Lipid Mobilization and Food Intake in Experimentally Obese Mice Bearing Transplanted Tumors[1] (35924)

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Tumor-bearing animals characteristically show a striking depletion of body lipid stores and a subsequent hyperlipemia during the course of tumor growth (1). However, the physiological mechanisms involved in this tumor–host relationship are unknown. It has been suggested that the neoplastic cells are elaborating a "lipid-mobilizing" substance and the resulting hyperlipemia during the depletion of the lipid component of the fat depots influences food intake regulation (2). Evidence supporting the existence of a lipid-mobilizing substance in various animals after fasting has been provided (3–5). Also an extract of an experimental tumor has demonstrated lipid-mobilizing activity (6). Rats made obese with electrolytic hypothalamic lesions were found to be more sensitive to the lipid-mobilizing substance extracted from the urine of fasting rats compared to normal rats (5).

It was the purpose of this study to follow the changes in body weight, total body lipid content, and food intake in normal, gold thioglucose-obese and genetically obese mice bearing either a transplanted stomach carcinoma or a fibrosarcoma.

Materials and Methods. All mice used in these studies were obtained from the Kirschbaum Memorial Laboratory (7) and included both sexes of the following inbred strains: CBA/Ki, (C57Bl/Ki × CBA/Ki), and (CBA/Ki × YBR/Ki)F1 hybrids. The latter hybrids included both the yellow ($A^{Y}a$) "obese" and brown (aa) "lean" litter mates (8).

Mice were kept in plastic cages upon wire-meshed platforms and Purina Lab Chow pellets were available at all times in special feeding hoppers in each cage to measure food intake of individual animals. CBA/Ki and (C57Bl × CBA)F1 hybrids were made obese by injecting 0.4 mg/g of body weight of gold thioglucose (GTG)[4] when the animals were 60–70 days of age (9). Body weights and food intake were measured at 2–3 day intervals with average daily food intake calculated on a grams per day basis. Total body lipid content was determined by chloroform–methanol extraction procedure (10).

Both tumors used in this study were transplanted subcutaneously by the trocar technique (11) and measurements of the tumors were made with vernier calipers. The stomach carcinoma (CBA No. 2663) used in these studies arose in a CBA male mouse that had received 9 feedings of 1 mg of methlycholanthrene in olive oil once each week for 9 weeks. The tumor has been carried by serial transplantation in CBA/Ki hosts for 219 transplant generations and has remained as an undifferentiated carcinoma. The fibrosarcoma (C57Bl 14) also used in these studies arose in a C57Bl female mouse which received a single injection of urethan at newborn age. It has been maintained by serial transplantation for over 122 transplant generations in C57Bl/Ki mice and has remained the same histological type since the first transplant generation.

The following four experiments were carried out to determine the effects of either the CBA or C57Bl tumors on body weight, total

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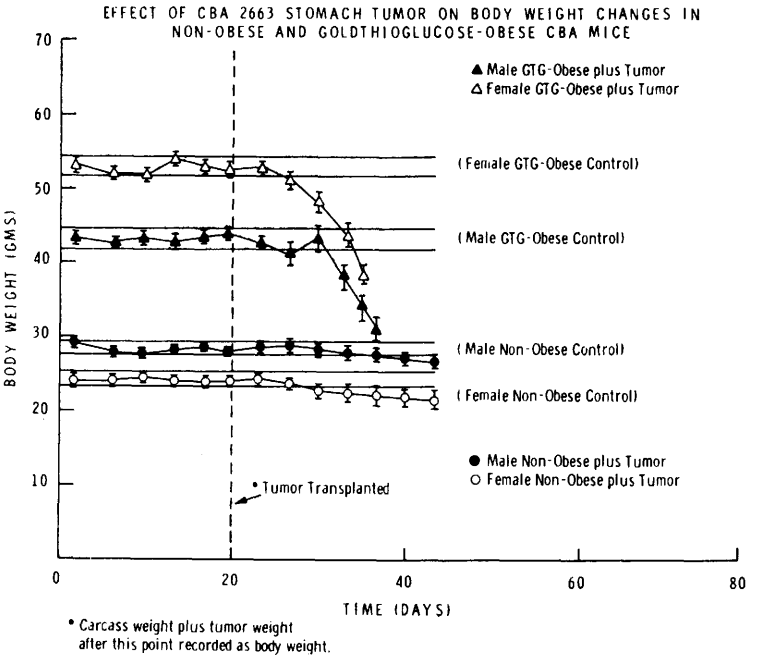

FIG. 1. Changes in body weight in nonobese and gold thioglucose-obese CBA mice bearing CBA 2663 stomach tumor.

body lipid content, and food intake in normal and either GTG-obese or genetically obese mice.

