Impairment of Feeding Response to Cold Exposure of Rats Bearing Walker 256 Carcinosarcoma

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ABSTRACT

The feeding response to cold exposure of male Sprague-Dawley rats bearing Walker 256 carcinosarcoma has been measured at 5°, 13°, and 17° exposure. The cold-specific feeding response of the host is estimated taking account of the food cost of the tumor and of changes in food intake induced by weight change of host produced by both cold exposure and tumor growth. The cold-specific feeding response to cold exposure is depressed by about 30% by presence of tumor but is only marginally influenced by tumor size. This tumor-induced impairment of feeding response contrasts with the eventual total abolition and tumor size dependency of feeding response of tumor bearers to caloric density of food.

INTRODUCTION

An approach to investigation of the anorexia induced by tumors is to find the extent to which normal feeding responses to feeding stimuli are impaired by tumor growth. If different feeding responses are affected differentially by tumor growth, then it should be possible, at least in principle, to identify the control site(s) of tumor-induced anorexia by comparison of the common and separate parts of the pathways of the normal feeding responses.

The feeding response to reduction of caloric density of the diet is progressively impaired and eventually abolished by growth of Walker 256 carcinosarcoma (14, 15) and of Morris 5123 hepatoma (16). The impairment of this normal feeding response is a function of tumor size. Feeding response to exogenous insulin does not seem to be impaired even by quite large Walker 256 tumors (15), but this response has not been investigated in detail. The feeding response to change in caloric density of the diet is probably extrahypothalamic (4), while the response to insulin is mediated, at least partly, by the hypothalamus (21). This, along with the findings that hypothalamic damage that produces the classic feeding abnormalities in normal rats does not block or affect the course of tumor-induced anorexia (2, 11, 13), indicates that the central mediation of tumor-induced anorexia is extrahypothalamic (18). This represents a first, tentative stage in identification of the site of tumor-induced depression of food intake.

The normal feeding response to reduced environmental temperature is also believed to be mediated extrahypothalaminically (18), and it is of interest to determine if the effect of tumor on this response can be used to dissect further the functional site of tumor-induced anorexia. The small amount of available information shows that the feeding response to 5° exposure persists qualitatively during growth of Walker 256 and several hepatomas (3, 25), but it is not possible to tell from the data, whether the response is quantitatively impaired or even quantitatively enhanced. A preliminary study in this laboratory appeared to confirm the qualitative response at 5° but indicated that, at more moderate cold exposure (17°), the response was seriously impaired (17).

In these studies, the cold-stimulated food intake was compared with pre-cold exposure intake or with non-cold-exposed rats with approximately equal tumor burden. However, the tumor itself progressively reduces food intake and host weight (and hence, food requirement of host) to a degree that is only coarsely related to tumor burden, and, the growth and maintenance of the tumor preempts a progressively greater amount of the food eaten. In addition, cold exposure reduces body weight (or rate of gain of body weight) as well as raising food intake, so that the gross, observed change in food intake during cold exposure is the result of a weight-specific depression of food intake and a cold-specific elevation (19). The size of the cold-specific elevation of food intake in the tumor-bearing animal is the necessary measure of the impairment by tumor of the feeding response to cold.

In this paper, the weight changes of the host due both to tumor and to cold and the food preemption by tumor costs are simultaneously and continuously taken into account to permit valid evaluation of the effect of tumor on the cold-specific increment in food intake on exposure to 3 reduced environmental temperatures.

