Saturated fatty acid diet prevents radiation-associated decline in intestinal uptake

A. B. R. THOMSON, M. KEELAN, T. LAM, C. I. CHEESEMAN, K. WALKER, AND M. T. CLANDININ
Nutrition and Metabolism Research Group, Departments of Medicine, Laboratory Medicine and Physiology, University of Alberta and University of Alberta Hospitals, Edmonton, Alberta T6G 2C2, Canada

THOMSON, A. B. R., M. KEELAN, T. LAM, C. I. CHEESEMAN, K. WALKER, AND M. T. CLANDININ. *Saturated fatty acid diet prevents radiation-associated decline in intestinal uptake.* Am. J. Physiol. 256 (Gastrointest. Liver Physiol. 19): G178–G187, 1989.—Adult female Sprague-Dawley rats were fed isocaloric semipurified diets containing a high content of either polyunsaturated (P) or saturated (S) fatty acids; these diets were nutritionally adequate, providing for all known essential nutrient requirements. On *day 3* after beginning S or P, one group of animals was exposed to a single 6-Gy dose of abdominal radiation, and the other half was sham irradiated. S or P diets were continued for a further 14 days. Brush-border membrane purification and sucrase-specific activities were unaffected by diet or by abdominal irradiation. In rats fed P, irradiation was associated with an increase in jejunal brush-border membrane total phospholipid and the ratio of phospholipid to cholesterol; these changes were not observed in animals fed S. In irradiated rats, ileal brush-border membrane phospholipid per cholesterol was high in animals fed S compared with P. In irradiated animals fed P, there was reduced jejunal and ileal uptake of several medium- and long-chain saturated and unsaturated fatty acids and cholesterol, and the ileal uptake of higher concentrations of glucose was reduced in irradiated animals fed P. In contrast, lipid uptake was similar in control and irradiated animals fed S except for cholesterol uptake, which was reduced. Ileal uptake of higher concentrations of glucose was increased in irradiated animals fed S. Quantitative autoradiography failed to demonstrate any change in the distribution of leucine or lysine transport sites along the villus 1 or 2 wk after abdominal irradiation or in response to feeding S or P. Also, these differences in transport achieved by feeding S to radiated animals were not explained by variations in the animals' food consumption or intestinal mucosal surface area. Thus the use of short-term feeding with a saturated fatty acid diet in the prevention of acute irradiation damage to the intestine warrants further investigation in humans.

adaptation; dietary lipids; radioprotection; transport

ABDOMINAL IRRADIATION is associated with short- as well as long-term alterations in the active and passive intestinal transport of a variety of nutrients, including glucose, leucine, bile acids, fatty acids, and cholesterol (5, 16–19). These functional alterations are not explained by changes in the animals' food consumption, weight gain, or intestinal mucosal surface area. It is possible to divide the effects into two phases. The first is a short-term response that tends to result in a decline in transport function for only some substrates, whereas the later phase is much longer lasting and more subtle. Defining the mechanisms for these effects is difficult because often different studies have used different base lines to express their data. For instance, because the tissue mass may change postirradiation and may not be uniform for the various components of the intestinal wall, expressing uptake per unit of wet or dry weight may bias the data compared with data using surface area as a base line. What must be borne in mind when trying to interpret such findings is that the distribution of transporting enterocytes along the villus is not uniform. As enterocytes migrate up the villus from the crypts they mature and gain the ability to transport various substrates (4, 12). Without knowing the distribution of the transport capacity along the villus, it is not possible to say whether the responses observed after abdominal irradiation are the result of a change in the number of transporting cells or an alteration in the uptake capacity of individual cells. Thus it is necessary to appreciate the effects of abdominal irradiation on the distribution of transporting enterocytes along the villus.

The intestinal tract is capable of adaptation to alterations in the dietary content of protein, carbohydrate, and lipids (14, 25, 26). Isocaloric modifications in the dietary content of saturated (S) or polyunsaturated (P) fatty acids modify the intestinal uptake of nutrients (22, 23). Also, the feedng of P prevents the enhanced intestinal uptake of glucose and lipid, which occurs in the short-term in rats with streptozotocin-diabetes (24). These dietary manipulations are not associated with alterations in the brush-border membrane content of cholesterol or phospholipid (23), although dietary manipulations in the P-to-S ratio do produce changes in the fatty acyl components of the membrane lipids (3, 10). Abdominal irradiation is associated with an increase in the brush-border membrane content of total phospholipid in rats fed chow (10). Accordingly, the present study was undertaken to test the hypothesis that isocaloric modifications in the dietary content of fatty acids prevents the alteration in the absorption and membrane biochemical phospholipids that have been previously described in response to abdominal irradiation.

0193-1857/89 $1.50

METHODS

Animals and diets. The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Sciences and by the Council of the American Physiological Society, were observed in the conduct of this study. Female Sprague-Dawley rats weighing 220–225 g were allowed ad libitum access to water and food until the time of death. Animals were fed one of two diets for 3 days: a semipurified diet containing 20% (wt/wt) fat of either a high- or low-P/S ratio (23). The semipurified diets were nutritionally adequate, providing for all known essential nutrient requirements. The diet high in P provided ~22% of calories and 55% (wt/wt) of total fatty acids as 18:2w6, whereas the diet high in S provided ~2% of calories and 5% of total fatty acids as 18:2w6.

On the third day, half the animals were exposed to 6 Gy of abdominal irradiation from a ^{137}Cs source, as previously described, whereas the remaining animals were sham-irradiated and served as controls (5, 16–19). The animals continued on S or P for an additional 14 days. All the animals survived. The food consumption and body weight gain were measured before irradiation, as well as 1 and 2 wk postirradiation. Animals were killed by an anaesthetic overdose injection of ketamine (300 mg/kg body wt).

Chemicals. The medium- and long-chain fatty acids, cholesterol, D-glucose, and D-galactose were all 99% pure as supplied by the manufacturer (Applied Science Laboratories, State College, PA; Sigma Chemical, St. Louis, MO). The following fatty acid probe molecules were used: octanoic (8:0), decanoic (10:0), dodecanoic (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3). The ^{14}C-labeled isotopes were used as supplied by the manufacturer: 8:0 (New England Nuclear, Boston, MA), 10:0 (ICN Biochemical, Montreal, Quebec, Canada), 12:0, 14:0 (New England Nuclear), 16:0 (ICN), 18:0, 18:1, 18:2, 18:3 (New England Nuclear), cholesterol, D-glucose, and L-glucose (Amersham Canada, Oakville, Ontario, Canada). [^3H]inulin (ICN) had an approximate molecular weight of 5,000 and was used as a nonpermeant marker of the adherent mucosal fluid volume. All other compounds of reagent grade were obtained from Fisher Scientific.

