Characterization of a Transplantable Adenocarcinoma of the Mouse Colon Producing Cachexia in Recipient Animals [1,2]

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ABSTRACT—MAC16 is a chemically induced, transplantable adenocarcinoma of the colon passaged in inbred NMRI mice. At small tumor burdens (<1% of the host weight), weight loss was observed without a reduction in food intake. As the tumor mass increased, weight loss also increased and reached 33% of host body weight in females and 20% in males when compared with the weight of age-matched controls. The reduction in host body weight was directly proportional to the tumor size and was reversible when the tumor was excised. There was a preferential loss of body fat in tumor-bearing animals with an increase in the plasma level of free fatty acids, although there was a minimal elevation of ketone bodies. Tumor growth was accompanied by progressive hypoglycemia and a reduction in the plasma insulin levels. The decrease in plasma insulin may have contributed to the catabolic effects of progressive tumor growth.—JNCI 1987; 78:539-546.

Progressive wasting of the host is frequently associated with neoplastic diseases. This cancer cachexia increases as the tumor enlarges and is the major cause of mortality. De Wys et al. (*1*) showed that at the time of presentation 54% of patients with disseminated cancer had lost some weight. The frequency of weight loss ranged from 31% for patients with non-Hodgkin's lymphomas of favorable histology to 87% in patients with gastric carcinomas. In general, depletion of host muscle and adipose tissue occurs, whereas organs such as the liver, kidney, adrenal glands, and spleen tend to be spared and may actually enlarge. Two main mechanisms appear to be in operation in cachectic cancer patients (*2*). For some patients the primary phenomenon is lack of nutrient intake either through anorexia or intestinal malfunction, whereas other patients may continue to ingest food at an appropriate level, do not have intestinal malfunction, and yet continue to lose weight. Although the former group follows a pattern similar to that in starvation, the latter group has more complex metabolic problems (*3*). Most patients, however, have both mechanisms operating simultaneously, but to varying degrees.

Most experimental studies of cancer cachexia have used rapidly growing rodent tumors, where signs of cachexia begin only when the tumor may represent 30-40% of the total body weight. In humans, however, tumor growth is slower and the tumor mass does not normally exceed 5% of the body weight. Two xenograft models of cachexia have been described where weight loss occurs when the tumor mass contributes less than 5% of the total body weight (*4, 5*). However, in one of these models (*4*) weight loss was associated with some reduction in the food intake of the tumor-bearing mice, whereas in the other (*5*) food consumption was not monitored.

The mouse adenocarcinoma of the colon series of transplantable tumors has been extensively used in experimental chemotherapy studies (*6*). Transplantation of one of these tumor lines (MAC16) results in a significant loss of body weight without excessive tumor mass and without a concomitant reduction in food intake of the host when compared with body weight in non-tumor-bearing controls. The present study was undertaken to ascertain the suitability of this line as a model for cancer cachexia and to determine the biochemical changes underlying weight loss.

MATERIALS AND METHODS

Animals.—Pure strain NMRI mice (age 6-8 wk) from our inbred colony were used. They were fed on diet 86 (James Burnhill & Sons, Cleckheaton, England) or rat and mouse breeding diet (Pillsburys, Birmingham, England) and water ad libitum.

Tumor system.—The development of several transplantable adenocarcinomas of the large bowel in mice from primary tumors induced by prolonged administration of 1,2-dimethylhydrazine has been described elsewhere (*7*). MAC16 was first briefly described by Cowen et al. (*8*) but has subsequently been serially passaged in female mice for a period of 5 years. The tumor was excised from donor animals and placed in sterile 0.9% saline containing streptomycin and penicillin and cut into small fragments 1×2 mm in size. Fragments were implanted into the flank by means of a trocar. Take rates are variable with good rates being dependent on

ABBREVIATIONS USED: AB = Alcian blue; FFA = free fatty acid; PAS = periodic acid-Schiff; TNF = tumor necrosis factor.

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careful implantation of viable tumor fragments. Positive takes can only be identified 2-3 weeks after implantation.

Histology.—Tissues for light microscopical examination were fixed in buffered 10% Formalin. Sections were stained with hematoxylin and eosin, PAS, or AB.

Body weight loss.—Tumor fragments were implanted subcutaneously into 50 female and 50 male mice. Mice were weighed twice weekly, and tumor volumes were calculated. Twenty mice of each sex served as controls.