1. CBA 2663 stomach tumor transplanted into normal and GTG-obese CBA/Ki males and females. The CBA stomach tumor was implanted into 20 normal (10♂, 10♀) CBA/Ki mice at 4 months of age and into 20 GTG-obese (10♂, 10♀) CBA/Ki mice at the same age. The latter animals had received a single injection of GTG approximately 2 months earlier and had reached a plateau in body weight increase. Food intake levels had returned to approximately preinjection levels following the characteristic transient hyperphagia (9). The body weights and food intake were followed for a period of 2 weeks prior to implanting of the tumor and then continued to be measured until the tumors reached a size of approximately 1.5 cm in diameter. At this time the tumor-bearing animals were killed; and total carcass (minus tumor) lipid content was determined.

2. Relationship between CBA stomach tumor weight and total carcass lipid content of normal and GTG-obese CBA/Ki female mice. Thirty control CBA/Ki females at 4 months of age and 20 GTG-obese CBA/Ki females were used in this study. The latter animals had received a single injection of GTG at 2 months of age and had reached a plateau in weight gain. Control animals ranged in body weight from 25 to 27 g with a mean of 26.2 g and obese animals ranged in body weight from 50 to 52 g with a mean of 50.9 g at the time the tumor was implanted. Tumor-bearing control animals were killed when the tumors reached weights ranging from approximately 0.2 g to approximately 2.3 g. Obese tumor-bearing animals were killed when the tumor weights ranged between approximately 0.2 g to only 1.0 g. These obese animals became severely moribund and died when the tumors exceeded this latter size.

3. Comparative response of GTG-obese (C57Bl × CBA)F1 hybrids to CBA stomach tumor or C57Bl fibrosarcoma. Twenty GTG-obese (C57Bl × CBA)F1 hybrid males were used in this study at approximately 4 months of age. All animals had received a single injection of GTG at approximately 2 months of age and the animals had attained a plateau

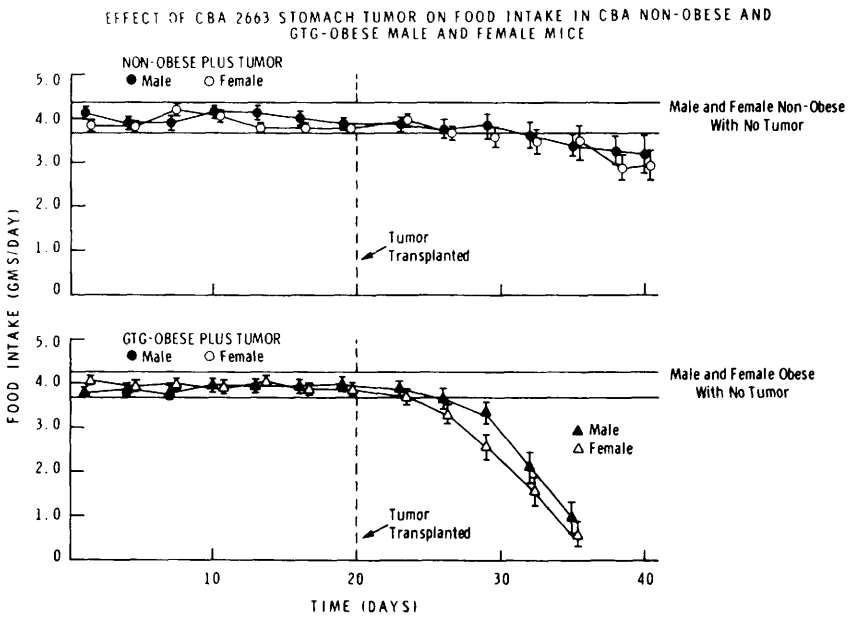

FIG. 2. Changes in food intake in nonobese and gold thioglucose-obese CBA mice bearing CBA 2636 stomach tumor.

in weight gain. Body weights and food intake were recorded for approximately 10 days prior to implantation of the CBA stomach tumor into 10 recipients and the C57Bl fibrosarcoma into the remaining 10 animals. Body weights and food intake were recorded for an additional 24 days in the group bearing the CBA tumor and for 40 days in the group bearing the C57Bl tumor. At these respective times all animals were killed and total carcass (minus tumor) lipid content was determined.

