

# Comparison Between Meal-eating and Nibbling Rats Fed Diets Containing Hard Red Spring Wheat Bran: Bioavailability of Vitamins A and E and Effects on Growth<sup>1</sup>

S. T. OMAYE and F. I. CHOW, Nutrients Research Unit, U.S. Department of Agriculture, Western Regional Research Center, Agricultural Research Service, Berkeley, CA 94710.

## ABSTRACT

Cereal Chem. 61(2):95-99

Semipurified diets containing 5 or 20% hard red spring wheat bran were fed to two groups of male Sprague-Dawley rats. One group, nibbling rats, was fed ad libitum; the other group (meal-eating) was fed for restricted 2-hr periods each day for 35 days. After 35 days on the dietary regimen, the following results were found: Body and body weight gain and food intakes were significantly ( $P < 0.05$ ) increased, and total intestinal tract, stomach, proximal colon, and distal colon weights were significantly decreased in nibbling rats compared to meal-eating rats fed either 5 or 20% wheat bran. Ratios of fat gain to body weight were  $5.7 \pm 0.5$  for meal-eating rats and  $8.0 \pm 0.6$  for nibbling rats fed 5% wheat bran; and  $5.6 \pm 0.4$  for meal-eating rats and  $7.1 \pm 0.5$  for nibbling rats fed 20% wheat bran. Wheat bran consumption did not influence body weight; however, the proximal

colon weights were significantly increased in meal-eating ( $28.0 \pm 8\%$ ) and in nibbling rats ( $39.8 \pm 5\%$ ) fed 20% wheat bran compared to rats fed 5% wheat bran. Distal colon weights were increased  $42.5 \pm 1\%$  ( $P < 0.05$ ) in nibbling rats. Plasma levels of vitamin E were  $27.5 \pm 2.8\%$  less ( $P < 0.05$ ) in meal-eating rats and  $28.1 \pm 3.7\%$  less ( $P < 0.05$ ) in nibbling rats fed 20% wheat bran than in corresponding rats fed 5% wheat bran. Similar trends were found for plasma vitamin A levels with changes in wheat bran in the diet. These results indicate that the frequency of meals and the dietary content of wheat bran markedly affect gastrointestinal weights and alter plasma fat-soluble vitamins. The trend is for lower plasma fat-soluble vitamins and larger intestinal tract weights with decreased meal frequency, high dietary wheat bran, or both.

The eating pattern whereby people of Western populations consume two to three meals per day is quite different from the eating pattern of the common domesticated laboratory rat (Leveille and Romsos 1974). Domestic rats are by nature "nibblers," eating small amounts of food at frequent intervals and reaching maximal intake during the night (Conn 1964). Most Western peoples have an intermittent eating behavior with fewer feedings and are labeled by some as "meal-eating" (Conn 1964). Extensive work has been done on the physiological (Kissileff and Van Itallie 1982) and metabolic (Leveille and Romsos 1974, Leveille 1970) relationship between the two extremes of eating patterns. Work on animals has shown that consumption of total food intake in fewer feedings induces adaptive changes that include alterations in energy metabolism, more rapid intestinal absorption of glucose and fat, enhanced lipogenesis, and increased glucose synthesis (Leveille and Romsos 1974, Leveille 1970). Several of these changes seem to have their counterparts in meal-eating Western populations, suggesting that the pattern of the meal-eating rat is an appropriate model for the Western eating pattern.

Our ongoing investigations characterize interactions between various constituents of mixed diets, such as dietary fiber (Cummings 1976) (including cereals and cereal by-products) with selected nutrients. The objective of our research was to determine whether or not the consumption of dietary fiber adversely affects the bioavailability of such nutrients. Whole grains and whole-grain products are good sources of vitamins and minerals. The possibility that the bioavailability of vitamins (Cullen and Oace 1977, Keltz et al 1978) or of other nutrients (Ranhotra et al 1978, 1979) is reduced in diets rich in whole-grain products or whole grains is a matter of considerable discussion. Zinc bioavailability improves in soy-fortified bread in which phytate hydrolysis is substantial (Ranhotra et al 1978) but does not improve when phytate hydrolysis is minimal (Ranhotra et al 1979). Significant reduction of magnesium absorption in boys fed hemicellulose has been noted (Drews et al 1979), although feeding cellulose had less influence. Utilization or absorption of vitamins B<sub>12</sub> and B<sub>6</sub> are adversely affected by diets high in fiber (Cullen and Oace 1977, Leklem et al 1980). Dietary fiber influences urinary excretion of ascorbic acid (Keltz et al 1978)

with increased intake of hemicellulose, resulting in increased ascorbic acid excretion. We recently found a significant plasma vitamin E reduction in rats fed high levels of pectin (DeLumen et al 1982). Further investigation, *in vitro*, suggests that other forms of dietary fiber potentially reduce the amount of free vitamin that might be available for utilization (Omaye et al 1982, 1983).