Tumor resection.—Tumor fragments were implanted subcutaneously into 10 mice of each sex. Tumor volumes and body weights were recorded twice weekly for 6 weeks. Tumor volumes were calculated as $A^2 \times B/2$ as described (*9*). Tumors were excised and body weights recorded for a further 4 weeks.

Microbiology.—Tumor tissue and blood from tumor-bearing animals were subjected to bacteriologic screening.

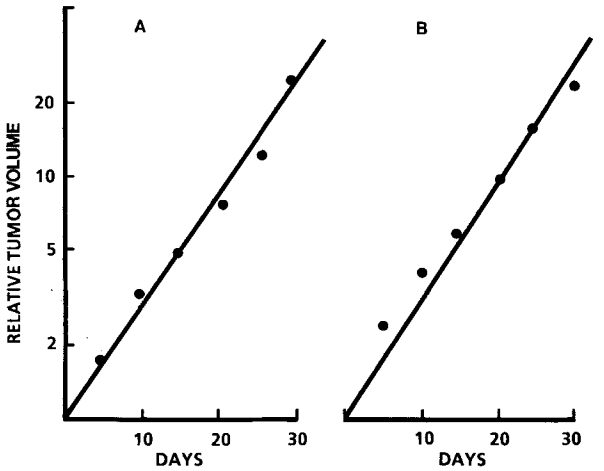
Metabolite determinations.—Blood glucose was determined with the use of the *o*-toluidine reagent kit (Sigma Chemical Co., St. Louis, MO). Acetoacetate and 3-hydroxybutyrate were measured by the method of Mellanby and Williamson (*10*) and Williamson and Mellanby (*11*), respectively. Lactate levels were determined by the method of Gutman and Wahlefield (*12*). FFA levels were measured with a Wako NEFA C kit (Alpha Laboratories). Plasma insulin levels were determined by radioimmunoassay, and pyruvate levels were determined by the method of Czok and Lamprecht (*13*).

Total carcass fat and water content.—The gastrocnemius muscles from the left hindleg were carefully dissected out and weighed. Each carcass plus dissected muscles were placed in an oven at 80°C until constant weight was reached. Carcasses plus muscles were then reweighed separately, and the total fat content was determined by the method of Lundholm et al. (*14*). Water content for the muscle and total carcass was calculated from the wet and dry weights.

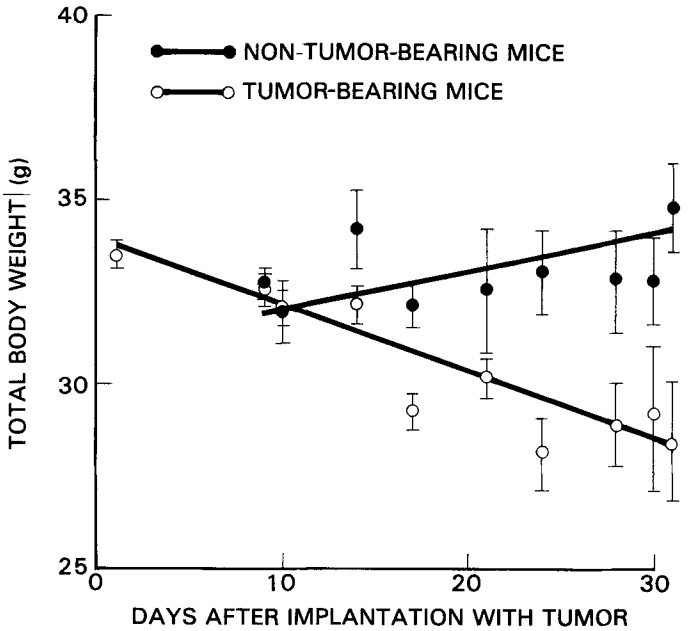
RESULTS

MAC16 is a moderately well-differentiated adenocarcinoma that has remained histologically unaltered during the course of these experiments and is typified by the development of necrotic areas as the tumor enlarges (fig. 1). Occasional PAS- and AB-positive mucosubstances were seen in the lumina of tumor acini. The amounts of connective tissue stroma and necrosis vary between individual tumors, and in larger tumors the necrosis becomes hemorrhagic. Distant metastases were not observed, but skin invasion with ulcerations and local leg muscle invasion sometimes occurred. Microbiologic screening indicated that neither the tumors nor the host was infected. The tumor has a volume doubling time of approximately 6 days (text-fig. 1) and has similar growth characteristics in either sex. The tumor takes 30 days to reach a size of 2.5-3.0 g and thus represents about 10% of the host weight at this time.