Preparation of incubation solutions. The technique used for the preparation of the test solutions containing hexoses, fatty acids, and cholesterol has been published (13, 27). For the preparation of the micellar solutions of fatty acids 14:0–18:3 and cholesterol (11), an appropriate amount of both a ^{14}C-labeled and unlabeled probe molecule was dissolved in 150 ml of Krebs-bicarbonate buffer to yield a final concentration of 20 mM taurodeoxycholic acid (TDC). The concentrations of D-glucose was 1.0–40 mM, and the concentration of the fatty acids were as follows (in mM): 8:0 1; 10:0 0.5; 12:0 0.1; 14:0 0.5; 16:0 0.5; 18:0 0.1; 18:1 0.1; 18:2 0.1; and 18:3 0.1; and the concentration of the cholesterol was 0.05.

Tissue preparation. As outlined in detail elsewhere (13, 15, 27), short segments of proximal jejunum, distal ileum, and transverse colon were rapidly removed, rinsed with 150 ml of cold saline, opened along the mesenteric border, and the mucosal surface was carefully washed with a stream of cold saline from a syringe to remove visible mucus and debris. Sheets of intestine were mounted in incubation chambers and clamped between two plastic plates so that the mucosal and serosal surfaces were exposed to separate incubation solutions with apertures in the plates exactly 0.5 cm in diameter. The chambers were transferred to beakers containing oxygenated Krebs-bicarbonate buffer at 37°C for a preincubation period of 15 min. The chambers were then transferred to other beakers for specific experiments.

The preincubation and incubation solutions were mixed at identical stirring rates with circular magnetic bars, and the stirring rates were precisely adjusted by means of a strobe light. Stirring rates were reported as the revolutions per minute (rpm) at which the stirring bar was driven. The bulk phase was stirred at 600 rpm to reduce the effective thickness of the intestinal unstirred water layer (27). This condition of stirring of the bulk phase was chosen to better demonstrate possible alterations in transport function arising from irradiation and dietary treatment.

Determination of rates of uptake. After preincubation in Krebs-bicarbonate buffer for 14 min, the chambers were transferred to other beakers containing [^3H]inulin and various probes, including ^{14}C-labeled D-glucose, L-glucose, cholesterol, and fatty acids in oxygenated Krebs-bicarbonate buffer at 37°C. After incubation for 6 min in these test solutions, the experiment was terminated by removing the chamber and rinsing the intestinal tissue in cold saline for ~5 s. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch, placed on glass slides, and dried overnight in an oven at 55°C. The dry weight was determined and the tissue transferred to counting vials. The sample was then saponified with 0.75 N NaOH, scintillation fluid added and radioactivity determined by means of an external standardization technique to correct for variable quenching of the two isotopes.

Expression of results. The rate of uptake was calculated after correcting the total tissue ^{14}C radioactivity for the mass of the probe molecule present in the adherent mucosal fluid. The adherent mucosal fluid volume was determined from the [^3H]inulin. The uptake rates were expressed as nanomoles of probe molecule taken up into the mucosa per minute per 100 milligrams dry weight of tissue ($nmol \cdot min^{-1} \cdot 100\ mg^{-1}$).

The values obtained for the different groups are reported as the means ± SEM of the results of 8 to 12 animals. The statistical significance of the difference between means was determined using a two-way analysis of variance procedure followed by a Duncan's multiple-range test to determine differences between individual treatment means.

Brush-border membrane isolation and analysis. The isolation and purification methods used to establish the marker enzymes and lipid content of the jejunal and ileal brush-border membrane (BBM) have been published (9, 11). After the rats were killed, a 40-cm segment of both jejunum and ileum was removed from each rat and placed into ice-cold saline. Each segment was irrigated three

TABLE 1. *Effect of altering dietary fat saturation and irradiation on animal characteristics*

Characteristic	Control		Irradiation (6 Gy)	
	High-saturated fatty acid diet	High-polyunsaturated fatty acid diet	High-saturated fatty acid diet	High-polyunsaturated fatty acid diet
	Food intake, g·rat^{-1}·day^{-1}			
Before irradiation	19.8±0.6	16.5±1.0§	17.8±1.0	17.5±0.7
Postirradiation				
1 wk	16.0±0.4*	15.8±0.6	11.9±0.6*‡	11.5±0.6*‡
2 wk	15.2±0.6*	15.2±0.6	14.6±0.6*†	13.4±0.6*
	Weight gain, g·rat^{-1}·day^{-1}			
Before irradiation	0.7±0.4	−0.3±0.6	0.4±0.4	0.0±0.4
Postirradiation				
1 wk	2.7±0.2*	3.4±0.6*	0.2±0.2‡	0.9±0.2*‡§
2 wk	1.4±0.3†	2.5±0.3*	2.3±0.2*†	1.7±0.2*†

Values are means ± SE. * $P < 0.05$, 1 wk postirradiation vs. before irradiation; 2 wk postirradiation vs. before irradiation. † $P < 0.05$, 1 wk postirradiation vs. 2 wk postirradiation. ‡ $P < 0.05$, irradiated vs. control. § $P < 0.05$, high-polyunsaturated fat diet vs. high-saturated fat diet.

times with ice-cold saline and placed on a prechilled glass plate. The mesentery was removed and the segments opened along the mesenteric border. The mucosal surface was blotted with lint-free tissue to remove excess moisture. Mucosal scrapings were obtained by gently scraping with a microscope slide and frozen at −80°C for later purification of the BBM.

Mucosal scrapings were homogenized and then filtered to remove mucus and debris. A 1.0-M solution of $CaCl_2$ was added to precipitate subcellular components. Differential centrifugation was used to remove contaminated membranes and obtain a pellet of BBM. After sonication the BBM preparation was layered onto a 40% Percoll gradient for further purification. A visible upper fraction contained the BBM. Percoll was removed from the BBM by ultracentrifugation. The final BBM preparation was suspended in the supernatant and aliquotted for immediate lipid extraction according to published methods (9, 11, 23).

Alkaline phosphatase, sucrase, phospholipid, cholesterol, free fatty acid, and bile acid content were assayed as previously reported (9, 11, 23).

Morphology. The methods used to determine the morphological characteristics of the jejunum and ileum of rats fed the semisynthetic saturated or polyunsaturated diets have been published (7, 9, 11).

Autoradiography and microdensitometry. These studies were performed in control and irradiated rats fed chow. L-[4,5-^{3}H]leucine (120 Ci/mmol), L-[4,5-^{3}H]lysine (96 Ci/mmol), and [methyl-^{3}H]thymidine (25 Ci/mmol) were obtained from Radiochemicals, Amersham. Amino acids and grade I glutaraldehyde were supplied by Sigma. NTB2 nuclear track emulsion was from Eastman Kodak, Rochester, N.Y.