MATERIALS AND METHODS

Experimental

The work was done on adult male Sprague-Dawley rats. The rats were housed individually in suspended wire cages with 1.25-cm wire grid floors and were allowed food and water ad libitum at all times. Body weight, food intake, and water intake were measured daily to the nearest g. A casein-based, semisynthetic diet, C21 (12), with a metabolizable energy of 4.85 kcal/g, was used throughout (Table 1). It supports good and consistent growth of normal adult male rats (5 to 7 g/day for Sprague-Dawley rats), and because it has been used in all previous studies from this laboratory it permits direct comparison with previous results. By comparison with current nutrient standards for rats (20), it is in excess for protein and fat. From nominal analysis of the salt mixture used (5, 8), zinc is absent, but rats eating this diet have never shown any sign of zinc deficiency. Cage trays were inspected daily for scattered food, and any scatter was collected and weighed. The diet used is a thick paste which minimizes food scattering, and very little scatter was observed. All rats were acclimated to the individual housing and diet for at least 7 days before recording was begun and, for the tumor bearers, for at least 3 weeks before transplant of tumor. A standard daily light cycle of 12 hr dark and 12 hr light obtained at all times.

Fragments of Walker 256 carcinosarcoma (1 to 2 mg), taken from the viable rim of a 2-week tumor, were transplanted by sterile trocar s.c. in the left flank of 8 groups of 15 rats with body weights of 300 to
Tumor and Feeding Response to Cold

Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin-free casein</td>
<td>21.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.5</td>
</tr>
<tr>
<td>Refined sucrose</td>
<td>14.7</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>35.4</td>
</tr>
<tr>
<td>Vegetable fat</td>
<td>24.0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>0.1</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.1</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4.0</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Crisco (Procter and Gamble, Cincinnati, Ohio).
*Premixed with sucrose (mg/100 g diet): thiamin-HCl, 0.5; riboflavin, 1.0; pyridoxine-HCl (Be), 0.5; calcium pantothenate, 0.5; niacin, 1.0; p-aminobenzoic acid, 30.0; inositol, 100.0; folic acid, 0.5; biotin, 0.1; methyl naphthoquinone, 0.2; \( \alpha \)-tocopherol acetate, 50.0; vitamin B12, 0.08.

Where weight is viable and \( T \) is total weight of tumor in g. For tumors weighing 50 to 100 g, this gives a viable compartment of about 60%, which agrees with earlier work on the necrotic-viable partition (24).

This relationship was applied to total estimated tumor weight \( \geq 5 \) g throughout the study to obtain individual, estimated, viable (respiring) tumor mass. Tumors less than 5 g were assumed to be nonnecrotic (6); any error from this assumption would be less than the least significant digit in the calculated tumor cost and can be ignored.

The daily food cost of the tumor was then estimated as:

\[
F_T = \frac{(0.08T_2 - T_1) + 0.13(0.88T_2^{0.92} \times 0.88T_1^{0.92})^{1/2}}{4.4}
\]

where \( F_T \) is daily food cost of tumor and \( T_1 \) and \( T_2 \) are estimated total weights of tumor on successive days, all in g. The value 4.4 is the absorbable, metabolizable energy of the diet in kcal/g. The food intake available to the host is then:

\[
F_n = F - F_T
\]

where \( F_n \) is effective food supply available to host and \( F \) is total observed food intake, all in g.

It is assumed that the metabolic rate of the viable tumor does not change with environmental temperature.

Food Requirement of Isogravimetric Host. The isogravimetric host is a computed control that is defined as being identical to the experimental host with respect to weight and rate of weight change but identical to a normal, unexposed, tumor-free animal in other respects. From data in unexposed, non-tumor-bearing experimental controls (19), the food intake for this computed isogravimetric control has been derived as:

\[
F_n = F_n + a(H_2 - H) + b(\Delta H - \Delta)
\]

where \( F_n \) is the expected food intake of the isogravimetric host; \( F_n \) is the mean food intake available to the host, \( H \) is the mean weight of the host, and \( \Delta \) is the mean daily weight change of the host during the 5-day pre-exposure period; \( H_1 \) and \( H_2 \) are successive daily weights of the host and \( \Delta \) is the weight change of the host \( H_2 - H_1 \) over that daily interval.