Tumor growth is accompanied by a considerable host body weight loss. In males this represents almost 20% of the total body weight 30 days after tumor transplantation (text-fig. 2), and in females it represents as much as 33% when compared with total body weight of age-matched controls. However, the tumor does not have to reach an extensive mass to produce weight loss, since a weight reduction occurs in tumor-bearing animals 14 days after transplantation at which time the tumor mass is only about 0.2 g (<1% of host weight). This weight reduction in tumor-bearing animals is clearly seen as a reduction in the lean body mass and fat mass (fig. 2). The decrease in carcass weight of the animals is directly related to the tumor size (text-fig. 3). This weight loss is not accompanied by a decrease in food intake when compared with food intake of non-tumor-bearing NMRI



TEXT-FIGURE 1.—Growth curves of the MAC16 tumor. Tumor volumes have been normalized to a value of 1 when the tumors first became apparent. A) Female mice. B) Male mice.



TEXT-FIGURE 2.—Mean body weight of male NMRI mice either implanted with MAC16 tumor on day 0 (○) or non-tumor-bearing (●).

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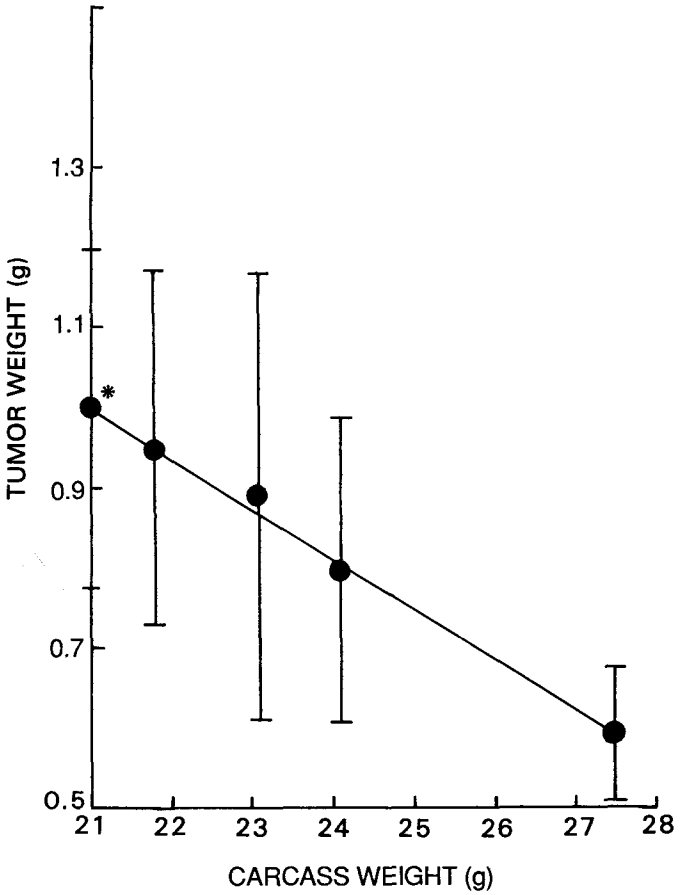
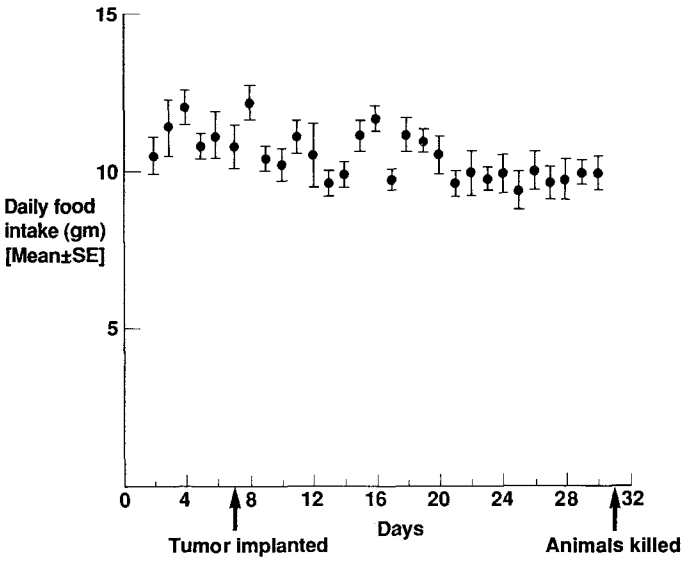
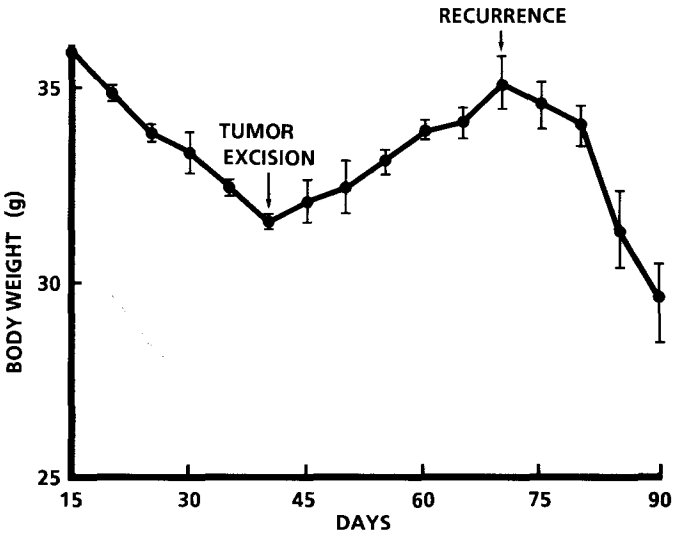


