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Effects of Three Feeding Schedules on Tumor and Host of Lewis Lung Carcinoma-Bearing Mice[1] (41898)

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Abstract. The effect of three feeding schedules on tumor and host were examined in Lewis Lung bearing (TB) and nontumor bearing (NTB) C57/Bl mice. Both NTB and TB animals were divided into three groups: the control groups which were fed *ad libitum;* the intermittent fed (IMF) groups were fed for 32 hr and fasted for 16 hr in each 48-hr cycle, and the alternate day fed (ADF) groups were fed for a 24-hr interval in each 48-hr cycle. The animals were killed at the end of the fifteenth day, following a *fed* day for all groups. In the NTB groups, only the ADF group showed decreased food intake and lower body weight gain as compared to their control group. In the TB mice, as compared to their control group, the IMF group showed a significant reduction in the mean tumor weight with no change in the mean host weight, even though the daily food intakes of these two groups were the same over the experimental interval. In contrast, the ADF group showed reductions in both host and tumor weights as compared to their control group. The tumor to host weight ratios were significantly reduced for both the IMF and ADF groups as compared to the ratios found for the control groups, which suggests a differential effect on the tumor and on the host due to the feeding schedule. As assessed by the protein, RNA, and DNA concentrations, no compositional differences were noted for the tumors obtained from the animals that were maintained on each of the three different feeding schedules. In the NTB mice, no differences in tissue leucine (Leu) oxidation occurred between the groups for liver and skeletal muscle, whereas in the TB animals *in vitro* Leu oxidation capability by skeletal muscle specimens was markedly enhanced in the ADF group, but no difference was noted for the IMF group of the TB mice when compared to the control group. Taken together, these results suggest that the 32-hr fed:16-hr fast schedule (IMF) was beneficial and the 24-hr fed:24-hr fast schedule was detrimental compared to the *ad libitum* feeding schedule with respect to tumor and host relationships.

Restriction of caloric or nutrient intake has been shown in several experimental models to decrease tumor growth, but the effects on the host were generally not assessed (1). In a recent study, Goodgame *et al.* showed that animals fasted from 1–4 days had reduced body weights without altered tumor size (2). Moreover, in the same study it was reported that there was actually an increased *in vitro* [^{3}H]thymidine incorporation by tumor tissue in fasting animals. Stragand *et al.* (3) have shown that both tumor, host weights and tumor volumes were decreased as a result of fasting. Although fasting can reduce the tumor size, tumor growth can continue in fasting animals (4). On the other hand, adequately nourished animals have been found to support optimal tumor growth in some studies, and hyperalimentation has actually been shown to stimulate tumor growth (5). The present study was designed to determine whether the primary Lewis Lung carcinoma size could be decreased without adversely affecting the host by simply altering the feeding pattern and not the level of alimentation.

Methods. Female C57/Bl mice were housed in a temperature and humidity controlled room with a 12-hr light:12-hr dark cycle in which the dark phase was from 6 PM to 6 AM as shown in Fig. 1. At the start of the experimental interval the TB animals were inoculated in the flank with 5×10^5 viable Lewis Lung carcinoma cells (LL), and both the TB and NTB animals were placed randomly in individual wire bottom cages. Both the TB

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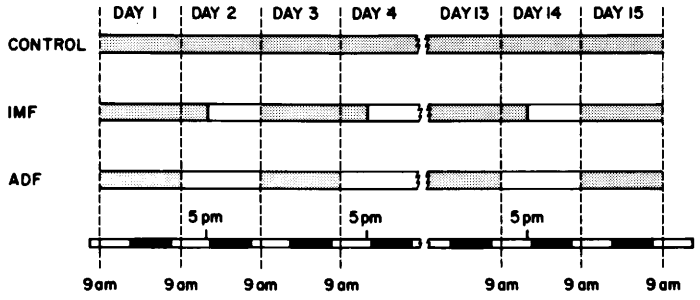

FIG. 1. Feeding schedule protocols and light–dark cycles. This figure illustrates the several sets of 48-hr cycles for the fed (stippled bars) and the fast phases (open bars) of the three feeding schedule protocols. The control (*ad libitum*), IMF (32-hr fed:16-hr fast), and the ADF (24-hr fed:24-hr fast) plans are indicated for each 2-day cycle. The corresponding light (6 AM–6 PM) and dark (6 PM–6 AM) phases are identified in the bottom bar.