The autoradiography technique previously described by Cheeseman (4) was employed. The middle fifth of the small intestine was removed from the rats under anesthesia after flushing out the luminal contents with saline at room temperature. The tissue was opened up along the antimesenteric border and mounted, mucosal surface uppermost, over stainless steel pins on a Lucite block. The serosal surface was kept moist with a piece of filter paper soaked in saline. This block was then clamped to a second with 12 identical ports, each of which exposed a disk of mucosa of 0.2 cm^2 surface area (area equivalent to serosal surface, no correction made for villi). Each port could be rapidly filled or emptied by suction, and the bulk phase could be rapidly stirred with a small propeller to reduce unstirred layers. The entire apparatus was maintained at 37°C. After mounting the tissue it was preincubated for 10 min to allow it to equilibrate, two chambers at a time were drained, and the radiolabelled solution was added containing either 1 or 20 mM substrate and a minimum of 100 μCi/ml. The incubations lasted 45 s, during which time the bulk phase was stirred at 900 rpm. The ports were then drained and immediately filled with fixative, 4% glutaraldehyde and 2% sucrose in phosphate-buffered saline, pH 7.3. This was left in contact with the tissue until all 12 ports had been incubated. The tissue was then punched out and kept in fixative for 30 min. After washing in phosphate-buffered saline overnight to remove the glutaraldehyde the tissue was fixed in glycomethacrylate. Sections (10 μm) were coated with NTB2 nuclear track emulsion and left to expose in the dark at 4°C for 14–21 days.

Scanning microdensitometry of unstained sections was carried out at a final magnification of ×400 using a Vickers M85 microdensitometer. Scanning was performed on villi at a wavelength of 650 nm in discrete 30-μm steps. All optical density readings were converted to a percentage of the maximum density for each villus which was invariably at the villus tip. Values for 30-μm steps were averaged for the villi analyzed and expressed as a means ± SE.

Measurement of cell migration rates. Chow-fed animals were injected intraperitoneally with [^{3}H]thymidine (1 μCi/g body wt) and killed 10, 18, 26, 34, or 42 h later. The tissue was then processed for autoradiography as described above. The distance of the silver grain front from the crypt-villus junction was measured using a micrometer eyepiece on a microscope.

RESULTS

Animal characteristics. Food intake and weight gain were decreased 1 wk after 6-Gy abdominal irradiation

TABLE 2. *Effect of altering dietary fat saturation and irradiation on BBM morphology and lipid composition of rat jejunum*

Phospholipid	Control		Irradiation (6 Gy)	
	High-saturated fatty acid diet	High-polyunsaturated fatty acid diet	High-saturated fatty acid diet	High-polyunsaturated fatty acid diet
		Choline		
Spingomyelin				
%	23.6±1.2	24.1±2.4	21.8±3.1	23.0±1.8
nmol/mg protein	99.2±9.3	91.8±9.8	94.7±11.6	111.6±8.1
Lysolecithin				
%	0.3±0.2	0.4±0.3	0.7±0.3	0.1±0.1
nmol/mg protein	1.4±0.8	0.7±0.6	1.9±1.0	0.6±0.3
Lecithin				
%	40.7±1.1	43.0±1.7	46.2±2.0*	48.4±2.3
nmol/mg protein	178.0±13.3	168.4±16.8	236.8±16.3*	247.8±27.1*
Total choline				
%	64.5±1.5	67.5±3.2	70.6±3.2	71.6±1.9
nmol/mg protein	278.6±20.8	260.9±24.2	344.9±17.0*	360.1±30.0*
		Amine		
Phosphatidyl serine				
%	1.3±0.3	1.4±0.6	1.5±0.6	1.2±0.4
nmol/mg protein	5.4±1.3	5.2±2.1	6.8±2.5	5.7±2.1
Lysophosphatidylethanolamine				
%	4.2±1.0	3.6±0.8	4.0±1.0	2.2±0.5
nmol/mg protein	20.6±5.0	12.4±2.3	20.1±5.5	10.2±2.1
Phosphatidylethanolamine				
%	20.5±1.9	21.3±2.8	18.6±1.9	19.4±1.5
nmol/mg protein	90.2±6.3	88.3±11.2	93.8±13.5	106.4±11.8
Total amine				
%	30.3±1.9	27.4±2.9	24.1±2.7	23.8±1.2
nmol/mg protein	127.9±9.4	114.1±12.0	120.7±17.4	120.9±11.7
		Other		
Phosphatidic acid				
%	0.5±0.2	0.9±0.4	0.8±0.3	0.6±0.3
nmol/mg protein	2.5±1.0	2.0±1.2	2.8±1.2	0.8±0.8
Phosphatidylinositol				
%	4.0±0.6	4.3±0.8	3.8±0.4	3.0±0.4
nmol/mg protein	16.9±2.5	16.6±3.1	18.8±2.7	15.3±3.1
Choline Amine	2.2±0.2	2.8±0.4	3.4±0.5	3.1±0.2
Total phospholipid	418±28	371±37	493±26	504±41*
Cholesterol				
Total	337±26	385±32	315±14	360±22
Free	301±17	367±32	311±15	353±22
Esters	14±5	18±6	4±2	7±3
Phospholipid Cholesterol	1.25±0.05	1.13±0.10	1.58±0.09*	1.41±0.11
Mucosal surface area, mm^2/mm^2 serosa	7.8±0.5	9.3±0.6	6.0±0.4	6.2±0.6

Values are means ± SE. * $P < 0.05$, irradiated vs. control.

(Table 1). By 2 wk postirradiation, food intake, and weight gain returned to control values when animals were fed S, but remained depressed when animals were fed P. Dietary fat saturation did not influence food consumption in either control or irradiated animals. Weight gain was greater in control animals fed P when compared with those fed S, but reduced in irradiated animals fed P as compared with those fed S.

The jejunal mucosal surface area was lower in irradiated than in control animals and was similarly reduced in P and S (data not shown), whereas in the ileum, mucosal surface area was unaffected by abdominal irradiation. The mucosal surface area of the jejunum and ileum was unaffected by abdominal irradiation or by manipulation in the type of lipid in the diet.

BBM protein and enzyme markers. The wet weight of mucosal scrapings and BBM protein content were not affected by diet or irradiation (data not shown). BBM purification was not influenced by diet or irradiation, as illustrated by similar values for sucrase and alkaline phosphatase activity and low levels of β-glucuronidase activity and DNA content.