TEXT-FIGURE 3.—Relationship between carcass weight and tumor weight in male NMRI mice bearing the MAC16 tumor. Carcass weights at 21 g were significantly lower than those at 27.5 g. *, $P<.05$.



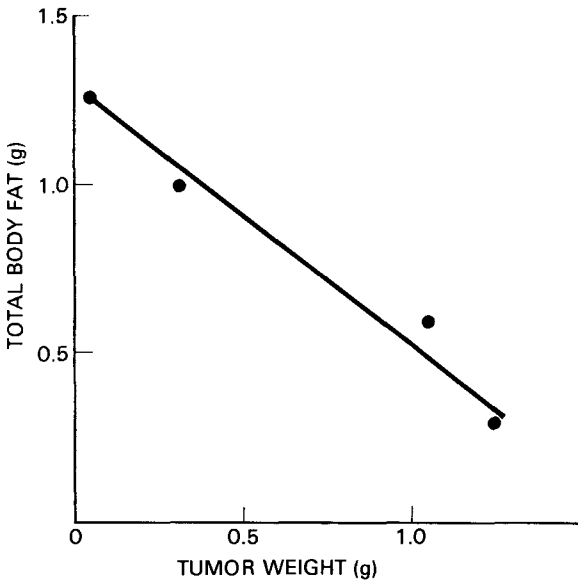
TEXT-FIGURE 4.—Food consumption by male NMRI mice after implantation with the MAC16 tumor. Data from non-tumor-bearing mice have not been included because of duplication of data. *Each point* is the cumulative food intake by 2 mice. The total food consumption during the 30 days of the study was 156 g/mouse for tumor-bearing mice and 159 g/mouse for non-tumor-bearing controls.



TEXT-FIGURE 5.—Body weight changes in mice following tumor growth, excision, and recurrence.

mice (text-fig. 4). Tumor excision results in cessation of weight loss with subsequent weight gain upon recovery from surgery (text-fig. 5). Occasional local recurrence of tumor growth is again accompanied by loss of body weight. There is no significant change in the total body water, although both the dry weight of the carcass and the total carcass fat decrease in proportion to the tumor size (text-fig. 6, table 1). Carcass fat, which contributes 6% of the total body mass in non-tumor-bearing mice, decreases to 1.2% of the total body mass in mice with a tumor burden of 1.25 g.

Concomitant with the reduction in body fat, the plasma level of FFAs increases after tumor transplantation, although the blood glycerol, acetoacetate, and 3-hydroxybutyrate levels do not differ significantly from



TEXT-FIGURE 6.—Relationship between total-body fat and tumor weight in MAC16 tumor-bearing male NMRI mice.

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TABLE 1.—*Effect of the MAC16 tumor on body composition of male NMRI mice*

Day after transplantation	Carcass wet weight, g, ± SEM	Dry weight, g, ± SEM	Water, %, ± SEM	Carcass fat, g, ± SEM	Carcass fat, % body wt
		Tumor-bearing mice			
7	32.0±0.9	10.1±0.7	66.5±0.8	1.3±0.1	4.1
14	31.6±1.1	10.5±0.7	66.0±1.5	1.2±0.3[a]	4.1
21	24.5±1.4[b,c]	8.0±1.2[d]	68.4±2.2	0.5±0.1[c,e]	2.0
28	24.3±0.9[b]	7.7±0.8[d]	67.3±1.7	0.3±0.1[e]	1.2
Day	Carcass wet weight, g, ± SEM	Dry weight, g, ± SEM	Water, %, ± SEM	Carcass fat, g, ± SEM	Carcass fat, % body wt
		Non-tumor-bearing mice			
7	31.5±1.5	10.2±0.4	67.3±1.5	1.9±0.3	6.0
14	32.7±1.0	11.2±0.9	65.3±3.7	2.0±0.2	6.1
21	32.7±1.4	11.1±0.9	66.0±2.8	1.7±0.2	5.1
28	33.8	10.5	68.7	1.5	4.4