4. CBA stomach tumor transplanted into "yellow obese" and "brown lean" (CBA × YBR) F1 hybrid female mice. Ten "yellow obese" (CBA × YBR) F1 hybrid females and 10 "brown lean" females of the same hybrid combination were approximately 5 months of age at the time this study was started. All animals were placed into individual cages 15 days prior to transplantation of the tumor. During this time body weight and food intake were recorded. These measurements were continued after tumor implantation for 20 additional days in the obese animals and 30 additional days in the lean animals. At these respective times the animals were killed and total carcass (minus tumor) lipid content was determined.

Results. 1. CBA 2663 stomach tumor transplanted into normal and GTG-obese CBA/Ki males and females. Nonobese animals showed relatively stable body weights and food intake values prior to tumor implantation although individual animals showed fluctuations within a relatively narrow range, especially for food intake values (Figs. 1, 2). Following tumor implantation, body weight (carcass plus tumor) showed a gradual decline with the nonobese females reaching a significantly decreased body weight by approximately 35 days after tumor implantation. Nonobese males also showed the gradual decline in body weight but even after 40 days posttransplantation they did not decrease significantly from that of nontumor bearing hosts.

The obese tumor-bearing animals also manifested relatively stable body weights and food intake values as a group before tumor implantation (Figs. 1, 2). Following inoculation of the tumor fragments, the female group of mice began to show a rapid decrease in body weight at approximately 5 days after implantation; whereas a comparable decrease in body weights was noted in the

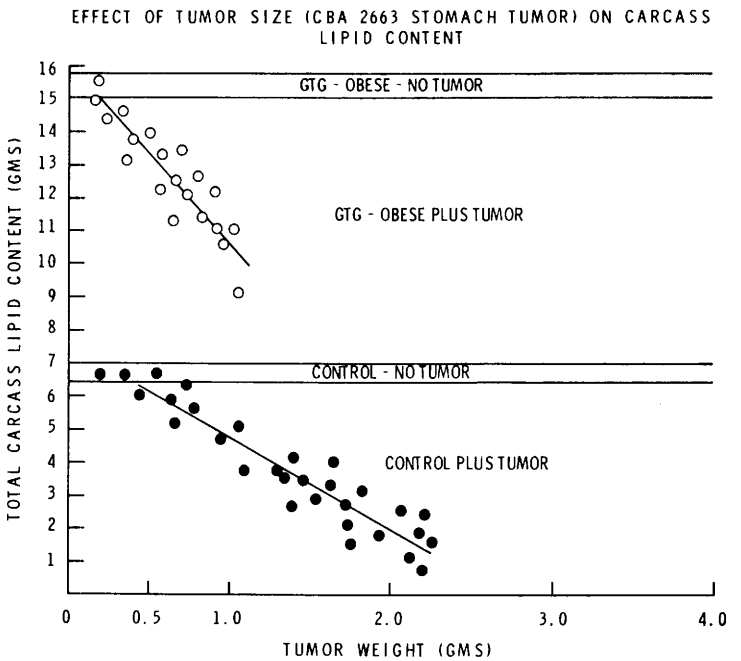

FIG. 3. Relationship between total body lipid content in nonobese and gold thioglucose-obese CBA mice and size of CBA stomach tumor.

males at approximately 12 days postimplantation. A decrease of approximately 29% in body weight was recorded by 15 days after implanting the tumor in the females and a comparable decrease of approximately 30% was recorded in the males after a period of approximately 17 days following tumor implantation. All animals were killed at these respective times because of the onset of debilitation.

The food intake of both nonobese males and females prior to tumor implantation fell within the range previously found for mice of this strain (10). Although not significantly different from control levels, nevertheless, food intake in both sexes showed a gradual decline starting at approximately 12 days postimplantation (Fig. 2). Females showed a significant decrease in food intake from that of controls at approximately 18 days after tumor implantation. Males, on the other hand, showed a decline in food intake but to a lesser degree.

Food intake during the pretreatment period for the obese animals was similar to that of nonobese controls as previously described (10). A striking decrease in food intake was noted at approximately 7 days after tumor implantation with the decrease being slightly earlier in the females compared to the males. Food intake continued to decrease to a point where it was less than 1 g/day for each group although several animals in both groups stopped eating completely by 15–18 days after tumor implantation.