BBM lipid composition. Jejunal and ileal BBM lipid composition was not influenced by diet in control animals, and there were no differences in BBM lipid composition between irradiated rats fed P or S (Tables 2 and 3). Compared with nonirradiated rats fed P, irradiation was associated with an increase in jejunal and ileal BBM total bile acids, total phospholipid, and the ratio of phospholipid to cholesterol in animals fed P. Irradiation was not associated with any changes in BBM lipid composition of rats fed S compared with irradiated rats fed P, except for an increased total phospholipid and ratio of phospholipid to cholesterol in the ileal BBM. Irradi-

TABLE 3. *Effect of altering dietary fat saturation and irradiation on BBM morphology and lipid composition of rat ileum*

Phospholipid	Control		Irradiation (6 Gy)	
	High-saturated fatty acid diet	High-polyunsaturated fatty acid diet	High-saturated fatty acid diet	High-polyunsaturated fatty acid diet
	Choline			
Sphingomyelin				
%	25.6±2.9	25.9±4.1	27.8±1.8	25.0±3.0
nmol/mg protein	56.5±7.0	51.5±7.3	90.2±7.8*	106.7±20.3*
Lysolecithin				
%	0.2±0.2	0.9±0.6	1.4±0.9	0.9±0.4*
nmol/mg protein	0.6±0.4	2.0±1.4	4.0±2.8	1.9±0.9
Lecithin				
%	42.8±4.0	38.4±4.7	46.0±5.1	49.0±3.3
nmol/mg protein	119.6±22.6	82.0±16.3	155.9±36.0	176.0±16.8*
Total choline				
%	68.7±3.9	65.2±3.3	75.2±3.7	75.0±3.1
nmol/mg protein	157.5±14.6	135.4±14.8	250.2±40.4*	274.9±31.5*
	Amine			
Phosphatidyl serine				
%	1.0±0.7	0.9±0.4	0.7±0.6	2.3±0.8
nmol/mg protein	2.0±1.7	2.0±1.0	1.8±1.3	6.9±2.9
Lysophosphatidylethanolamine				
%	5.1±2.0	3.9±1.4	4.2±2.2	4.4±1.6
nmol/mg protein	12.3±5.0	8.0±2.6	14.2±7.3	12.4±5.9
Phosphatidylethanolamine				
%	21.3±2.2	21.2±1.3	15.4±2.6	14.8±1.8*
nmol/mg protein	56.1±5.5	44.5±5.6	50.3±8.8	52.0±7.3
Total amine				
%	27.4±3.6	26.0±1.0	20.4±3.3	21.4±2.5
nmol/mg protein	70.4±7.4	60.2±8.6	66.2±11.2	72.3±7.9
	Other			
Phosphatidic acid				
%	1.0±0.5	1.4±0.6	0.8±0.5	0.4±0.2
nmol/mg protein	2.1±1.2	3.2±1.6	2.4±1.4	1.4±0.8
Phosphatidylinositol				
%	3.0±0.6	4.7±1.5	3.6±1.0	2.1±0.3
nmol/mg protein	7.8±1.3	9.2±2.7	11.7±3.2	9.2±2.5
Choline amine	2.9±0.6	2.4±0.3	4.1±0.8	4.0±0.6*
Total phospholipid	308±39	236±23	289±28	422±56*†
Cholesterol				
Total	414±14	394±21	377±18	382±17
Free	388±20	368±23	382±25	372±19
Esters	13±7	22±7	10±4	10±4
Phospholipid Cholesterol	0.74±0.08	0.62±0.07	0.73±0.06	1.10±0.13*†
Mucosal surface area, mm^2/mm^2 serosa	6.2±0.5	4.7±0.3	5.9±0.3	5.4±0.5

Values are means ± SE. * $P < 0.05$, irradiated vs. control. † $P < 0.05$, high-polyunsaturated fatty acid diet vs. high-saturated fatty acid diet.

ation was associated with a quantitative (nmol/mg protein) increase in jejunal BBM lecithin content (Table 2) as well as increased ileal BBM choline (Table 3).

Glucose uptake. No difference was noted in the jejunal uptake of varying concentrations of D-glucose in control or in irradiated animals fed S or P (Figs. 1, *A* and *C*). In contrast, the uptake of higher concentrations of glucose into the ileum was lower ($P < 0.05$) in irradiated than in control animals fed P (Fig. 1*B*), whereas uptake was higher ($P < 0.05$) in irradiated than in control animals fed S (Fig. 1*D*). The uptake of 1 mM L-glucose into the jejunum and ileum was unaffected by irradiation or by the dietary content of P or S (data not shown).

Lipid uptake. The jejunal uptake of several fatty acids (8:0, 12:0, 14:0, 18:2, and 18:3) was lower ($P < 0.05$) in irradiated than in control animals fed P (Fig. 2), whereas these alterations in jejunal uptake were not observed in irradiated as compared with control animals fed S (Fig. 3). Ileal uptake of 8:0, 14:0, and 18:1 was lower ($P < 0.05$) in irradiated than in control animals fed P, whereas these differences were not observed in ileal uptake in irradiated animals fed S (Figs. 2 and 3). Cholesterol uptake was reduced after irradiation regardless of diet or intestinal site (Figs. 2 and 3).

Cell turnover and villus morphology. There was a linear progression of the silver grain front up the jejunal villi in the chow-fed control animals and for each of the time periods after irradiation (Fig. 4). In each case the labeled cells arrived at the crypt-villus junction close to 14 h after the thymidine injection, a delay that corresponds

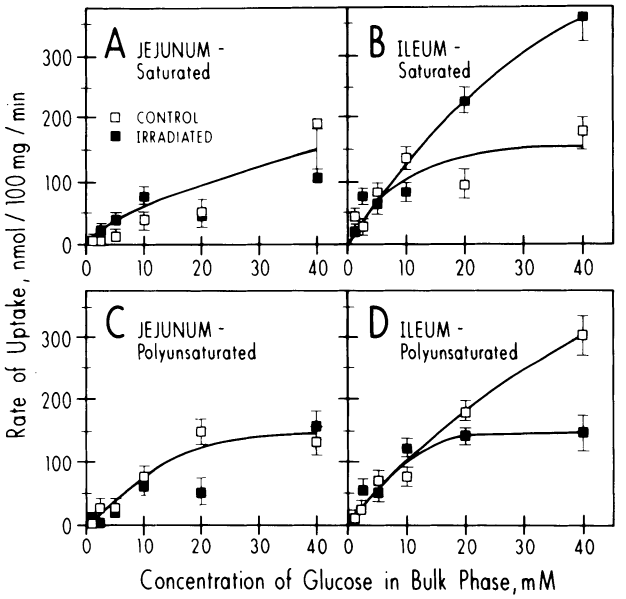

FIG. 1. Effect of abdominal irradiation and diet on intestinal uptake of D-glucose. Animals were fed for 3 days on isocaloric semipurified diets high in saturated or polyunsaturated fatty acids. Abdomen was then exposed to 6 Gy of irradiation from a ^{137}Cs source, saturated or polyunsaturated fatty acid diets were continued for a further 14 days, and rates of uptake of glucose into jejunum or ileum were determined from bulk phase concentrations of 1–40 mM. Bulk phase was stirred at 600 rpm to reduce effective resistance of unstirred water layer. Results represent means ± SE and were obtained from 8–12 animals.