[a] $P<.05$ from corresponding non-tumor-bearing animals.
[b] $P<.0005$ from 7-day weight.
[c] $P<.001$ from corresponding non-tumor-bearing animals.
[d] $P<.05$ from both 7-day weight and corresponding non-tumor-bearing animals.
[e] $P<.005$ from 7-day weight.

those of non-tumor-bearing mice (table 2). At no time is there significant ketosis because the plasma level does not exceed 0.2 m*M*.

There is a decrease in the blood glucose level in tumor-bearing mice with increasing time after tumor transplantation, which is significantly lower ($P<.01$) than that of non-tumor-bearing mice 21 days after tumor transplantation (text-fig. 7). Plasma insulin levels also decrease with increasing time after tumor transplantation and increasing tumor weights (text-fig. 8). There is also a trend toward decreased lactate levels with increasing tumor size, although the lactate-to-pyruvate ratio does not change significantly (table 2).

DISCUSSION

MAC16 is a slow-growing, transplantable adenocarcinoma of the colon producing weight loss in tumor-bearing animals with a small tumor mass (<1% of the host weight). Weight loss appears to be directly related to the tumor size and occurs without a reduction in food intake by the host, suggesting a metabolic basis for the ensuing cachexia. Weight loss is reversible since tumor excision results in weight gain in the host. Recent results with the use of parabiotic rats provide evidence that cancer-associated cachexia is mediated via the circulation (*15*); weight loss appears to be greater in female than in male mice, probably because of the more extensive fat reserves of the latter. The weight loss is associated with a significant reduction in both fat and nonfat mass. The proportion of carcass lipids varies inversely with the tumor size. On a percentage basis the decrease of carcass fat is greater than that of the lean body mass, which appears to decrease in proportion to the overall body weight. Lipemia has frequently been observed in

TABLE 2.—*Effect of the MAC16 tumor on blood levels of FFA, glycerol, acetoacetate, 3-hydroxybutyrate, lactate, and pyruvate in male NMRI mice*

Day after transplantation	FFA, m*M*	Glycerol, μ*M*	Acetoacetate, μ*M*	3-Hydroxybutyrate, μ*M*	Lactate, μ*M*	Pyruvate, μ*M*
			Metabolite levels, ± SEM			
			Tumor-bearing mice			
7	0.166±0.039	108±21	47±5	72±7	7.4	0.11
14	0.179±0.036	1,150±12	70±17	67±13	3.6±0.8	0.09±0.01
21	0.265±0.071	130±15	52±16	92±19	5.1	0.10±0.004
28	0.298±0.094[a]	155±17	57±15	55±6	3.5	0.01
Day	FFA, m*M*	Glycerol, μ*M*	Acetoacetate, μ*M*	3-Hydroxybutyrate, μ*M*	Lactate, μ*M*	Pyruvate, μ*M*
			Non-tumor-bearing mice			
7	0.176±0.024	107±25	95±19	118±19	4.0±0.4	0.13±0.03
14	0.176±0.024	140±27	67±8	54±1	5.0±0.7	0.12±0.01
21	0.176±0.024	118±14	56±8	84±11	4.8±1.0	0.09±0.03
28	0.176±0.048	132±16	62±8	44±4	6.5	0.10

[a] $P<.02$.