As an attempt to define the usefulness of the meal-eating rat for our research, and because inconsistency and disagreement exist among the past studies regarding the physiology and metabolism of the meal-eating rat (Kissileff and Van Itallie 1982, Adams and Morgan 1981), the following preliminary experiment was initiated. The meal-fed rat model is appropriate for future *in vivo* bioavailability research because a pulse ingestion of a specific nutrient (vitamin) can be given at the same time interval of the meal. In addition, as part of our ongoing interest in the influence of various dietary fibers on vitamin bioavailability (DeLumen et al 1982; Omaye et al 1982, 1983), we also examined the effect of ingesting wheat bran incorporated into the diet and compared the results with our previous *in vitro* findings (Omaye et al 1982, 1983).

## MATERIALS AND METHODS

### Animals and Diets

Thirty young male Sprague-Dawley rats (Charles River, Wilmington, MA), ages 30–35 days and weighing 90–125 g, were housed individually in stainless steel wire-bottom cages. The cage racks were equipped with an automatic watering system, and the animal room was maintained at 25°C, 65% relative humidity, with a 12-hr light-dark cycle (7:00 A.M. to 7:00 P.M.). On arrival from the supplier, the animals were fed a standard stock diet (Rodent Chow, Ralston Purina Co., St. Louis, MO) for seven days. After the acclimation period, they were divided randomly into five groups and fed a standard stock diet or a semipurified AIN-76 diet (Bio. Serv. Inc., Frenchtown, NJ) containing either 5 or 20% (w/w) hard red spring (HRS) wheat bran (American Association of Cereal Chemists, St. Paul, MN) (total sample milled through 30 mesh) diets 1 and 2, respectively. Tap water was allowed ad libitum throughout the experiment. The modified AIN-76 diet is described in Table I (AIN 1977). The stock diet contained 60 mg of  $\alpha$ -tocopherol per kilogram.

Two groups of rats conditioned to being fasted from 11:00 A.M. on one day to 9:00 A.M. on the consecutive day hereafter will be known as meal-eating rats. Rats fed the stock diet serve only as a comparison between a commercial diet and the semipurified diet. Stock diet was not construed as a control diet. We did not include a

<sup>1</sup>The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

fiber-free diet because normal diets of animals are not devoid of fiber. The other three groups, including the group of rats fed the standard stock diet, were fed ad libitum and will hereafter be referred to as nibbling rats. Body weights were measured two to three times each week throughout the 35 days. Total food intake

and food spillage were measured over a 24-hr period several times each week throughout the study.

### Blood and Tissue Preparation

At 9:00 A.M. after 35 days on the dietary regimen, the rats were weighed and anesthetized with methoxyflurane (Metofane, Pitman-Moore, Inc., Washington Crossing, NJ). The thorax was opened, and blood was collected into heparinized tubes by cardiac puncture until exsanguinated. Liver, the entire gastrointestinal tract, and total abdominal fat (epididymal fat pads and renal fat pads) were excised, and the cleaned organs and tissues resected of extraneous material were weighed. The gastrointestinal tract was sectioned into the stomach, the upper 10 cm of the small intestine (from the pylorus), the proximal 5 cm of colon (not including the cecum), and the distal 5 cm of colon. After the gastrointestinal tract was divided, the contents of segments of gastrointestinal tracts were removed by thoroughly flushing the lumen with 5 ml of ice-cold 0.84% NaCl (saline). The sections were pat-dried with two layers of cheesecloth and weighed. The segments of colon were laid out on a flat ice-cold glass surface and a microscopic slide was used to scrape the mucosal layer with constant pressure. The mucosal layer and the remaining muscles plus the serosa layer were then reweighed. Care was taken not to damage the tissues.

Hematocrit values were determined on an aliquot of blood. The remaining blood was centrifuged at  $1,400 \times g$ ,  $4^\circ\text{C}$  for 15 min, and the deproteinized plasma was used to determine the vitamin A and vitamin E concentrations by high performance liquid chromatography.<sup>2</sup>

### Vitamin A and Vitamin E Assays

One hundred microliters of plasma were added to an equal volume of ethanol containing internal standard and butylated hydroxytoluene (BHT). This was extracted with 200  $\mu\text{l}$  of heptane containing BHT, the heptane phase was evaporated in a vacuum centrifuge, and the remaining lipid was redissolved in methanol for injection. Analysis was by reverse-phase chromatography using a  $\mu\text{Bondapak C-18}$  column equipped with a guard column and developed with a mobile phase of methanol-water (93:7). The chromatogram was completed in 25 min with a flow rate of 1.0 ml/min (UV 292 nm). Quantitation was by peak area, and recovery was greater than 96% in all cases, as determined by vitamin addition.

### Statistical Evaluation

All data were analyzed by analysis of variance procedures. Orthogonal contrasts as well as Fisher's least significant difference (LSD) comparisons were used to test treatment differences (SAS 1979). The harmonic means of treatment groups sample sizes were used to calculate LSD values when data were missing.

<sup>2</sup> Presented in part at the regional meeting of the American Chemical Society, 1982. F. I. Chow and S. T. Omaye. Simultaneous determination of  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and retinol in human and various animal species plasma by high performance liquid chromatography. Pacific Conference on Chemistry and Spectroscopy, San Francisco, CA.