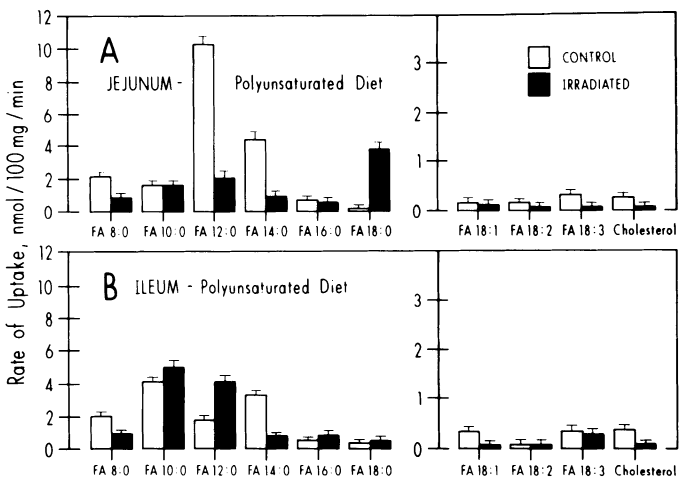

FIG. 2. Effect of abdominal irradiation and polyunsaturated fatty acid diet on intestinal uptake of lipids. Animals were fed for 3 days on isocaloric semipurified diets high in saturated or polyunsaturated fatty acids. Abdomen was then exposed to 6 Gy of irradiation from a ^{137}Cs source, saturated or polyunsaturated fatty acid diets were continued for a further 14 days, and rates of uptake of glucose into jejunum or ileum were determined from bulk phase concentrations of 1–40 mM. Bulk phase was stirred at 600 rpm to reduce effective resistance of unstirred water layer. Results represent means ± SE and were obtained from 8–12 animals.

to the time taken by the cells to migrate up the crypts after being produced by cell division. The slopes of the lines drawn throughout the points indicate the rate of migration up the villi, and the horizontal lines show the average height of the villi for each condition. The lines were fitted by linear regression analysis. In the case of control, 1, and 2 wk postirradiation, the lines were not significantly different so the data were pooled, and the

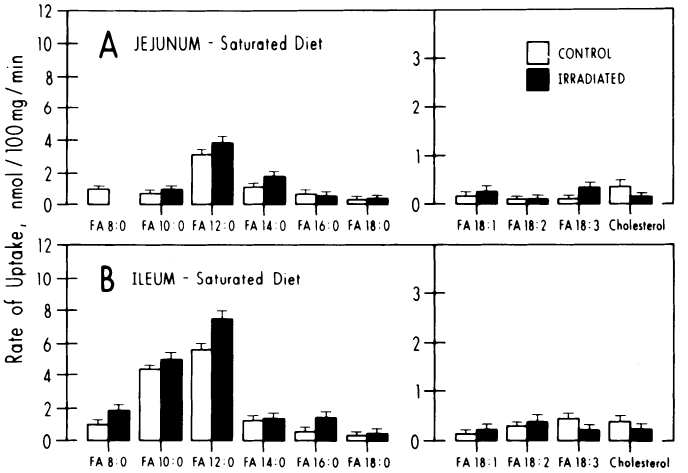

FIG. 3. Effect of abdominal irradiation and saturated fatty acid diet on intestinal uptake of lipids. Animals were fed for 3 days on isocaloric semipurified diets high in saturated or polyunsaturated fatty acids. Abdomen was then exposed to 6 Gy of irradiation from a ^{137}Cs source, saturated or polyunsaturated fatty acid diets were continued for a further 14 days, and rates of uptake of glucose into jejunum or ileum were determined from bulk phase concentrations of 1–40 mM. Bulk phase was stirred at 600 rpm to reduce effective resistance of unstirred water layer. Results represent means ± SE and were obtained from 8–12 animals.

line was fitted. Clearly two features of the cell migration and morphology are markedly altered at 3 days postirradiation. The rate of migration is accelerated from 10 to 15 μm/h (Table 4), whereas the villus height is reduced from 420 to 300 μm. However, by correlating position up the villus with the time after the thymidine injection it is possible to determine the age of the cells at any point along the villus. It is interesting to note that although the villi are shorter in the 3-day postirradiation animals, the cells appear on the villus at the same age as in the control, 1-, and 2-wk postirradiation animals. Also, although the rate of migration is faster up the 3-day villi, the cells at the tip are only 37 h old, whereas those for the other conditions are 55–60 h old.

Distribution of amino acid transport along the villus. Figure 5*A* shows the distribution of leucine and lysine transport along the villus measured in control animals. The optical density of the silver grains is expressed as a percentage of the maximum density found at the villus tips. Two features are apparent. First, there is very little amino acid found in the cells between the crypt-villus junction and a point ~250 μm up the villus. The density of silver grains corresponding to amino acid in the cells then rapidly increases all the way up to the villus tip. This is believed to represent a greater transport capacity in these cells, which in turn is a function of their maturity. Consequently, this data has also been plotted against the age of the cells on the upper *X*-axis, as well as against the distance from the crypt-villus junction on the lower *X*-axis (Fig. 5, *A–D*). Cell age was computed from their position up the villus. For the control animals, the point of inflection of the curve where cells start to express significant transport function corresponds to a distance from the crypt-villus junction of 180 μm for both leucine and lysine, and to a cell age of ~35 h.