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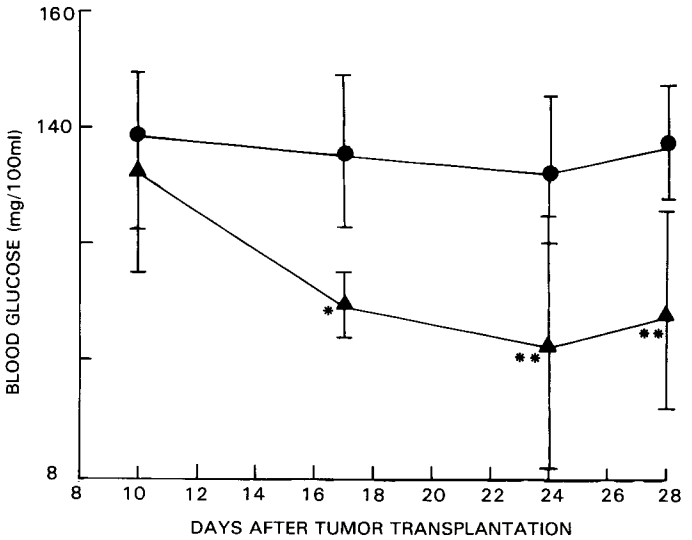


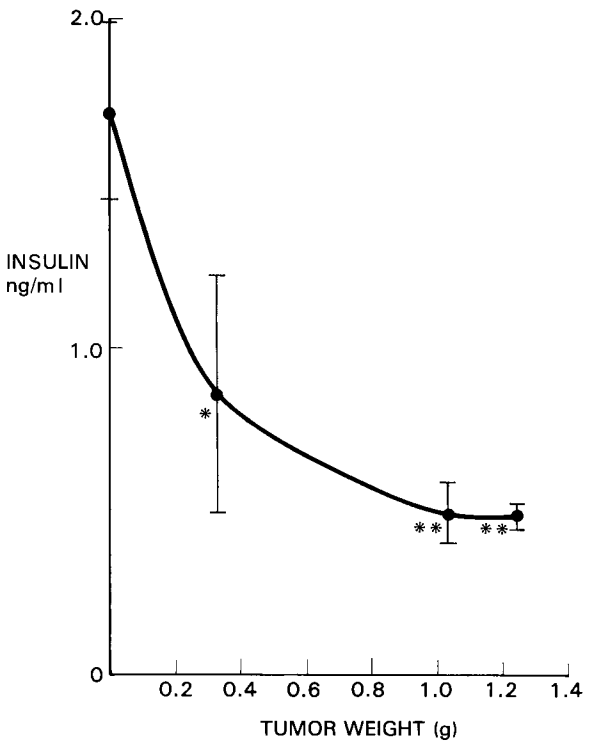
TEXT-FIGURE 7.—Blood glucose concentration in tumor-bearing (▲) and non-tumor-bearing (●) male NMRI mice after tumor transplantation. Tumor-bearing mice, $n=12$. Non-tumor-bearing mice, $n=12$. *, $P<.05$ from corresponding non-tumor-bearing mice. **, $P<.01$ from corresponding non-tumor-bearing mice.

tumor-bearing animals (*16*), perhaps reflecting an increased mobilization of lipids to meet increasing energy demands.

Despite the loss of carcass fat and the progressive increase in the plasma level of FFA, ketosis does not occur. There is a transient elevation in 3-hydroxybutyrate at day 21, although this is not greater than the normal range of less than 0.2 m*M*. Ketonuria has been reported to be an uncommon phenomenon both in cancer patients (*17*) and tumor-bearing rats (*16*), possibly due to an elevated basal metabolic rate and utilization of ATP from the oxidation of FFAs in liver to support an enhanced gluconeogenesis. However, there appears to be no impairment of the ability of the liver to synthesize ketone bodies, since ketonemia is observed in tumor-bearing animals given an exogenous supply of fatty acids (*18*).

Growth of the MAC16 tumor is accompanied by hypoglycemia, which increases with increasing tumor size. The hypoglycemia may result from excessive glucose consumption by the tumor. Separate experiments have indicated a high glucose consumption by MAC16 tumor cells in vitro (Tisdale MJ: Unpublished results). However, both the lipid mobilization and hypoglycemia may be due to production by endotoxin-stimulated macrophages of an acidic protein cachectin, which has been shown to exhibit a high degree of homology to TNF (*19*). Cachectin has been shown to inhibit lipoprotein lipase activity in peripheral tissues resulting in a marked elevation of plasma very-low-density lipoprotein (*20*) and production of TNF by macrophages after lipopolysaccharide challenge is also accompanied by hypoglycemia (*21*). Despite the tendency of a number of tumors to act as a trap for glucose, normal blood glucose concentrations have been observed in most, but not all (*22*), tumor-bearing animals and patients due to an increased gluconeogenesis from noncarbohydrate sources (*23, 24*). Increased gluconeogenesis from glycerol (*24*) may explain the lack of elevation of blood glycerol levels by the MAC16 tumor, despite increased mobilization of triglycerides from adipose tissue. Gluconeogenesis from lactate is an energy-requiring process that may play a role in excessive energy expenditure by the host (*25*).