2. Relationship between CBA stomach tumor weight and total carcass lipid content of normal and GTG-obese CBA/Ki female mice. A linear relationship between tumor weight and total carcass lipid content was seen for both nonobese and obese hosts (Fig. 3). Whereas the tumors in the nonobese animals reached a size of approximately 2 g, before the hosts showed signs of debilitation, the tumor-bearing obese hosts became moribund when the tumor reached a size of approximately 1 g. The tumor in both groups grew at approximately the same rate, *i.e.*, the tumor reached a size of approximately 1×1 cm in 15 days whether the host was obese or nonobese. The total carcass lipid decreased by approximately 28% when the tumor reached a size of 1 g in nonobese hosts. On the other hand, total carcass lipid content

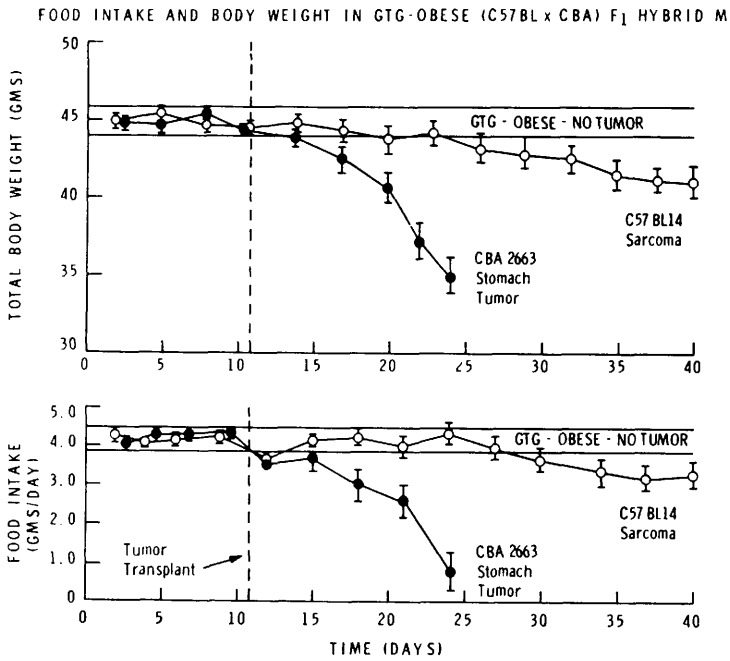

FIG. 4. Changes in body weight and food intake in gold thioglucose-obese (CBA × C57Bl) F1 hybrids bearing either CBA stomach tumor or C57Bl sarcoma.

was decreased by approximately 36% when the tumor reached a size of 1 g in obese hosts. Thus approximately 5 g of lipid were mobilized in obese hosts as compared to approximately 2 g in nonobese hosts for tumors of similar weight and during the same period of time. In the nonobese hosts the decrease in total carcass lipid approached 90% as the tumor reached a weight of 2 g.

3. Comparative response of GTG-obese (C57Bl × CBA)F1 hybrids to CBA stomach tumor or C57Bl fibrosarcoma. Animals bearing the CBA stomach tumor began to show a significant decrease in body weight (carcass + tumor) at approximately 7 days after tumor transplantation (Fig. 4). Body weight continued to decline rapidly until 15 days after implantation of the tumor at which time it had decreased by 23% compared to preimplantation weights. A significant decrease in body weight did not occur in the animals bearing the C57Bl tumor until approximately 22 days after tumor transplantation and it continued to decrease at a much more gradual rate. Thirty days after tumor transplantation body weights of hosts bearing the C57Bl tumor had decreased by approximately 5% compared to control weights. Both tumors grew at approximately the same rate reaching a size of approximately 1 cm in diameter by 17 days posttransplantation. Animals bearing the CBA tumor became moribund when the tumor reached a size of approximately 1 cm, but the C57Bl tumor reached a size twice as large before the hosts became moribund.

Food intake levels followed the rate of decrease in body weights in animals bearing either tumor (Fig. 4). A rapid decline in food intake occurred in the animals bearing the CBA tumor within 5 days after transplantation of the tumor. By 15 days after transplantation food intake had decreased by 80% compared to control levels. Animals bearing the C57Bl sarcoma did not show a significant decrease in food intake until approximately 20 days after tumor implantation. Food intake was reduced by only 23% when the tumor reached a size of 2 cm at which time the tumor began to ulcerate and contained necrotic material.