The coefficient \( a \) (0.02) defines the maintenance cost in g food per g rat for a normal unexposed rat, and the coefficient \( b \) (0.77) defines the energy density of body weight change in g food equivalent per g weight change. These coefficients were derived from the experimental control rats used in this study; the derivation has been reported in detail previously (19).

In summary, for each day on each animal, we have: \( F = \) total observed food intake; \( F_T = \) food intake preempted to grow and maintain tumor; \( F_n = \) food intake available to host; \( F_n = \) expected food intake of isogravimetric host; \( F_n - F_n = \) cold-specific compartment of food intake available to host. During maintenance at room temperature, \( F_n \) and \( F_n \) are equal. Since different tumors and different stages of growth of tumors deplete the host at different rates and thus produce different host weights, the cold-specific food intake for each day is expressed per 100 g host weight \( [100(F_n - F_n)/H] \). The sum of these daily values over the 7 days of cold exposure gives a total cold-specific availability and requirement are not considered here. The energy value (heat of combustion) of tumor substance is 0.8 kcal per g wet tumor for Walker 256 (22). The energy cost of tumor maintenance from tissue slice respiration is 0.17 kcal per g fresh viable tissue per day (1) and from whole tumor, measured by arterial-venous differences of metabolites, 0.09 kcal per g fresh viable tissue per day (6, 7). The mean of these values (0.13) has been used. Only the viable (nonnecrotic) part of the tumor is assumed to respire. The data for total tumor weight and necrotic-viable partition found at tumor excision, over a range of final tumor weight of 12 to 208 g (74 tumors), yielded the relationship:

\[
V = 0.88T^{0.92}
\]

where \( V \) is viable weight and \( T \) is total weight of tumor in g.

Three orthogonal lineal dimensions of each tumor were measured with calipers to the nearest mm twice a week, from the time that the tumor-bearing rat less estimated tumor weight for each day of experiment.

Host Weight. Host weight was derived as measured total weight of tumor-bearing rat less estimated tumor weight for each day of experiment.

Host Food Intake. The host food intake is that part of observed daily intake that is available to the host after the cost of tumor growth and maintenance is met for that day. All intakes and costs are expressed in terms of available food equivalent of energy (g food that contain the appropriate amount of metabolizable, absorbable energy); nitrogen
compartment of food intake per 100 g host weight for the total exposure period for each animal. The same calculations are made for the experimental tumor-free animals (in this case, \( F_r = 0 \)).

RESULTS

The exposure of one-half of the rats to reduced temperature during the third week of tumor growth and one-half during the fourth week was intended to allow examination of any progression of tumor effect with size of tumor. Because of the wide spontaneous variation in growth rates of individual tumors, tumor size during exposure showed great overlap between the 2 growth periods, and it was not possible to discriminate effect of tumor size on the basis of tumor age. Accordingly, the data for each exposure temperature were divided, after the fact, into 2 groups based on tumor weight at the start of exposure < or ≥1.5% of total body weight (Chart 1).

Tumor Weight. Average tumor growth for large and small initial tumors at the 4 exposure temperatures is shown in Chart 1. Growth rates among individual tumors were very variable. Crude weight gains of tumor during the 7-day exposure period were 34.5 ± 5.3 (S.E.), 18.3 ± 3.6, 48.2 ± 11.7, and 39.7 ± 6.3 g at 5°, 13°, 17°, and 24°, respectively. The weight gain of tumor over any interval is strongly influenced by tumor size at the beginning of the interval. Reduction of tumor weight gain to a constant initial tumor size of 15 g (the overall mean initial tumor size) by analysis of covariance yielded tumor gains of 33.1 ± 4.5, 38.5 ± 5.7, 53.8 ± 4.7, and 30.7 ± 8.1 g during 5°, 13°, 17°, and 24° exposure, respectively. The growth rate at 17° was significantly higher \((p < 0.05)\) than at any other temperature. There was no statistically significant difference in growth rates among any other groups. Notwithstanding some statistically significant differences, there was no systematic change in rate of tumor growth produced by cold exposure in this experiment, but the variability in tumor size was too great to allow a satisfactory assessment of this effect.