and NTB animals were subsequently fed on one of the three schedules: the control groups were fed *ad libitum* throughout the experimental interval; the intermittently fed groups (IMF) were maintained on a 32-hr fed:16-hr fast schedule in which the food cups were removed from 5 PM of the second day to 9 AM of the following day; and the alternate day fed groups (ADF) were maintained on a 24-hr fed:24-hr fast schedule in which the food cups were removed at 9 AM every other day for 24 hr (giving a period of 48 hr). These feeding patterns were repeated each successive 48-hr period throughout the experimental period and each experimental day began at 9 AM. Food consumption was measured and recorded for each 2-day interval and the animals fed on IMF and ADF schedules were provided the same quantity of rations for each 2-day period that their control group consumed for the previous 2-day interval. All animals were fed a synthetic amino acid diet, the composition of which is indicated in Table I, and water was available *ad libitum* throughout the entire experimental period. At the end of Day 15 following a fed day for all groups, the animals were killed by cervical dislocation and their body and tumor weights determined. This overall procedure was performed once using the NTB animals and was performed three separate times using the TB animals. The results obtained were combined and analyzed using standard statistical methods (7). In the tables, the means of various parameters presented for the IMF and ADF groups were tested against the values obtained for the control group using a two-tailed Student's t test with a $P < 0.05$ as the level of significance.

For one experiment with the TB animals, the homogenate of a specimen of the tumor was prepared in distilled water using a Techmar tissue homogenizer. These homogenates were used for the determination of tumor DNA, RNA, and protein, using the method of Wannemacher *et al.* (8) with the modifications suggested by Munro and Fleck (9). Leu oxidation capabilities of liver and skeletal muscle specimens were estimated by incubating 100-mg minced samples of tissue in 3.0 ml of complete tissue culture media (Difco TC 199) containing [1-^{14}C]Leu with a final specific radioactivity in the incubation media

TABLE I. COMPOSITION OF SYNTHETIC AMINO ACID-DEFINED DIET

Ingredients	g/kg Diet
L-Amino acid mixture[a]	170.0
Corn starch	100.0
Sucrose	564.0
Corn oil	100.0
Cellulose	15.0
Vitamin mixture[b]	10.0
Mineral mixture[c]	40.0
Chromium and selenium supplement[d]	1.0

[a] Amino acid composition as reported by Rogers and Harper (6).

[b] Vitamin mixture-Teklad No. 40060, Madison, Wisc.

[c] Bernhart–Tomarelli mineral mixture Teklad No. 17050, Madison, Wisc.

[d] Supplementation with mixture of $CrK(SO_4)_2 \cdot 12H_2O$ (19.25 mg/kg diet) and $Na_2Se_3 \cdot 5H_2O$ (0.35 mg/kg diet).

of 22.5 Ci/m*M* Leu. The reactions were performed in closed vessels equipped with plastic center wells containing 0.2 ml of hyamine hydroxide as the trapping agent. At the end of 90 min, the reaction was stopped and the CO_2 evolved by addition of 1.5 ml 2 *N* citric acid and trapped in the center wells, which were then placed into scintillation vials containing 10 ml of a toluene-based scintillation cocktail and the $^{14}CO_2$ radioactivity counted.

Results and Discussion. All animals used in the experiments were drawn from a homogenous pool and the initial body weights between all three feeding schedule groups were the same for both the TB and NTB experiments. The results obtained for food intakes, as well as host and tumor weight parameters using the three feeding schedules are presented in Table II. In the NTB animals, the IMF schedule did not affect the average daily food intake, but the ADF schedule significantly decreased ($P < 0.05$) the food intake to 80% of the control group's intake. Reflecting the food intake, the change in host weight (terminal body weight–initial body weight) was significantly reduced ($P < 0.01$) in the ADF group to 35% of the control group. As for the TB animals, the net body weight changes show that the mean body weight of the control group (*ad libitum fed*) was increased, the IMF group (32-hr fed:16-hr fast) was virtually unchanged, and the ADF group (24-hr fed:24-hr fast) was markedly decreased over the experimental period. These net changes represent the sum of alterations in the host weight plus the tumor weight at the time the experimental period was ended. The mean terminal host weights were not different for the control and IMF groups, but were reduced ($P < 0.001$) to 84% of the control groups level at the end of 15 days for the animals fed on the ADF schedule. The mean tumor weights were reduced ($P < 0.001$) in both the IMF and ADF groups to 69 and 51%, respectively, of those found for the control group. Thus, the host weight was only adversely affected by the ADF group schedule, but the tumor weight was decreased by both feeding patterns.