Growth of the MAC16 tumor is also associated with a decreased plasma insulin level, which may be a response to the falling blood glucose. Decreased plasma insulin levels have also been observed in rats bearing the Walker 256 carcinoma (*26*), and in rats bearing 3-methylcholanthrene-induced sarcomas levels of insulin were decreased and glucagon increased (*27*). Cachectic cancer patients exhibit an abnormal diabetic-type glucose tolerance, which has been correlated with a marked resistance to administered insulin (*28*) or may be due to reduced insulin production. Insulin has an inhibitory role in adipocyte lipolysis, and the decreased insulin levels may be partly responsible for the increased mobilization of FFA, particularly if glucagon levels are increased (*27*). Some evidence for this has been provided by the fact that insulin, to some extent, reduces the loss of fat in tumor-bearing animals (*29*). Insulin has also been shown to increase amino acid uptake into muscle and subsequent incorporation into protein (*30*) as well as decreasing proteolysis. Thus reduced insulin concentration may have contributed to the decrease in muscle and dry carcass weight in MAC16 tumor-bearing mice.



TEXT-FIGURE 8.—Plasma insulin levels with increasing tumor weight in male NMRI mice. Levels of plasma insulin were significantly lower in the tumor-bearing groups of mice compared to those in the non-tumor-bearing controls. *, $P<.05$; **, $P<.01$.

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The results presented here show that the MAC16 tumor system is an excellent tool to study the factors involved in the production of cancer cachexia. A knowledge of these factors may lead to therapeutic manipulations to control or even reverse the cachectic process, which would represent significant advantage in the management of many patients with advanced cancer.

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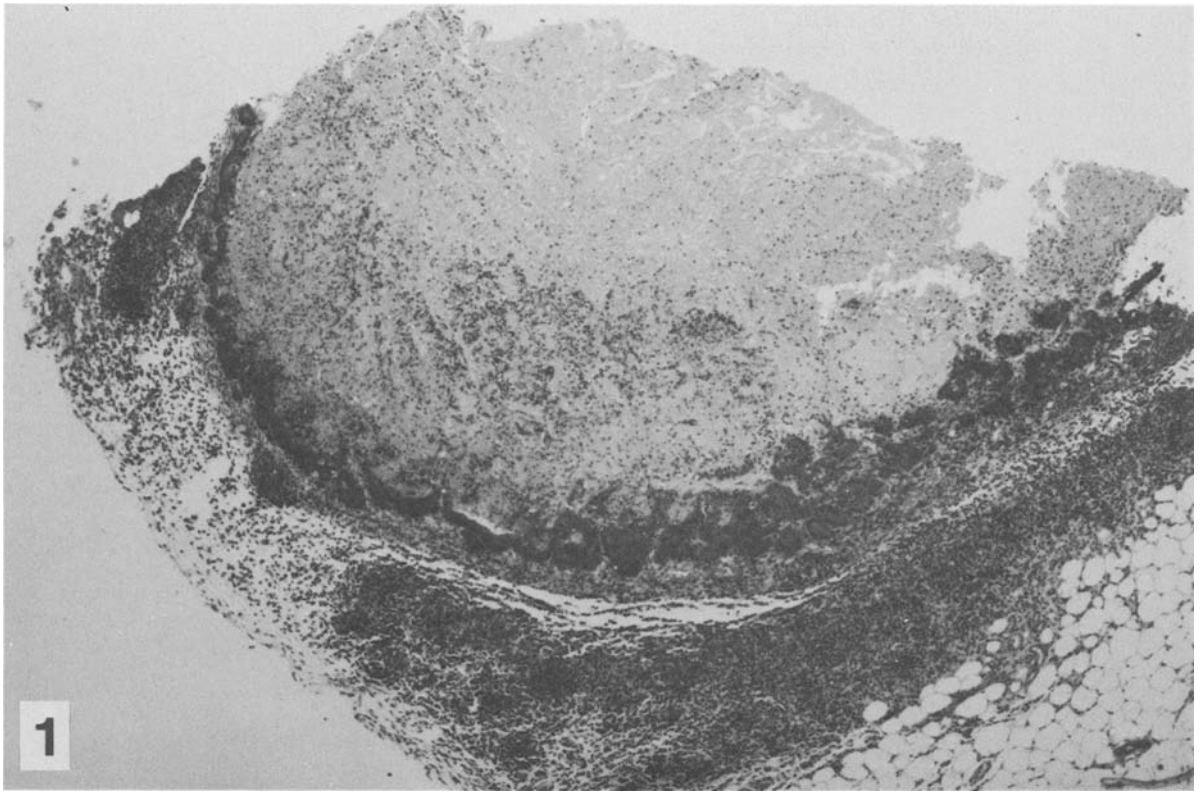

FIGURE 1.—Section through the MAC16 adenocarcinoma (×5) at 28 days after tumor transplantation. The tumor is a well-differentiated colon adenocarcinoma and contains a centrally necrotic region.