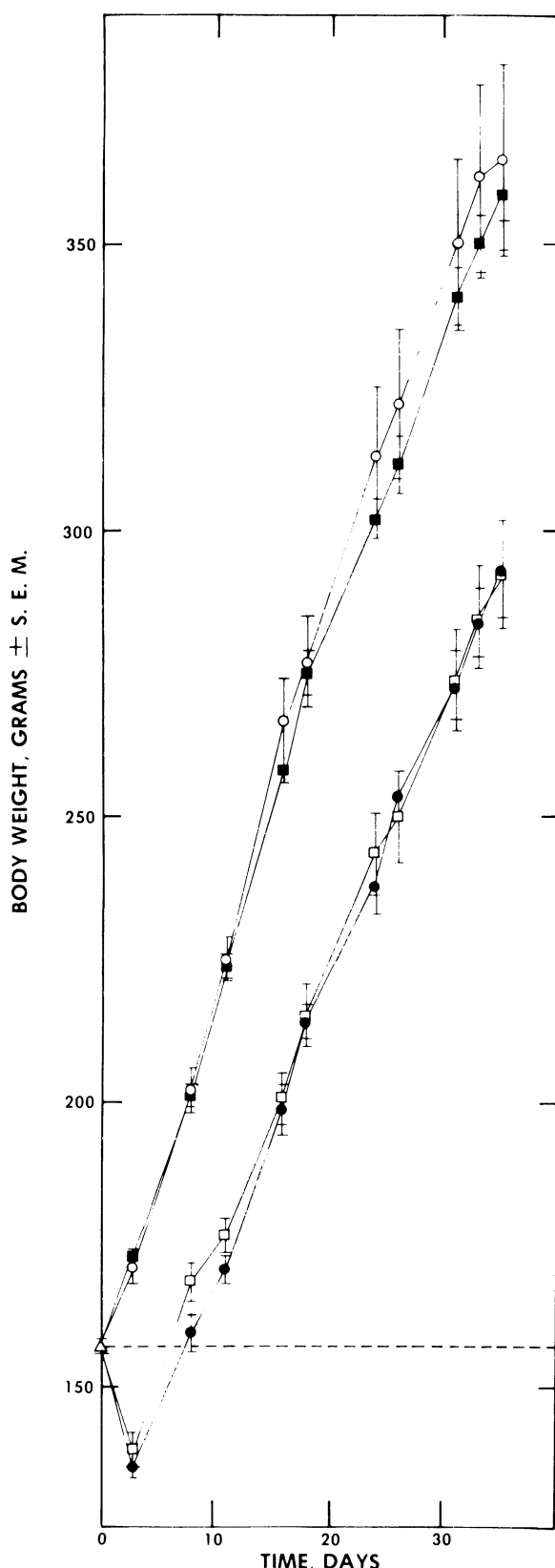


Fig. 1. Growth of rats fed diets containing hard red spring wheat bran. Meal-eating rats were fed a diet containing either 5% wheat bran (●) or 20% wheat bran (■). Nibbling rats were fed a diet containing either 5% wheat bran (□) or 20% wheat bran (○).

TABLE I  
Composition of the Semipurified Diet<sup>a</sup>

| Ingredients              | Diet 1 | Diet 2 |
|--------------------------|--------|--------|
| Casein                   | 20.0   | 20.0   |
| DL-Methionine            | 0.3    | 0.3    |
| Cornstarch               | 15.0   | 15.0   |
| Sucrose                  | 50.0   | 35.0   |
| Wheat bran               | 5.0    | 20.0   |
| Corn oil                 | 5.0    | 5.0    |
| AIN mineral <sup>b</sup> | 3.5    | 3.5    |
| AIN vitamin <sup>b</sup> | 1.0    | 1.0    |
| Choline bitartrate       | 0.2    | 0.2    |

<sup>a</sup> Dietary constituents were obtained from Bio. Serv. Inc., Frenchtown, NJ.  
<sup>b</sup> As specified by American Institute of Nutrition. Diets contained (per kilogram) 8 mg of retinyl palmitate and 200 mg of dl- $\alpha$ -tocopheryl acetate.

## RESULTS

### Body Weights and Food Intake

Figure 1 demonstrates that initial body weight dropped in meal-eating rats restricted to consuming their food in a 2-hr period from 9:00 A.M. to 11:00 A.M. Thereafter, the growth of meal-eating rats paralleled the growth of nibbling rats; however, the meal-eating rats always weighed significantly less. Meal-eating rats were noticeably more aggressive, more active, and exhibited considerably more curiosity than nibbling rats regardless of the level of wheat bran in the diet.

Body weights and final food intakes are shown in Table II. Body weights were similar at the start of the experiment. Final body weights and food intakes are significantly ( $P < 0.005$ ) lower in meal-eating rats than in nibbling rats. A slight discrepancy in calorie density and in protein existed between the two bran diets because of the wheat bran additions; this was not adjusted because the magnitude of the differences was relatively low. Body weight and food intakes were not influenced by changing the concentration of dietary wheat bran; however, nibbling rats fed the stock diet had lower final body weights than nibbling rats fed wheat bran. Final food intake of nibbling rats fed the stock diet was not determined. No evidence of interaction was seen between the effects of wheat bran and meal frequency on body weights and food intake.