Data for the transport in animals 3 days postirradia-

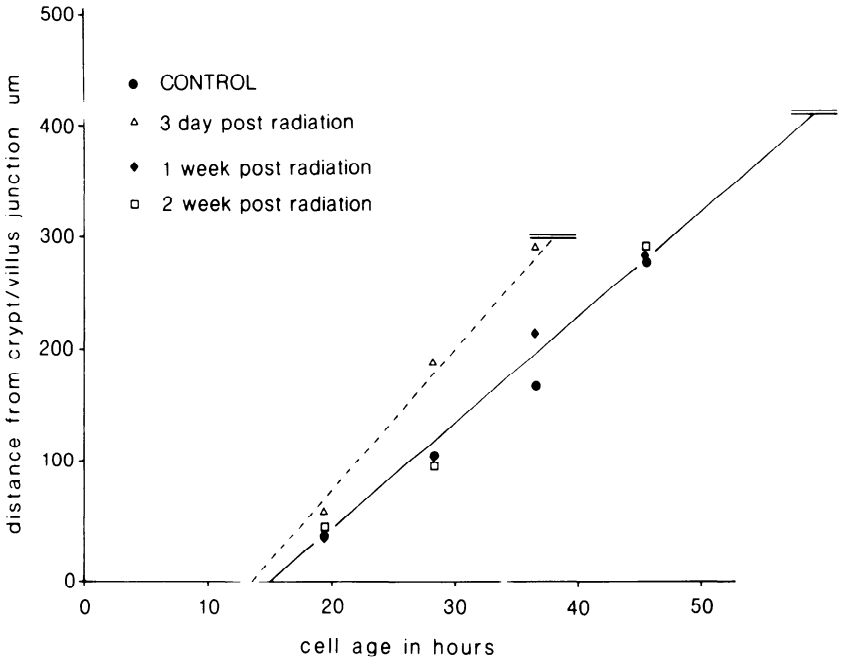

FIG. 4. Cell migration along small intestinal villi in control and irradiated rats. This figure shows progression of silver grain front up villi in control and irradiated animals. Points represent average distance of silver grain front from crypt-villus junction as measured with a micrometer plotted against time interval between injection of [^{3}H]thymidine and death of animals. Lines were fitted by linear egression analysis and horizontal double lines represent mean villus heights. Fronts were measured on 10–15 villi taken from 3 animals for each time point.

TABLE 4. *Villus height and cell migration rate along villi for control and irradiated animals*

	Villus Height, μm	Migration Rate, μm/h	Initial Expression	
			μm	Age, h
Control	420	10	180	34
3 days	300	15	90	18
1 wk	420	10	120	26
2 wk	420	10	210	35

Initial expression represents point on optical density curves (Fig. 6) where density starts to rise rapidly. This point was correlated with distance from crypt villus junction and estimated age of cells at that distance.

tion shown in Fig. 5*B* has very different characteristics. The point of inflection where the uptake of the amino acids into the enterocytes starts to increase is far more difficult to determine, particularly for leucine. The lysine curve starts to climb at ~140 μm from the crypt-villus junction, corresponding to a cell age of only 21 h. Leucine uptake does not really show two components to the curve but may start its steeper component even lower down the villus, 75 μm at 17 h. Clearly the normal distribution has changed.

Figure 5, *C* and *D*, show the data for transport distribution in villi from animals 1 and 2 wk postirradiation. These data are much closer to the control data; at 1 wk the inflection point corresponds to ~220 μm and a cell age of 36 h, whereas at 2 wk the values are 220 μm and 34 h. All of the positions and cell ages have been collected in Table 4 along with the corresponding data obtained using a concentration of 20 mM amino acids. The changes postirradiation can be summarized as being dramatic at 3 days, whereas recovery appears to be essentially complete by 2 wk.

DISCUSSION

Single doses of 3, 6, or 9 Gy of abdominal irradiation are associated with alterations in jejunal and ileal active and passive nutrient uptake and with changes occurring as early as 3 days postirradiation and persisting for at least 33 weeks (5, 16–19). These changes have been confirmed with a variety of in vivo and in vitro techniques (2, 16–19) and with the use of fractionated doses of abdominal irradiation (21). The transport alterations are not due to differences in the animals' food consumption or mucosal surface area, but the transport alterations are associated with increases in the brush-border membrane content of phospholipids in animals fed chow (10). Thus the mechanism of altered intestinal transport following sublethal doses of abdominal irradiation remains unclear, but the association with alterations in brush-border membrane phospholipid content raises the possibility that the changes in membrane lipids may contribute to these functional aberrations.

In irradiated animals fed S or P, there were no differences in the brush-border membrane content of phospholipid (Tables 2 and 3). Thus the differences in transport observed in irradiated animals fed S or P could not be explained by differences in the major membrane phospholipids. It is possible, although as yet unproven, that the mechanical basis for the transport alterations was due to changes in the fatty acyl constituents of the brush-border membrane.

In this study, we did not determine the effect of irradiation and dietary manipulation on the brush border membrane fatty acyl constituents. Therefore, we are unable to comment on whether the irradiation-associated alterations in brush-border membrane phospholipids were also associated with changes in brush-border membrane fatty acyl constituents or whether feeding S prevented these possible alterations in brush-border membrane fatty acids. Lipid peroxidation has been postulated to be one mechanism of irradiation-associated membrane damage (1, 4, 11). It is possible that if lipid peroxidation occurs in the brush-border membrane of irradiated ani-