Host Weight. Cold exposure produced depletion of host weight that was progressively more severe with increasing tumor weight. This is shown for the 5° exposure in Chart 2. Exactly the same pattern, but with all depletion rates being less severe, occurred with moderate exposure temperatures. Superficially, this suggests that tumor burden progressively impairs the host's response to cold. However, the change in rate of change of host weight \((d^2H/dt^2)\) from preexposure to exposure \((i.e., the angle through which the weight gradient turns)\) is approximately the same for tumor-free and both tumor-bearing groups (change in rate of body weight change at 5°; tumor free, −11.1 ± 0.7; small tumor, −10.0 ± 1.9; large tumor, −12.9 ± 2.1 g/day) showing that the differences in host loss are predominantly a function of effect of tumor size on preexposure host loss and that there is little or no interaction between exposure and tumor size.

Food Intake. The partition of observed food intake among food cost of tumor, isogravimetric host (weight-specific) intake, and cold-specific compartment of intake ("Materials and Methods") is shown in Chart 3 for 5° exposure. The total food intake \((F)\) and the host-available intake \((F_H)\) increase with cold in the absence of tumor and with small tumors and are maintained with the large tumors. The food cost of tumor \((F_r)\) increases with size of tumor, being, for 5° exposure, 0.4 ± 0.06, 1.6 ± 0.25, 2.4 ± 0.37, and 3.2 ± 0.43 g/day at tumor size of 5.1 ± 0.8, 25.6 ± 4.3, 50.2 ± 7.8, and 95.1 ± 13.0 g, respectively. This change in food cost of tumor can be seen in Chart 3 as a wider difference between total intake and host-available intake for the large than for the small tumors and as a progressive widening of this band with time within each tumor group. In all cases, the calculated isogravimetric intake \((F_{H})\) falls as a consequence of the cold-induced decline in host weight (see above).

This way of expressing the data (Chart 3) shows no obvious effect of presence or size of tumor on the cold-specific intake \((F_{H} - F_r)\). The partitions for 13°, 17°, and 24° exposure are similar but with reduced cold-specific compartment at the moderate cold exposure and no significant cold-specific compartment at 24° exposure (Table 2).
The total cold-specific food compartment (summed across 7 days of exposure) showed no significant difference between large and small tumor groups at any exposure level (Pdm = 0.2, >0.05, >0.1, >0.5 for 5°, 13°, 17°, and 24°, respectively). For individual animals at 13°, there was a small but statistically significant decrease in cold-specific food intake with increasing initial size of tumor (Table 2). There was no other significant correlation between cold-specific intake and any of 3 measures of tumor burden: tumor size at start of exposure; tumor weight gain during exposure; fractional relative tumor growth rate per day during exposure (Table 2).

The cold-specific food intake of tumor bearers was significantly depressed below that of tumor-free rats for the 5° and 13° exposures (5°, depression of 23%, p < 0.001; 13°, depression of 25%, p < 0.01) (Table 2; Chart 4). The gradient of cold-specific intake against environmental temperature was significantly (p < 0.001) depressed for tumor bearers relative to tumor-free controls (-0.096 ± 0.006 for tumor bearers and -0.136 ± 0.005 for controls, both in g per 100 g host weight per degree of temperature) (Chart 4).

DISCUSSION

To assess the effect of a treatment on food intake, it is necessary to take into account the effects of the treatment on body weight, since change in body weight alters the expected requirement for food (19). In the case of the tumor bearer, this is further complicated by the depression, due to tumor, of host weight and rate of weight change and by the cost of the tumor itself. Although the existence of this complex of problems has been recognized qualitatively because of its liability to produce false-positive assessments of antitumor activity (9), it has not previously been expressed or used quantitatively or taken into account in examination of the mechanism of production of cachectic effects.