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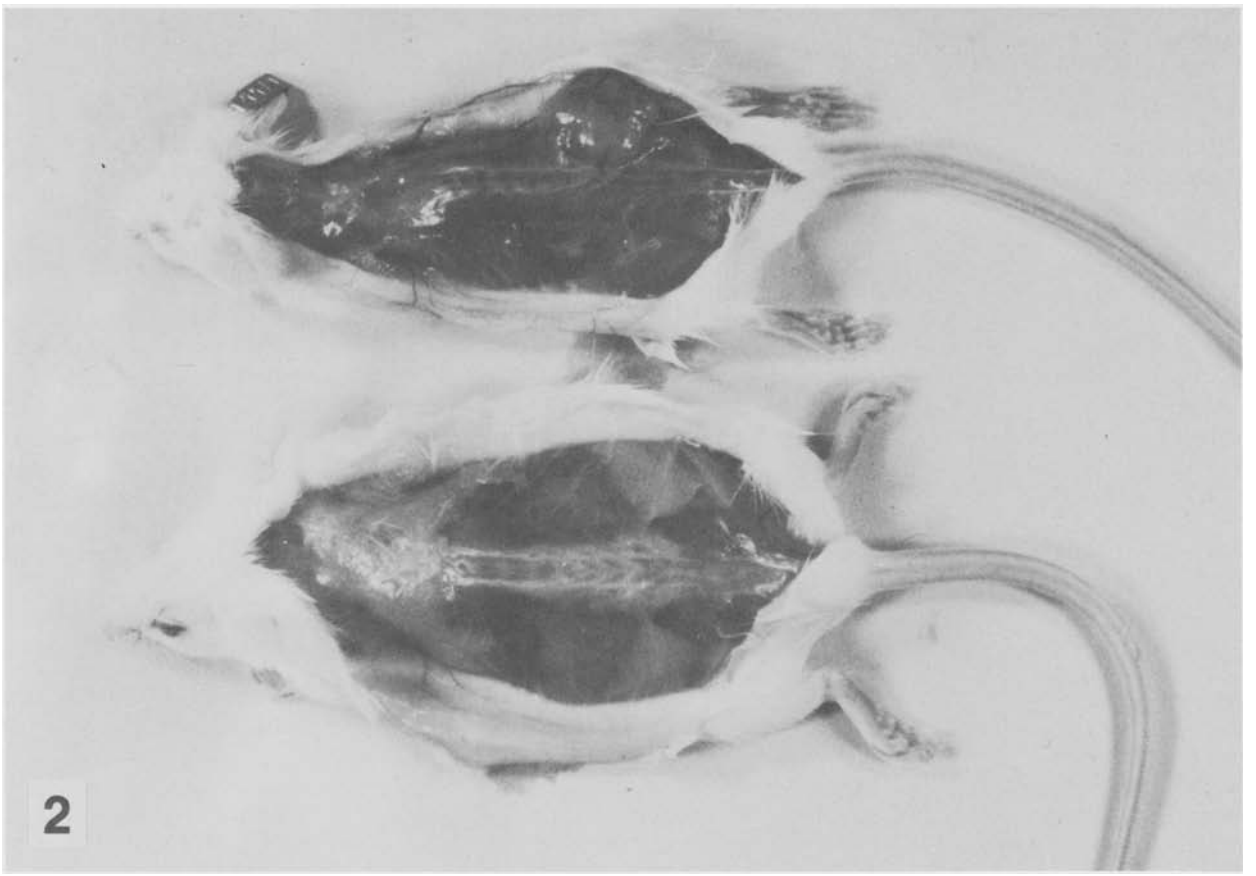

FIGURE 2.—Effect of the MAC16 tumor on the growth and fat content of NMRI mice. Both mice started at the same weight and consumed equal amounts of food, but the mouse *on the top* had a fragment of the MAC16 tumor implanted 28 days prior to the photograph being taken. There is a reduction in the overall size of the mouse bearing the MAC16 tumor in addition to loss of adipose tissue and muscle mass.

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