Recipients bearing either the CBA stomach tumor or the C57Bl sarcoma showed essentially the same decrease in body weight (14 and 11%), as well as the same total carcass lipid content (26 and 25%) at the time the animals were killed (Table I). It should be

TABLE I. Lipid Mobilization in GTG-Obese (C57Bl × CBA)F1 in Hybrid Female Mice Bearing Either CBA 2663 Stomach Tumor or C57Bl 14 Sarcoma.

Group	Body wt at time of autopsy	Change (%) in body wt at autopsy	Tumor growth rate (days required to reach 1 × 1 cm)	Total lipid content at time of autopsy	Change (%) at autopsy	Calc amount of lipid mobilized/day (mg)
Control (no tumor)	45 ± 0.8[a]	—	—	16.0 ± 0.7	—	—
CBA stomach tumor	39 ± 1.8	14	16 ± 1	11.9 ± 1.1	26	260 ± 8.2
C57Bl 14 sarcoma	40 ± 1.3	11	17 ± 2	12.1 ± 0.8	25	114 ± 3.2

[a] Mean ± SE.

noted, however, that those animals bearing the CBA tumor had to be killed at approximately 15 days after implantation compared to 30 days following implantation of the C57Bl sarcoma. At the time the latter animals were killed the tumor was approximately 2 cm in diameter; whereas the former group of animals were killed when the tumors were approximately 1 cm in diameter. No hosts were killed when the C57Bl sarcoma reached the size of 1 cm in diameter.

For comparative purposes, the rate of the decrease in lipid content was calculated on a daily basis assuming that the rate of mobilization was linear and began immediately after tumor implantation. This assumption is justified in part on the basis of the findings in Expt. 2. Viewed in this manner, animals bearing the CBA tumor mobilized lipids at a rate of 260 ± 0.2 mg/day compared to 114 ± 3.6 mg/day for those bearing the C57Bl tumor. More detailed studies are in progress concerned with the kinetics of this lipid mobilization and sites of utilization.

4. CBA stomach tumor transplanted into "yellow obese" and "brown lean" (CBA × YBR)F1 hybrid female mice. The "yellow obese" mice showed a decrease in body weight during the pretumor period following placement into individual cages. This phenomenon of weight loss following "isolation" has been noted before with this particular strain of mouse (2). Whereas the yellow obese animals receiving the tumor transplant continued to show a decrease in body weight, the nontumor animals body weights leveled off and remained stable, for the remaining 30 days of the study. The tumor-bearing obese animals had to be killed at approximately 20 days after tumor implantation as they became moribund, and the body weight had decreased 32% (Fig. 5). No significant changes were noted in the body weights of the brown lean animals during the pretumor period. Following tumor implantation the tumor-bearing animals showed no significant change in body weight until approximately 25 days after transplantation. At which time they showed a 21% decrease in body weight compared to controls.

The food intake pattern in the tumor-

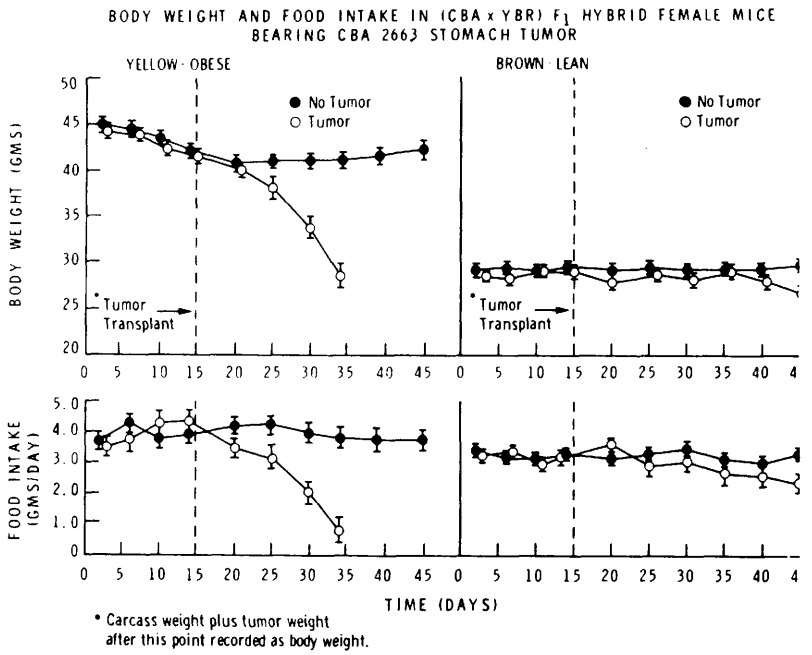

FIG. 5. Changes in body weight and fool intake in genetically "obese" and "lean" (CBA × YBR)F1 hybrids bearing CBA stomach tumor.

