded growth and spread of the tumor, and in prolongation of the life of the host. This is not suggested as a practical measure for cancer in man, since it probably in no way compares with the results and potentialities of surgery and irradiation.

SUMMARY

The influence of restricted food intake on the survival of mice with spontaneous mammary carcinoma was investigated in three experiments. Strain C3H mice with small single tumors were paired according to age and body weight, as well as size and location of the neoplasms. One of each pair was full-fed (15 Calories daily), the other restricted (7.4 Calories). Limitation of food intake
was achieved by either proportionate reduction of all dietary components (underfeeding) or by decrease of carbohydrate only (caloric restriction). A total of 163 pairs of mice was used in the study.

The average survival time of the tumor-bearing mice on the low-calorie rations was about 20 percent longer than that of the full-fed controls. Furthermore, in two-thirds of the pairs the restricted mouse outlived its respective mate. The limitation of food intake also resulted in a decreased rate of growth of the tumors, reduced incidence of additional mammary carcinomas, and lower frequency of grossly visible metastases to the lungs.

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REFERENCES

Effect of Limited Food Intake on Survival of Mice Bearing Spontaneous Mammary Carcinoma and on the Incidence of Lung Metastases

Albert Tannenbaum and Herbert Silverstone