### Organ Weights and Hematocrit Values

Blood hematocrit values are shown in Table III. No significant difference in liver weights was demonstrated. Nibbling rats generally had higher hematocrit values than did meal-eating rats, regardless of whether the nibbling rats were fed semipurified diet containing wheat bran or simply a stock diet. There was no interaction between wheat bran and meal frequency for liver weights or hematocrit values.

Total intestinal wet weights and segments of upper intestine and stomach weights are tabulated in Table III. Intestinal tract weights per 100 g of body weight of meal-eating rats were significantly higher than the intestinal tract weights of nibbling rats. No significant alterations observed in the weights of intestinal tract were attributable to different amounts of dietary wheat bran. Likewise, there was no evidence of interaction between wheat bran and meal frequency on the weights of the intestinal tract.

The effect of wheat bran diets and meal frequency on the colonic weights per 100 g of body weight is shown in Table IV. Similar to the upper and total intestinal tract weights, the weights of the proximal colon and distal colon were significantly greater in meal-eating rats than in the nibbling rats. In addition, both proximal and distal colon weights increased in the 20% wheat bran-fed rats when compared to the 5% wheat bran. Significant alterations occurred in the weights of colonic muscle plus serosa in rats fed 20% wheat bran compared to rats fed 5% wheat bran. No significant alteration in the weights of colonic mucosa was due to changes in wheat bran content of the diet, although rats fed one meal per day exhibited larger distal colonic mucosa weights than nibbling rats. The rats fed the stock diet had significantly heavier lower intestinal weights than rats fed the semipurified diet. There was no wheat bran-meal frequency interaction for colon weights.

Table V gives the effect of wheat bran on epididymal and renal fat of rats on the different feeding systems. Generally, the total abdominal fat deposition was greater in the nibbling rats than in meal-eating rats. No significant difference in the deposition of abdominal fat existed between rats fed 5% wheat bran and rats fed 20% wheat bran. Deposition of fat in rats fed the stock diet generally was less than in rats fed the semipurified diet. There was no evidence of interaction between the effects of wheat bran and meal frequency on fat deposition.

### Plasma Vitamin A and Vitamin E

The effect of meal frequency and diets containing wheat bran on plasma vitamin A and vitamin E levels is shown in Table VI. In spite of equally adequate amounts of dietary vitamin E (Table I), nibbling rats had higher levels of vitamin E than meal-eating rats. A

similar trend was found for the plasma levels of vitamin A. Vitamin E levels were significantly less in rats fed 20% wheat bran than in rats fed 5% wheat bran, regardless of whether the rats were nibblers ( $28.1 \pm 3.7\%$ ) or meal fed ( $27.5 \pm 2.8\%$ ). Likewise, the plasma vitamin A levels of nibbling rats fed 20% wheat bran was significantly less than that of nibbling rats fed 5% wheat bran ( $20.3 \pm 6.3\%$ ). There was no effect of wheat bran on the plasma levels of vitamin A in the meal-eating rats. Table VI also demonstrates that nibbling rats fed a stock diet had very significantly less plasma vitamin E than any of the other treatment groups. This last finding may be the result of nonspecific binding due to the large content of dietary alfalfa in the stock diet (Rodent Chow, Ralston Purina Co., St. Louis, MO). The lower plasma vitamin A or vitamin E was not the result of wheat bran-meal-frequency interactions.

## DISCUSSION

Feeding patterns, or the number of meals consumed per day, have been reported to influence intestinal organ weight, lipogenesis, blood serum lipid, and body weights (Leveille and Romsos 1974, Conn 1964, Kissileff and Van Itallie 1982, Leveille 1970, Cummings 1976, Adams and Morgan 1981). Coincidentally, the ingestion of diets high in fiber has resulted in similar findings (Kelsay 1978, Tasman-Jones 1980, Story 1980, Heaton 1980, Eastwood et al 1980). Our intent is to eventually use the meal-fed rat model for our vitamin bioavailability research. Therefore, we

TABLE II  
Effect of Wheat Bran Diet on Body Weight and Food Intake  
in Meal-eating and Nibbling Rats<sup>a</sup>

|                  | Final Body Weight <sup>a,b</sup><br>(g) | Final<br>Food Intake <sup>b</sup> |
|------------------|-----------------------------------------|-----------------------------------|
| 5% Wheat bran    |                                         |                                   |
| Meal-eating      | 284.9 b                                 | 16.9 c                            |
| Nibbling         | 362.1 a                                 | 22.1 a                            |
| 20% Wheat bran   |                                         |                                   |
| Meal-eating      | 284.0 b                                 | 19.1 c                            |
| Nibbling         | 350.8 a                                 | 23.6 a                            |
| Stock diet       |                                         |                                   |
| Nibbling         | 318.8 b                                 |                                   |
| LSD <sup>c</sup> | 29.18                                   | 3.80                              |

<sup>a</sup> Mean values where  $n = 6$ . Data with different letters within each column differ significantly at  $P < 0.05$ .

<sup>b</sup> Food intake = grams per day during final week of the study. Meal frequency effect significant ( $P < 0.005$ ).

<sup>c</sup> Least significant difference between means.  $P < 0.05$ .