In order to assess the relative effects of the two schedules on the tumor and the host weights, the mean tumor weight to host weight ratios (TW/HW%) were determined to be 15.3 ± 1.0, 10.6 ± 0.7, and 9.5 ± 1.1 for the control, IMF, and ADF groups, respectively. These results show that the TW/HW% for both the IMF and ADF groups were decreased ($P < 0.001$) when compared to the control group, suggesting that in each case, the tumor was affected to a greater extent than was the host as a result of following the two test feeding schedules. Although the tumor weights were reduced in both of the test groups, the host weight was not compromised in the IMF group, whereas, the host weight for the ADF

TABLE II. EFFECT OF THREE FEEDING SCHEDULES ON FOOD INTAKE, TUMOR AND HOST WEIGHT PARAMETERS

	Group (Feeding schedule)		
	Control (*ad lib*)	IMF (32 fed:16 fast)	ADF (24 fed:24 fast)
Non-tumor bearing			
Body weight change (g)	+3.1 ± 0.4[a]	+2.0 ± 0.5	+1.1 ± 0.4**
Food intake (g/day)	3.3 ± 0.2	3.2 ± 0.1	2.5 ± 0.1*
N	5	5	5
Tumor bearing			
Body weight change (g)	+1.22 ± 0.23	+0.16 ± 0.25**	−2.70 ± 0.35***
Terminal host weight (g)	16.3 ± 0.4	16.1 ± 0.4	13.7 ± 0.5***
Tumor weight (g)	2.42 ± 0.13	1.67 ± 0.10*	1.22 ± 0.11***
Food intake (g/day)	2.54 ± 0.05	2.46 ± 0.03	1.83 ± 0.09**
N	30	30	20

[a] Mean ± SEM.
* $P < 0.05$ as compared to control.
** $P < 0.01$ as compared to control.
*** $P < 0.001$ as compared to control.

group was lowered when compared to the control group. The more favorable TW/HW% obtained for the IMF group, relative to that found for the control group, could not be ascribed to altered food consumption, since the daily food intakes were the same when these groups were compared. Availability of the diet during each successive 2-day period for these two test groups was regulated externally but, the pattern of eating within each 2-day segment may have varied also, but no data on the rates of food intake within the 2-day cycles was obtained. Thus, the 31% reduction in tumor weight found for the IMF group below that obtained for the control group cannot be explained by caloric or nitrogen intake differences, but can be ascribed to the feeding schedule plan. Although the food intake of the ADF group was significantly ($P < 0.001$) lowered to 72% of the control group's daily intake, decreased TW/HW%, relative to that obtained for the control group cannot unambiguously be explained on the basis of the schedule of food availability. In the case of the ADF schedule, either the fasting period and/or the lowered food intake could have been responsible for the decreased weights of both the tumor and the host relative to those noted for the control group. The results also showed that the LL bearing animals were anorexic and cachexic, since both the food intakes and the body weight changes were decreased for the TB animals as compared to their corresponding groups of NTB animals.