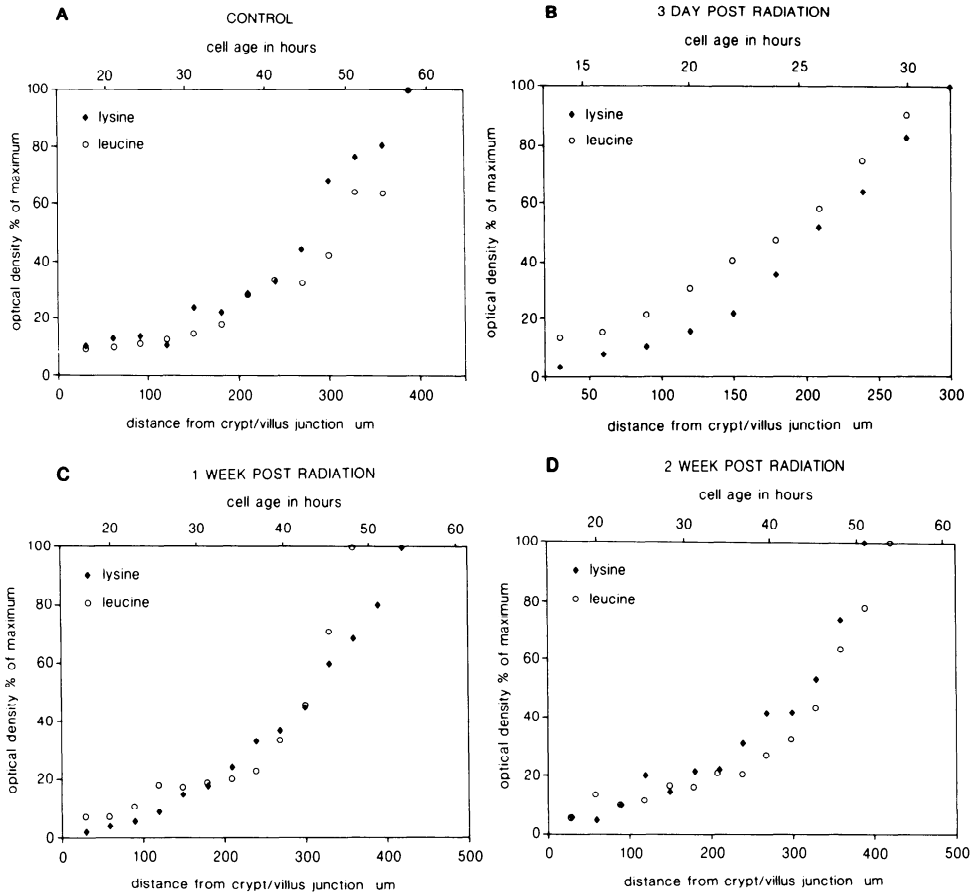

FIG. 5. Distribution of silver grain density along rat jejunal villi corresponding to leucine or lysine intracellular concentration. This figure shows distribution of leucine and lysine transport along the villus in control animals (*A*), 3 days after abdominal irradiation (*B*), as well as 1 wk (*C*), and 2 wk postirradiation (*D*). Integrated optical density is expressed as a percentage of the maximum (normally found at villus tip). Bottom *X* axis related density to distance from crypt villus junction while top axis is age of cells as calculated from data in Fig. 1. Each point represents mean % density measured in 8–14 villi taken from 3–4 animals.

mals, feeding a saturated fatty acid diet might provide a radioprotective effect against damaging ionizing irradiation. This possibility needs to be directly tested, and the use of short-term feeding with a saturated fatty acid diet in the prevention of acute irradiation damage to the intestine warrants further investigation in humans.

The form and function of the intestinal tract is capable of adaptation in response to dietary manipulations (20). Manipulations in the dietary content of fatty acids are not associated with alterations in mucosal surface area or brush-border membrane cholesterol or phospholipid content (23), although the fatty acyl component of the lipids in the brush-border membrane is influenced by dietary manipulations (11). The inhibiting effect of feeding P on nutrient uptake has found an application in streptozotocin-diabetic rats: feeding P before the injection of streptozotocin and the induction of hyperglycemia prevents the enhanced uptake of glucose and lipids observed in diabetic animals fed S or chow (24). From the observations of the enhanced nutrient uptake in control animals fed S (22, 23), it was predicted that the reduced nutrient uptake observed in irradiated animals could be prevented by the feeding of the saturated fatty acid diet. This hypothesis was proven to be correct; the reduced jejunal and ileal uptake of several lipids (Figs. 2 and 3) and the reduced ileal uptake of higher concentrations of glucose (Fig. 1) in irradiated animals fed P was not observed in irradiated animals fed S. Thus feeding a nutritionally adequate isocaloric diet high in S prevented the irradiation-associated alterations in intestinal transport observed in this study in radiated rats fed a diet high in P or, as observed in previous studies, in animals fed chow (5, 16–19). However, the clinical significance of this observation must be viewed with caution, since the major protective effect of S was on the uptake of medium-chain fatty acids, and these are not of major dietary importance. Also, this diet contained palmitic, stearic, and oleic acids, and it is unknown what constitutes the optimal amount or ratio of these nonessential fatty acids to achieve this effect on the intestine.

There have been numerous studies on the effects of irradiation on intestinal morphology and transport function, but comparison has often been difficult because of the different ways in which the results were expressed. Clearly, because the morphology changes so dramatically in the first few days after irradiation, the choice of how the uptake of solutes is expressed can significantly affect the interpretation of how the intestine is responding. This has already been shown in the case for the adaptation of the intestine to a low-protein diet, which also produces a reduction in villus height (4). If the uptake of amino acids is related to the tissue weight, then transport appears to be increased, yet when expressed per unit of intestinal length the transport stays the same. A further example of this was an earlier study of ours that employed isolated intestinal cells to assess changes in amino acid and hexose transport postirradiation (5). When glucose uptake in rat enterocytes at 3 days after exposure to 6 Gy was expressed per cell, the transport appeared to be elevated yet all previous studies had shown a reduction

in transport for the intestine as a whole. At the same time the transport of leucine did decline when expressed per individual cell. What must be borne in mind when trying to interpret these findings is that the distribution of transporting cells along the villus is not uniform (4, 12). As enterocytes migrate up the villus from the crypts they mature and gain the ability to transport various substrates. Without knowing the distribution of the transport capacity along the villus it is not possible to say whether these responses are the result of a change in the number of transporting cells or an alteration in the uptake capacity of individual cells. Although it is the overall transport by the intestine that is important to the animal, these data tell one nothing of the mechanisms by which the intestine is adapting. What must be kept in mind is that not all of the epithelial cells on the villi contribute significantly to the uptake of solutes from the lumen. The control data in this study confirm previous findings (4, 12) that it is the cells at the top one-third of the villus, which normally transport the majority of substrates. More recently, it has been shown that the major factor that determines the sudden increase in transport capacity is not the position of the enterocytes up the villus but the age of the cells (4). Changes in villus height in animals fed a low-protein diet did not influence the age of the cells that started to express transport although their distance from the crypt-villus junction changed considerably.

Similarly, without knowing the distribution of transporting enterocytes in irradiated animals, it is not possible to ascertain how the intestine is responding to sublethal doses of radiation. The data presented here indicate that 3 days postirradiation there are major changes in the enterocyte population. The cells start to express transport at an earlier age and at a position much closer to the base of the villus. This means that although the total number of enterocytes on the villi is decreased because the villi are shorter, nevertheless a greater portion of those enterocytes on the villus are involved in transport. This would appear to be an adaptive response that allows the enterocytes to express transport function far earlier in their life than they do normally. Consequently, although the cells are migrating faster up a shorter villus, the proportion of cells that take up substrate has increased (Fig. 4). If the normal age of 32–35 h for the start of transport function had still applied, then the cells would have been lost from the villus tip before they could become involved in substrate transport. Instead, at 3 days after irradiation, there is still ~150 μm of transporting cells on the villus. However, because the cells start to transport much sooner it would appear that the reduction in the number of transporting cells is minimized. The villi are slightly different in shape 3 days postirradiation, but a simple calculation would indicate that the number of transporting cells in control animals is ~45 on villi from control nonirradiated rats and 34 on those exposed 3 days before to 6 Gy.

Seven days after the irradiation the villi appear to have recovered, the cell migration rate returns to normal, and the height of the villi is close to that seen in nonirradiated animals. The distribution of transporting cells along the villus also reverts to that found before radiation exposure, so any lasting alterations in transport function must result from an alteration in the transport capacity of individual cells (Fig. 5, *A–D*). It must be stressed that this speculation rests on the observation of [^{3}H]leucine and [^{3}H]lysine labeling in the jejunum of rats fed chow and may not necessarily apply to those changes observed for lipids and glucose in animals fed the semisynthetic diets, since the three classes of solute transport may respond differently.