TABLE III  
Effect of Wheat Bran Diet Blood Hematocrit Values on the Weights  
of the Total Intestinal Tract, Upper Small Intestine, and Stomach  
in Meal-eating and Nibbling Rats<sup>a</sup>

|                  | Total<br>Intestinal<br>Tract<br>(g/100 g<br>of body wt) | Upper<br>Intestine<br>(10 cm)<br>(mg/100 g<br>of body wt) | Stomach<br>(mg/100 g<br>of body wt) | Hematocrit <sup>b</sup><br>(%) |
|------------------|---------------------------------------------------------|-----------------------------------------------------------|-------------------------------------|--------------------------------|
| 5% Wheat bran    |                                                         |                                                           |                                     |                                |
| Meal-eating      | 5.79 a                                                  | 358 ab                                                    | 566 ab                              | 44.5 b                         |
| Nibbling         | 4.65 b                                                  | 291 bc                                                    | 451 c                               | 46.5 ab                        |
| 20% Wheat bran   |                                                         |                                                           |                                     |                                |
| Meal-eating      | 6.28 a                                                  | 363 a                                                     | 617 a                               | 45.5 b                         |
| Nibbling         | 4.86 b                                                  | 290 c                                                     | 445 c                               | 47.8 a                         |
| Stock diet       |                                                         |                                                           |                                     |                                |
| Nibbling         | 5.75 a                                                  | 301 ab                                                    | 548 b                               | 46.3 ab                        |
| LSD <sup>c</sup> | 0.680                                                   | 0.0496                                                    | 0.0569                              | 2.06                           |

<sup>a</sup> Mean values where  $n = 6$ . Data with different letters within each column differ significantly at  $P < 0.05$ .

<sup>b</sup> Meal frequency effect significant ( $P < 0.002$ ).

<sup>c</sup> Least significant difference between means.  $P < 0.05$ .

**TABLE IV**  
Effect of Wheat Bran Diet on Colonic Weights in Meal-eating and Nibbling Rats<sup>a</sup>

|                | Proximal Colon (5 cm) (mg/100 g of body wt) |        |                                  | Distal colon (5 cm) (mg/100 g of body wt) |                     |                                |
|----------------|---------------------------------------------|--------|----------------------------------|-------------------------------------------|---------------------|--------------------------------|
|                | Total <sup>b,c,d</sup>                      | Mucosa | Muscle + Serosa <sup>b,c,d</sup> | Total <sup>b,d</sup>                      | Mucosa <sup>c</sup> | Muscle + Serosa <sup>b,d</sup> |
| 5% Wheat bran  |                                             |        |                                  |                                           |                     |                                |
| Meal-eating    | 150 cd                                      | 33.3 a | 107.4 c                          | 133 bc                                    | 33.0 a              | 93.3 cd                        |
| Nibbling       | 108 d                                       | 27.9 a | 83.7 d                           | 106 c                                     | 22.0 b              | 87.8 d                         |
| 20% Wheat bran |                                             |        |                                  |                                           |                     |                                |
| Meal-eating    | 192 a                                       | 35.8 a | 155.7 a                          | 146 b                                     | 32.9 a              | 115.3 bc                       |
| Nibbling       | 151 bc                                      | 33.8 a | 117.5 bc                         | 151 b                                     | 30.3 ab             | 121.8 b                        |
| Stock diet     |                                             |        |                                  |                                           |                     |                                |
| Nibbling       | 172 ab                                      | 37.4 a | 134.9 ab                         | 182 a                                     | 32.5 a              | 147.6 a                        |
| LSD            | 0.028                                       | 0.009  | 22.8                             | 0.021                                     | 0.007               | 19.7                           |

<sup>a</sup>Mean values where  $n = 6$ . Data with different letters within each column differ significantly at  $P < 0.05$ .

<sup>b</sup>Wheat bran effect significant ( $P < 0.002$ ).

<sup>c</sup>Meal frequency effect significant ( $P < 0.05$ ).

<sup>d</sup>Effect of stock diet treatment versus other treatments was significant ( $P < 0.05$ ).

<sup>e</sup>Least significant difference between means.  $P < 0.05$ .

**TABLE V**  
Effect of Wheat Bran on Epididymal and Renal Fat Deposits in Meal-eating and Nibbling Rats<sup>a</sup>

|                  | Epididymal Fat <sup>b,c</sup><br>(g/100 g of body wt) | Renal Fat <sup>b</sup><br>(g/100 g of body wt) | Total                                                |                                              |
|------------------|-------------------------------------------------------|------------------------------------------------|------------------------------------------------------|----------------------------------------------|
|                  |                                                       |                                                | Abdominal Fat <sup>b,c</sup><br>(g/100 g of body wt) | Fat Fat Gain/<br>Body Wt Gain <sup>b,c</sup> |
| 5% Wheat bran    |                                                       |                                                |                                                      |                                              |
| Meal-eating      | 1.32 b                                                | 1.21 b                                         | 2.52 b                                               | 5.73 bc                                      |
| Nibbling         | 1.88 a                                                | 2.49 a                                         | 4.49 a                                               | 7.99 a                                       |
| 20% Wheat bran   |                                                       |                                                |                                                      |                                              |
| Meal-eating      | 1.30 b                                                | 1.29 b                                         | 2.58 b                                               | 5.64 bc                                      |
| Nibbling         | 1.75 a                                                | 2.13 a                                         | 3.85 a                                               | 7.06 ab                                      |
| Stock diet       |                                                       |                                                |                                                      |                                              |
| Nibbling         | 1.17 b                                                | 1.27 b                                         | 3.11 a                                               | 4.86 c                                       |
| LSD <sup>d</sup> | 0.325                                                 | 0.650                                          | 8.98                                                 | 1.54                                         |

<sup>a</sup>Mean values where  $n = 6$ . Data with different letters within each column differ significantly at  $P < 0.05$ .