Feeding schedules with repeating periods of nutrient availability have been shown to influence several metabolic activities of Morris hepatomas in rats (10) and intermittent periodic starvation and refeeding resulted in significant reductions in the growth of Ehrlich Ascites cells in mice (11), although both tumor and host responses to alternating feeding and fasting schedules have not been reported previously. The effect of intermittent fasting was first studied by Carlson and Hoelzel in 1946 (12) followed by Fabry and co-workers (13). The reports of Tepperman and Tepperman (14) on the metabolic effects of a single 2-day fast followed by 24 hr of feeding, stimulated the subsequent studies by Potter and co-workers (10) on the metabolic consequence of different feeding schedules. However, the feeding schedules used by all these investigators varied widely and therefore, it is difficult to compare the results between studies. In general, those investigators used intermittent fasting as a means of stress to study metabolic adaptation and so the fasting intervals were longer than the ones used in the present study. The studies on the metabolic effect of restricted feeding schedule have shown that there is a circadian rhythm for most of the parameters measured ranging from tryosine transaminase activity (15) to amino acid uptake by tissues (16) and that a deviation of feeding schedules from *ad libitum* feeding can alter the rhythm of most of the parameters measured. Takahashi and Zatz have extensively reviewed the interrelationships between the endocrine systems and the regulation of circadian rhythm, concluding that recent advances in locating the circadian "pacemaker" in the brain could reveal the cellular and biochemical mechanisms in controlling circadian rhythm (17).

Since the molecular basis of circadian rhythmicity is not fully understood it is difficult to ascribe the contribution to our findings of the systematic oscillation in metabolic and/or hormonal changes as compounded by the effect of fast/fed and light/dark stimuli. Furthermore, most of the studies from Potter's group reported synchronized food availability of rats within the dark phase. Although rodents eat mostly during the daily dark phase, they do consume some food in the light phase. In the experimental design of the present study, both of the test groups were repeatedly fasted during the dark phase. Therefore, the result observed cannot be solely attributed to the periodic fasting–refeeding cycles per se without conceding to a possible effect brought about by the perturbation of the normal hormonally–metabolically driven fast–fed rhythm and as well as the effects of the light–dark cycles. Finally although mice consume more food in the dark than in the light, their energy expenditure is also considerably elevated during that phase. Nevertheless, the present experimental design demonstrated that periodic fasting affects the TW/HW% and that the IMF schedule employed, reduced tumor weights but did not lower host weights. The influence of the factors producing circadian rhythms on these findings cannot be assessed from this study, but the IMF feeding plan produced a model in which the tumor was proportionally

TABLE III. EFFECT OF THREE FEEDING SCHEDULES ON TUMOR PROTEIN AND NUCLEIC ACID CONCENTRATIONS

Concentration (mg/g tumor)	Group (Feeding schedule) Control (*ad lib*)	IMF (32-hr fed:16-hr fast)	ADF (24-hr fed:24-hr fast)
DNA	15.9 ± 0.2[a]	14.8 ± 0.5	16.5 ± 0.6
RNA	13.9 ± 0.8	12.5 ± 0.4	12.6 ± 0.9
Protein	266 ± 8	274 ± 11	277 ± 11
N	10	10	8

[a] Mean ± SEM.

smaller with respect to the host within the context of equivalent food intakes.

The reduced mean tumor weights of both the IMF and/or the ADF groups, relative to the control group, may be simply reflections of internal compositional changes in the tumor resulting in a smaller tumor cell size with either increased, decreased or similar cell numbers. In order to establish whether this was the case, RNA, DNA, and protein contents of tumor homogenates were determined. Table III showed that no significant differences were found for the tumor concentrations of these macromolecules when the results from the IMF and ADF groups were compared to those obtained from the control tumors. Since tumor DNA, RNA, and protein concentrations were not affected by the feeding schedule, the decreased tumor size of the IMF and ADF groups, relative to the control group, was apparently a hypoplastic response brought about by the altered feeding schedules.

Finally, Table IV illustrates the relative capabilities for Leu oxidation by liver and skeletal muscle samples from animals maintained on each of the three feeding schedules for 15 days. As measured by the $^{14}CO_2$ produced/g tissue-min, the rates for Leu oxidation by the liver and skeletal muscle tissues specimens of the ADF group were markedly elevated compared to the rates for their respective control group tissues. However, there were no significant differences between the IMF and control group results for either host tissue.