It is conceivable that the apparent depression of cold-specific intake could be due to overestimate of tumor cost at reduced temperature. It is assumed throughout that the metabolic rate of the tumor is unaltered by environmental temperature change,
but the tumor is s.c., and severe cold may depress its temperature and hence its metabolic rate. However, such an effect would vary with tumor size, and the cold-specific intake of tumor-bearers was independent of tumor mass at 5°C exposure, when such an effect would be most likely; thus, this explanation is unsupported. The significant depression of slope of the cold-specific intake against exposure temperature for tumor bearers as compared with non-tumor bearers, across all the data, indicates that the feeding response of the host to cold is indeed depressed by tumor by about 30%. Further, the results indicate that this depression is produced by presence of tumor and is rarely, if at all, influenced by tumor burden.

This depression of feeding response to cold is markedly different, both qualitatively and quantitatively from the depression of response to caloric density of diet. In the latter case, the response was eventually abolished by the tumor and was heavily influenced by size of tumor (14, 15). This indicates that there are at least 2 separate sites at which tumor impairs control of food intake: the function of one site is attenuated by presence of tumor; the function of the other is attenuated, and eventually blocked, by size of tumor.

The degree of separation of impairment of these 2 responses would, possibly, be sufficient to allow coarse identification of the portions of the control pathways that are being affected, if there were detailed information on the routes of these pathways in normal animals. Unfortunately, this information is not available. Research on the normal control of food intake has not been active in this direction. However, it is reasonable to suppose that the response to caloric density has a peripheral gastrointestinal component that is not invoked for the response to cold and that the total block part of the impairment lies in this component.

The existing data allow the hypothesis of 2 distinct sites of impairment to be made, but to support this hypothesis adequately it will be necessary to identify the sites and mechanisms. Possible places to look for the gastrointestinal component might be: the liver, which has been suggested to play a major part in the peripheral control of food intake (23) and in which a wide spectrum of abnormalities develops during growth of many tumors (26); abnormal control responses to gut distension associated with the attenuation of gut wall and abnormalities of mucosal architecture that are known to develop progressively with tumor growth (26); or failure to synthesize or sense any of the gastrointestinal hormones that are believed to play a part in the peripheral control of food intake (26). All such effects would be expected to become progressively more severe with increasing tumor size.

From the very early appearance of impairment of feeding response to caloric density (14), it seems possible that this impairment also contains the attenuation component which is the sole component of the impairment of the feeding response to cold. A likely common component of the 2 response pathways is the state of lipid reserves which is believed to be a major determinant of food intake (10, 11). This component of feeding impairment might arise from tumor-induced abnormalities in lipid mobilization (11) and in distribution of metabolic activity between carbohydrate and fat (27).

It should be noted that if these comparisons are made without adjustment for food costs of tumor and for isogravimetric control intake (i.e., are made on the basis of observed food intakes only) there appears to be a paradoxical response of tumor bearers to moderate cold and a strong dependence of response on tumor size. Using that simple but unacceptable procedure, the feeding response to cold in the presence of small tumors is depressed by about 30% at all exposure temperatures, while in the presence of large tumors the response is only slightly depressed at 5°C exposure (16%) but is totally abolished at both moderate temperatures. It was the use of this simple procedure in preliminary findings that led to the suggestion that tumor bearers responded normally at severe cold exposure (25) and paradoxically at moderately reduced temperatures (17); that, in turn, led to this series of experiments.

The fairly elaborate analysis procedure used here is necessary to expose the partial but quantitatively important impairment in the response to cold exposure. Previous studies on control of food intake during tumor growth have not made these adjustments. It is possible that reexamination of, for example, the interaction of tumor and hypothalamic damage or response to caloric dilution or response to insulin, using these adjustments, might modify present views.

REFERENCES


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