<sup>b</sup>Meal frequency effect significant ( $P < 0.002$ ).

<sup>c</sup>Effect of stock diet treatment versus other treatments was significant ( $P < 0.05$ ).

<sup>d</sup>Least significant difference between means.  $P < 0.05$ .

examined what effects, if any, feeding large amounts of dietary fiber would have on the meal-eating rat. We selected wheat bran as an example of a form of dietary fiber because of our current research interests (Drews et al 1979; Leklem et al 1980; DeLumen et al 1982; Omaye et al 1982, 1983).

Studies involving the effect of intermittent fasting over extended periods in rats have shown a variety of morphological adaptation (Leveille and Romsos 1974, Kissileff and Van Itallie 1982). Changes include marked enlargement of both the stomach and the glandular portion of the stomach with meal-eating animals. Total body weight for the rat was reduced, yet absolute weight of the stomach and its portions was increased with marked hypertrophy of the mucosa and musculature. Overall, our results (Tables II–IV) are consistent with the findings of these previous reports. Reduction of meal frequency (Leveille and Romsos 1974, Kissileff and Van Itallie 1982, Adams and Morgan 1981) may result in compensatory growth change in the gastrointestinal tract, which may be necessary for the efficient absorption of nutrients. We also found that increasing the content of wheat bran in the diets of the meal-eating rats superimposed an additional increase in weights of the large intestine compared to rats fed lower concentration of wheat bran; however, our results do not suggest that this increased weight is due to a wheat bran–meal frequency interaction. Recent work demonstrated that a diet containing 20% wheat bran fed to rats for two weeks led to colonic hyperplasia and muscle hypertrophy (Jacobs and Schneeman 1981). Dietary bulk,

**TABLE VI**  
Effect of Wheat Bran Diet on Plasma Vitamin A and Vitamin E in Meal-eating and Nibbling Rats<sup>a</sup>

|                  | Vitamin A <sup>b</sup><br>( $\mu\text{g/ml}$ ) | Vitamin E <sup>b,c,d</sup><br>( $\mu\text{g/ml}$ ) |
|------------------|------------------------------------------------|----------------------------------------------------|
|                  | 5% Wheat bran                                  |                                                    |
| Meal-eating      | 0.43 $\pm$ 0.03 b                              | 10.9 $\pm$ 0.4 b                                   |
| Nibbling         | 0.64 $\pm$ 0.05 <sup>a</sup>                   | 13.5 $\pm$ 1.9 a                                   |
| 20% Wheat bran   |                                                |                                                    |
| Meal-eating      | 0.44 $\pm$ 0.04 b                              | 7.9 $\pm$ 0.3 c                                    |
| Nibbling         | 0.51 $\pm$ 0.04 b                              | 9.7 $\pm$ 0.5 bc                                   |
| Stock diet       |                                                |                                                    |
| Nibbling         | 0.43 $\pm$ 0.05 b                              | 3.7 $\pm$ 0.3 d                                    |
| LSD <sup>c</sup> | 0.1204                                         | 2.620                                              |

<sup>a</sup>Mean values where  $n = 6$ . Data with different letters within each column differ significantly at  $P < 0.05$ .

<sup>b</sup>Meal frequency effect significant ( $P < 0.02$ ).

<sup>c</sup>Wheat bran effect significant ( $P < 0.001$ ).

<sup>d</sup>Effect of stock diet treatment versus other treatments was significant ( $P < 0.001$ ).

<sup>e</sup>Least significant difference between means.  $P < 0.05$ .

especially dietary fiber, stimulates colonic growth, but the mechanism is still poorly understood. Studies of diets containing alfalfa (Addis 1931), wood cellulose (Fischer 1957), and powdered kaolin (Dowling et al 1967) demonstrated that colonic weight increases in rats fed diets containing high amounts of residue. Alternatively, strict parenteral nutrition or a chemically defined diet excluding dietary fiber results in a loss of colonic mass (Ryan et al 1979, Morin 1980). Dietary manipulations such as the addition of wheat bran to the diet can alter the excretion of fecal constituents (Adams and Morgan 1981, Borside 1978), as well as the colonic flora (Eastwood et al 1980) or the metabolic activities of intestinal bacteria (Goldin et al 1978). Subsequently, this may influence intestinal cell turnover (Ranken et al 1971).