bearing obese hosts paralleled the loss of body weight, and at approximately 20 days posttransplantation daily food intake had been reduced by 83% from control levels. On the other hand, daily food intake was reduced by only about 20% in the "brown lean" animals at 30 days after tumor transplantation.

Comparison of total carcass lipids between the yellow obese and brown lean animals at the time the tumors reached a size of approximately 1 cm and 1.5 cm, respectively, indicated that the former groups lost approximately 32% compared to 21% in the latter group (Table II). Expressed in terms of the amount of lipid lost compared to respective control values this becomes approximately 4.6 g for the "obese" hosts and 1.5 g for the "lean" hosts or calculated as amount of lipid mobilized per day the values become 287 ± 12.6 mg/day in the former and 98 ± 1.3 g for the latter group.

Discussion. The "wasting" of the body that accompanys the growth of both spontaneous and transplanted tumors in experimental animals is a common laboratory observation. This weight loss can be accounted for in part by a translocation of nitrogenous substances from the normal to the neoplastic tissues (12). However, the progressive diminution in the body lipid stores eventually accounts for the rest of the wasting in carcass mass (13). Numerous investigators have described a hyperlipemia in these same animals bearing tumors (1). It has also been shown that the decline in food intake of a tumor-bearing animals is a function of tumor growth (13). Whether or not these three findings of lipid depletion, hyperlipemia, and decreased food intake in tumor-bearing animals are interdependent and important in our understanding of the interrelationships between the adipose tissue system (2) and food intake regulation remains to be determined.

The striking differences in the rate of body weight loss and decrease in food intake in the GTG-obese hosts compared to nonobese hosts bearing the same tumor (CBA 2663 stomach tumor) was unexpected. It had been previously shown that the destruction of the ventromedial region of the hypothalamus with GTG produced a permanent alteration in food intake regulation establishing a new "set-point" at the increased body weight (9), and it was anticipated that the tumor-bearing GTG-obese hosts would compensate for the tumor-induced "wasting" by a return to a hyperphagic state as seen in GTG-obese

TABLE II. Lipid Mobilization in "Yellow Obese" and "Brown Lean" (CBA × YBR)F1 Hybrid Female Mice Bearing CBA 2663 Stomach Tumor.

	Body wt at time of autopsy (gms)	Change (%) at time of autopsy	Total lipid content at time of autopsy (gms)	Change (%) at time of autopsy	Calc amount of lipid mobilized/day (mg)
Yellow obese (no tumor)	45.1 ± 1.1	—	14.3 ± 0.6	—	—
Brown lean (no tumor)	28.7 ± 0.9	—	6.8 ± 0.2	—	—
Yellow obese (plus tumor)	32.2 ± 2.4	29	9.7 ± 1.8	32	287 ± 12.6
Brown lean (plus tumor)	27.5 ± 1.2	3	5.3 ± 0.9	21	98 ± 1.3

animals that had been starved for 7 days then returned to *ad libitum* feeding (9). However, rats made hyperphagic and obese with electrolytic lesions in the hypothalamus showed a continued loss of appetite during the dynamic phase of weight gain with progressive growth of the Walker tumor (14). Studies are underway in which the effect of the CBA tumor is being determined during the pre- and postinjection period following GTG.

This precipitous decrease in body weight of the tumor bearing GTG-obese hosts paralleled the comparable decrease in food intake of these same hosts. A similar rapid decrease in body weight and food intake did not occur in the nonobese hosts bearing the same tumor. The basis for the unique response of the GTG-obese as contrasted to the nonobese hosts to the same tumor is not known. One possibility includes the difference in amount of stored lipids in the adipose tissue system between the two groups. Support for this concept is derived from the similar behavior of yellow obese hosts and brown lean hosts bearing the same CBA stomach tumor, namely, a similar striking decrease in body weight and food intake in the yellow obese hosts as contrasted to the brown lean hosts. This also would tend to diminish the significance of the GTG-induced lesions as being a major difference between the nonobese and GTG-obese CBA mice in their response to the CBA tumor.