As for the NTB animals, there were no differences in Leu oxidation rates observed among the three groups for both the liver and skeletal muscle samples. The distinct difference in the effect of the ADF schedule on Leu oxidation correlated with the changes in body weights when the TB and NTB groups were compared. In the NTB animals, the ADF group showed neither weight loss nor a change in Leu oxidation as compared to the NTB control group, whereas there was a loss in host weight in TB animals fed on the ADF schedule

TABLE IV. EFFECT OF THREE FEEDING SCHEDULES ON LEUCINE OXIDATION BY LIVER AND SKELETAL MUSCLE TISSUE

Tissue	Control (*ad lib*)	IMF (32-hr fed:16-hr fast)	ADF (24-hr fed:24-hr fast)
Nontumor bearing			
Liver	20.4 ± 2.4[a]	23.4 ± 2.9	28.7 ± 3.9
Skeletal muscle	9.5 ± 1.1	11.0 ± 0.8	10.2 ± 0.9
N	5	5	5
Tumor bearing			
Liver	25.0 ± 2.4	22.3 ± 1.0	42.3 ± 4.0*
Skeletal muscle	8.6 ± 0.8	8.8 ± 4.0	38.9 ± 4.9**
N	6	6	6

[a] Mean ± SEM (dpm/g tissue-min).
*$P < 0.01$ as compared to control.
**$P < 0.001$ as compared to control.

and the Leu oxidation of this group was significantly increased, when compared to that of the TB control group. These results indicated that tissue leucine oxidation may be stimulated by a factor associated with net body weight loss owing to the presence of the tumor. The control of Leu oxidation is not fully understood, but, accelerated Leu oxidation has been associated with catabolic states such as experimental diabetes and starvation (18, 19). Therefore, the results for Leu oxidation may be extrapolated to support the notion that the IMF schedule did not produce the catabolic response in the TB host that was noted for the host of the animals on the ADF schedule. Since the skeletal muscle is the site where the branched-chain amino acids are predominantly catabolized for energy (20), then the 4.5-fold increase in the metabolic activity observed for the skeletal muscle tissues of the ADF compared to the control group would clearly indicate a marked elevation of this tissue's *capability* to oxidize this essential amino acid owing to the ADF feeding schedule in the tumor bearing animals. Due to the magnitude of this increase in skeletal muscle Leu oxidation capacity, it is unlikely that this result could be solely accounted for as a procedural artifact due to equally large differences in the endogenous Leu pool sizes of the skeletal muscle between the ADF and the control groups. Finally, there were no differences between the control and IMF groups in the rates for hepatic Leu oxidation, although a significant increase of 1.7-fold for the liver of the TB ADF group compared to its TB control group was noted. Leu is catabolized by a transamination reaction followed by an irreversible oxidative decarboxylation catalyzed by the branched-chain oxo acid dehydrogenase (21). The former reaction is higher in the skeletal muscle and lower, but present, in the liver (20). The latter enzyme, however, is found to be mostly activated in the liver, but largely inactivated in the skeletal muscle tissue under normal conditions (21). This finding may explain the observed 1.7-fold increase in the liver tissue Leu oxidation as compared to the 4.5-fold increase in the skeletal muscle in the TB–ADF group, since the muscle has greater capacity for further activation. Odessey (21) also showed that the activity of the branched-chain oxo acid dehydrogenase varied as much as 5–20-fold under different experimental conditions. Thus, the 1.7- and 4.5-fold differences observed in this study are not out of range. The fact that we observed a higher Leu oxidation activity in the liver, on a per gram tissue basis, than the skeletal muscle does not negate the fact that muscle is the major site for branched-chain amino acid catabolism if we consider the total mass of skeletal muscle as compared to that of liver. Furthermore, Ichihara *et al.* (22) have noted that even though rat skeletal muscle showed higher activity of branched-chain amino acid transferase, the liver actually exhibited higher combined transamination and decarboxylation activity, again on a per gram tissue basis. Thus, these results are in concert with a general activation of the oxidation of Leu in the ADF group bearing the LL tumor as compared to its control, whereas no change in the parameter in the two tissues studied was found for the IMF group of the tumor bearing animals.

Even though our findings may be unique to the rapidly growing Lewis Lung carcinoma model, they provide a useful framework to contemplate the importance of feeding schedules in the nourishment of the host in the presence of a growing tumor. Furthermore, the results with respect to tumor growth were confined to the primary tumor at this point and cannot be extrapolated to include metastatic growth.

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