In agreement with other reports (Romsos and Leveille 1972, Ozceli et al 1978, Carlson and Arnrich 1978), deposition of fat was greater in the nibbling rats (Table V). We did not determine the total fat composition of our animals, but rather utilized abdominal epididymal and/or renal fat weights as a fairly reliable index. A high correlation has been found between accumulation of body lipid and epididymal lipid (Carlson and Arnrich 1978). Whether meal frequency restriction is manifested by a greater or lesser accumulation of body fat is a subject of considerable discussion (Leveille and Romsos 1974, Kissileff and Van Itallie 1982, Adams and Morgan 1981). It does appear that three criteria have a marked influence on the outcome of total body fat composition in meal-fed rats: the amount of caloric restriction in the diet; the duration of the study; and the initial adaptation period. There are also indications that immature, but not mature, animals can respond to decreased

feeding frequency by depositing larger amounts of fat than their nibbling control (Carlson and Arnrich 1978, Wardlan et al 1969).

We also found marked significant alteration in plasma vitamin E and vitamin A levels (Table VI). After 35 days on the dietary regimen, plasma levels of vitamin E were less in the meal-eating rats than in the nibbling rats fed the equal amount of wheat bran. A similar trend was shown for plasma vitamin A levels. At no time did the lower values of plasma vitamin E or vitamin A approach deficient conditions (DeLumen et al 1982). These findings would certainly be in line with the general suggestion that the frequency of meals influences the absorption of plasma cholesterol and other lipids (Leveille and Romsos 1974, Adams and Morgan 1981), the relationship being direct, ie, an increase in meal frequency results in increased plasma lipid levels. Several reports (MacMasters et al 1978) have documented that the tocopherol content of wheat bran is 25–87 ppm. Based on the intake of a growing rat, the contribution of vitamin E by wheat bran is insignificant.

Our finding that the inclusion of dietary bran in the diets of meal-eating or nibbling rats results in the reduction of plasma vitamin E also suggests that the bioavailability of selected fat-soluble vitamins could be affected adversely by the consumption of diets containing high amounts of bran. Pectin comprising 10% of the total rat diet significantly decreased levels of serum and red blood cell vitamin E and significantly increased red blood cell hemolysis (DeLumen et al 1982). Additionally, subjects consuming a diet high in fruits and vegetables excreted over twice the amount of total carotene compared to control subjects consuming a fiber-free diet (Kelsay 1982). Our findings are consistent with the hypothesis that dietary wheat bran, similar to other fibers (Kelsay 1978, Story 1980) may influence the absorption of certain lipid-soluble constituents. Alternatively, the turnover of vitamins E and A might be influenced by the composition of the fiber in the diet. Comments regarding mechanisms of wheat bran interaction with lipid-soluble vitamin would be premature at this time (Omeye et al 1982, 1983). The results of additional ongoing work must be considered before interpretations can be made.

In addition, we have found no complicated interaction between the number of meals consumed and the amount of wheat bran in the diet on any of the physiological or biochemical factors tested in this study. This supports our intention to utilize the meal-fed rodent for further investigation in nutrient (vitamin) bioavailability.

#### ACKNOWLEDGMENTS

The skillful technical assistance of Mara Hincenbergs, Sharon K. Sanui, and Yemaya Ponder is gratefully appreciated. The authors are also grateful to Antoinette A. Betschart for her advice with this research and Bruce E. Mackey for his consultation in the statistical evaluation of the data.