The nature of the body weight loss in all hosts obese or nonobese, bearing the CBA tumor proved to be primarily accounted for by a decrease in body lipid content which proved to be proportional to tumor size. However, body lipid content decreased at a more rapid rate in the GTG-obese animals compared to the nonobese hosts for a comparable tumor size. Whether this was related in some manner to the greater amount of lipid stored in the adipose tissue of these animals or an increased sensitivity of the lipid-mobilization mechanisms in GTG-obese hosts which facilitate a more rapid rate of mobilization remains to be determined.

Although both the CBA stomach tumor and the C57Bl sarcoma growing in identical GTG-obese hosts resulted in a comparable amount of lipid depletion at the time the hosts were autopsied, the rate of lipid mobilization was found to be more rapid in the hosts bearing the CBA stomach tumor. It should be noted that the two tumors grew at comparable rates in the two hosts. Thus it would appear that the CBA stomach tumor possessed some unique biological characteristic not related to growth rate that facilitated the mobilization of lipid compared to the C57Bl sarcoma.

Assuming a linear rate of mobilization with increasing tumor size as indicated at least in Expt. 2 for the CBA stomach tumor, the rate of lipid mobilization for this tumor on a milligram per day basis was more than twice that calculated for the C57Bl sarcoma. Thus it is possible that the striking decrease in food intake seen in hosts bearing the CBA stomach tumor and not seen in identical

hosts bearing the C57Bl sarcoma could be related to the differences in rate of lipid mobilization, which presumably was reflected in different levels in blood lipids. Preliminary unpublished studies reveal that the total plasma neutral lipids in the hosts bearing the CBA tumor at 1 cm in size was 67.6 mg/100 ml compared to 20.5 mg/100 ml in identical hosts bearing the C57Bl tumor at 1 cm in size.

The lipostatic theory of food intake regulation has emphasized the importance of metabolites of adipose tissue in influencing the amount of food eaten (15), and more recently it has been suggested that plasma steroid levels acting on a dilution principle basis served as a modulating factor in food intake regulation (16). Thus the increased levels of blood lipids due to mobilization of stored lipids in the obese hosts bearing the CBA tumor could account for the changes in food intake. Why these changes in food intake occurred in either the GTG-obese or yellow obese hosts bearing the CBA tumor but not in normal or lean hosts bearing the same tumor, even though both groups showed approximately the same percentage decrease in their lipid stores, is not known. Whether this is merely a quantitative difference in levels of plasma lipids between the two groups of animals or whether there is a qualitative difference in plasma lipids, or perhaps both, is presently being investigated. Another consideration is the possibility that the method of measuring food intake used in this study was not sensitive enough to detect food intake changes associated with only slight increases in plasma lipid, and only the "magnified" changes associated with higher levels of plasma lipids in the obese animals were measurable. This aspect of the study is also under further investigation.

The suggested biological uniqueness of the CBA tumor could be accounted for on the basis of a difference in the production of a postulated "lipid-mobilizing factor." Evidence has been presented for the existence of such a factor in the urine of starved experimental animals or man (3–5), and an extract of a transplanted mouse tumor injected into mice showed changes in lipid metabolism (6). Studies are in progress to determine whether such a substance can be isolated from the CBA stomach tumor.

Summary. A transplanted stomach tumor (CBA 2663) caused a striking decrease in total body lipid content and suppression of food intake while growing in GTG-obese and genetically "yellow obese" mice. Nonobese mice bearing the same tumor showed a comparable percentage of lipid depletion, but food intake decreased only as the host became moribund. The rate of lipid depletion was greater in the GTG-obese compared to nonobese hosts on a per gram tumor weight basis. Lipid depletion proceeded at a faster rate in GTG-obese (CBA × C57Bl)F1 hybrids bearing the CBA tumor compared to a sarcoma (C57B114) both having the same growth rate. Food intake was also rapidly depressed in the F1 hybrids bearing the CBA tumor, but not in those with the C57Bl sarcoma. It is suggested that the CBA tumor has some unique biological characteristics, which facilitates mobilization of body lipid stores which in turn influences food intake.

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