#### LITERATURE CITED

- ADAMS, C. E., and MORGAN, K. J. 1981. Periodicity of eating: Implication for human food consumption. *Nutr. Res.* 1:525.
- ADDIS, T. 1931. Hypertrophy of the gastrointestinal tract and high residue diets. *Am. J. Physiol.* 99:417.
- AMERICAN INSTITUTE OF NUTRITION. 1977. Ad Hoc Committee on Standards for Nutritional Studies. Report of the Committee. *J. Nutr.* 107:1340.
- BORSIDE, G. H. 1978. Stability of human fecal flora. *Am. J. Clin. Nutr.* 31:5141.
- CARLSON, S. E., and ARNRICH, L. 1978. Influence of dietary lipid and meal pattern on body composition and lipogenesis in adult rats. *J. Nutr.* 108:1162.
- CONN, C. 1964. Feeding patterns and some aspects of cholesterol metabolism. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 23:76.
- CULLEN, P., and OACE, S. 1977. Cellulose and pectin enhance vitamin B<sub>12</sub> depletion in rats. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 36:1118.
- CUMMINGS, J. H. 1976. What is fiber? Page 1 in: *Fiber in Human Nutrition*. G. A. Spiller and R. J. Amen, eds. Plenum Press, New York.
- DELUMEN, B. O., LUBIN, B., CHIU, D., REYES, P. S., and OMAYE, S. T. 1982. Bioavailability of vitamin E in rats fed a diet containing pectin. *Nutr. Res.* 2:78.
- DOWLING, R. H., RIECKEN, E. O., LAWS, J. W., and BOOTH, C. C. 1967. The intestinal response to high bulk feeding in the rat. *Clin. Sci.* 32:1.
- DREWS, L. M., KIES, C. and FOX, H. M. 1979. Effect of dietary fiber on copper, zinc, and magnesium utilization by adolescent boys. *Am. J. Clin. Nutr.* 32:1893.
- EASTWOOD, M. A., BRYDON, W. G., and TADESSE, K. 1980. Effect of fiber on colon function. Page 1 in: *Medical Aspects of Dietary Fiber*. G. A. Spiller and R. M. Kay, eds. Plenum Medical Book Co., New York.
- FISCHER, J. E. 1957. Effects of feeding diets containing lactose, agar, cellulose, raw potato starch or arabinose on the dry weights of cleaned gastrointestinal tract organs in the rat. *Am. J. Physiol.* 188:550.
- GOLDIN, B., DWYER, J., GORBACK, S. L., GORDON, W., and SWENSON, L. 1978. Influence of diet and age on fecal bacterial enzymes. *Am. J. Clin. Nutr.* 31:5163.
- HEATON, K. W. 1980. Food intake regulation and fiber. Page 223 in: *Medical Aspects of Dietary Fiber*. G. A. Spiller and R. M. Kay, eds. Plenum Medical Book Co., New York.
- JACOBS, L. R., and SCHNEEMAN, B. O. 1981. Effects of dietary wheat bran on rat colonic structure and mucosal cell growth. *J. Nutr.* 111:798.
- KELSAY, J. L. 1978. A review of research on effects of fiber intake on man. *Am. J. Clin. Nutr.* 31:142.
- KELSAY, J. L. 1982. Effects of fiber on mineral and vitamin bioavailability. Page 91 in: *Dietary Fiber in Health and Disease*. G. V. Vahouny and D. Kritchevsky, eds. Plenum Press, New York.
- KELTZ, F. P., KIES, C., and FOX, H. M. 1978. Urinary ascorbic acid excretion in humans as affected by dietary fiber and zinc. *Am. J. Clin. Nutr.* 31:1167.
- KISSILEFF, H. R., and VAN ITALLIE, T. B. 1982. Physiology of the control of food intake. *Ann. Rev. Nutr.* 2:371.
- LEKLEM, J. E., MILLER, L. T., PERERA, A. D., and PEFFERS, D. E. 1980. Bioavailability of vitamin B<sub>6</sub> from wheat bread in humans. *J. Nutr.* 110:1819.
- LEVEILLE, G. A. 1970. Adipose tissue metabolism. Influence of periodicity of eating and diet composition. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 29:1294.
- LEVEILLE, G. A., and ROMSOS, D. R. 1974. Meal eating and obesity. *Nutr. Today* 9:4.
- MACMASTERS, M. M., HINTON, J. J. C., and BRADBURY, D. 1978. Microscopic structure and composition of the wheat kernel. Page 51 in: *Wheat Chemistry and Technology*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.
- MORIN, C. L., LING, V., and BOURASSA, D. 1980. Small intestinal and colonic changes induced by a chemically defined diet. *Dig. Dis. Sci.* 25:123.
- OMAYE, S. T., CHOW, F. I., and BETSCHAT, A. A. 1982. In vitro interaction of 1-<sup>14</sup>C-ascorbic and 2-<sup>14</sup>C-thiamin with dietary fiber. *Cereal Chem.* 59:440.
- OMAYE, S. T., CHOW, F. I., and BETSCHAT, A. A. 1983. In vitro influence of dietary fiber on the availability of <sup>14</sup>C-vitamin D and <sup>14</sup>C-vitamin E. *J. Food Sci.* 48:260.
- OZELCI, A., ROMSOS, D. R., and LEVEILLE, G. A. 1978. Influence of a liquid diet and meal pattern on body weight and body fat in rats. *J. Nutr.* 108:1128.
- RANHOTRA, G. S., LEE, L., and GELROTH, J. A. 1978. Bioavailability of zinc in soy-fortified wheat bread. *Nutr. Rep. Int.* 18:487.
- RANHOTRA, G. S., LEE, L., and GELROTH, J. A. 1979. Bioavailability of zinc in cookies fortified with soy and zinc. *Cereal Chem.* 56:552.
- RANKEN, R., WILSON, R., and BEALMEAN, P. M. 1971. Increased turnover of intestinal mucosal cells of germ free mice induced by cholic acid. *Proc. Soc. Exp. Biol. Med.* 138:270.
- ROMSOS, D. R., and LEVEILLE, G. A. 1972. Effect of intermittent feeding on body fat content and in vitro lipogenesis in mice. *Nutr. Rep. Inter.* 5:131.
- RYAN, G. P., DUDRICK, S. J., COPELAND, E. M., and JOHNSON, L. R. 1979. Effect of various diets on colonic growth in rats. *Gastroenterology* 77:658.
- STATISTICAL ANALYSIS SYSTEM (SAS). 1979. SAS Institute Inc., Cary, North Carolina.
- STORY, J. A. 1980. Dietary fibers and lipid metabolism: An update. Page 137 in: *Medical Aspects of Dietary Fiber*. G. A. Spiller and R. M. Kay, eds. Plenum Medical Book Co., New York.
- TASMAN-JONES, C. 1980. Effect of dietary fiber on the structure and function of the small intestine. Page 67 in: *Medical Aspects of Dietary Fiber*. G. A. Spiller and R. M. Kay, eds. Plenum Medical Book Co., New York.
- WARDLAN, J. M., HENNYEY, D. J., and CLARK, R. H. 1969. The effect of decreased feeding frequency on body composition in mature and immature male and female rats. *Can. J. Physiol. Pharmacol.* 47:47.