What answers are not clear at present is how this adaptive response seen at 3 days postirradiation is achieved and how the height of the villi is determined under physiological or pathological conditions. The irradiation initially kills cells in the crypts, and so the villi become denuded; at 3 days the crypts are recovering and rapidly produce cells to replace those lost from the villi. Alterations in diet can markedly affect the villus height, particularly the protein content, and resection of the jejunum results in an elongation of the ileal villi. Variations in the dietary content of lipids do not influence the distribution of amino acid transport sites along the villus (unpublished observations), so we elected to use chow for these studies and to directly compare irradiated with nonirradiated rats. These observations have prompted the suggestion that luminal factors may be involved in determining the height of the villi. But it must also be borne in mind that the height of the villi alone does not determine the number of cells involved in transport, because they are not all mature transporting enterocytes. In addition, to finding what dictates the height of the villi, future studies will also need to ascertain what signals are involved in switching on the transport function of the enterocytes. Under physiological conditions the signal may well be primarily the age of the villus cells, but some pathological situations may produce a resetting of this parameter.

The authors thank S. Evans-Davies, S. Burdick, K. Charter, D. Luethe, Y. McIntyre, and M. Tavernini for secretarial and technical services.

We also express our appreciation for research funds kindly granted by the Medical Research Council of Canada. The Alberta Heritage Foundation for Medical Research kindly provided funds for the purchase of equipment used in these studies.

Received 18 November 1987; accepted in final form 12 August 1988.

REFERENCES

1. BAER, A. R., C. I. CHEESEMAN, J. RADZIUK, AND A. B. R. THOMSON. Effects of starvation or indomethacin on intestinal lipid peroxide formation following abdominal irradiation in rats (Abstract). *Clin. Invest. Med.* 10: B133, 1987.
2. BAER, A. R., C. I. CHEESEMAN, AND A. B. R. THOMSON. In vivo assessment of the effect of abdominal radiation on glucose and leucine transport in the rat small intestine (Abstract). *Clin. Invest. Med.* 6: 49, 1983.
3. BRASITUS, T. A., N. O. DAVIDSON, AND D. SCHACHTER. Variations in dietary triacylglycerol saturation alter the lipid composition and fluidity of rat intestinal plasma membranes. *Biochim. Biophys. Acta* 812: 460–472, 1985.
4. CHEESEMAN, C. I. Expression of amino acid and peptide transport systems in rat small intestine. *Am. J. Physiol.* 251 (*Gastrointest. Liver Physiol.* 20): G636–G641, 1986.
5. CHEESEMAN, C. I., A. B. R. THOMSON, AND K. WALKER. The effects of abdominal irradiation in intestinal transport as assessed

with isolated epithelial cells. *Radiat. Res.* 101: 131–143, 1985.
6. DE, A. K., AND A. S. AIYAR. Alterations in tissue lipids of rats subjected to whole body x-irradiation. *Strahlentherapie* 154: 134–138, 1978.
7. ECKNAUER, R., T. VADAKEL, AND R. L. WEPLER. Intestinal morphology and cell production rate in aging rats. *J. Gerontology* 37: 151–155, 1982.
8. HAMMER, C. T., AND E. D. WILLIS. The effect of ionizing radiation on the fatty acid composition of natural fats and on lipid peroxide formation. *Int. J. Radiat. Biol.* 35: 323–332, 1979.
9. KEELAN, M., C. T. CHEESEMAN, K. WALKER, AND A. B. R. THOMSON. Effect of external abdominal irradiation on intestinal morphology and brush border membrane enzyme and lipid composition. *Radiat. Res.* 105: 84–96, 1986.
10. KEELAN, M., M. T. CLANDININ, K. WALKER, AND A. B. R. THOMSON. Dietary fat saturation alters rat brush border membrane phospholipid fatty acid composition (Abstract). *Clin. Invest. Med.* 9: A54, 1986.
11. KEELAN, M., K. WALKER, AND A. B. R. THOMSON. Intestinal morphology, marker enzymes and lipid content of brush border membranes from rabbit jejunum and ileum: effect of aging. *Mech. Ageing Dev.* 31: 49–68, 1985.
12. KING, I. S., F. V. SEPÚLVEDA, AND M. W. SMITH. Cellular distribution of neutral and basic amino acid transport systems in rabbit ileal mucosa. *J. Physiol. Lond.* 319: 355–368, 1981.
13. LUKIE, B. E., H. WESTERGAARD, AND J. M. DIETSCHY. Validation of a chamber that allows measurement of both tissue uptake rates and unstirred layer thickness in the intestine. *Gastroenterology* 67: 652–661, 1974.
14. THOMSON, A. B. R. Influence of dietary modifications on uptake of cholesterol, glucose, fatty acids and alcohols into rabbit intestine. *Am. J. Clin. Nutr.* 35: 556–565, 1982.
15. THOMSON, A. B. R., AND J. M. DIETSCHY. Experimental demonstration of the effect of the unstirred water layer on the kinetic constants of the membrane transport process for D-glucose in rabbit jejunum. *J. Memb. Biol.* 54: 221–229, 1980.
16. THOMSON, A. B. R., C. I. CHEESEMAN, AND K. WALKER. Effect of abdominal irradiation on the kinetic parameters of intestinal uptake of glucose, leucine and gly-leucine in the rat. *J. Lab. Clin. Med.* 102: 813–827, 1980.
17. THOMSON, A. B. R., C. I. CHEESEMAN, AND K. WALKER. Effect of external abdominal irradiation on the dimensions and characteristics of the barriers to passive transport in the intestine. *Lipids* 19: 405–418, 1984.
18. THOMSON, A. B. R., C. I. CHEESEMAN, AND K. WALKER. Intestinal uptake of bile acids: effect of abdominal irradiation. *Int. J. Radiat. Oncol. Biol. Phys.* 10: 671–685, 1984b.
19. THOMSON, A. B. R., C. I. CHEESEMAN, AND K. WALKER. Late effects of variable doses of abdominal radiation on intestinal uptake of nutrients. *Radiat. Res.* 107: 344–353, 1987.
20. THOMSON, A. B. R., AND M. KEELAN. Effect of oral nutrition on the form and function of the intestinal tract. *Surv. Dig. Dis.* 3: 75–94, 1985.
21. THOMSON, A. B. R., M. KEELAN, C. I. CHEESEMAN, AND K. WALKER. Fractionated low doses of abdominal irradiation alters jejunal uptake of nutrients. *Int. J. Radiat. Oncol. Biol. Phys.* 12: 917–925, 1986.
22. THOMSON, A. B. R., M. KEELAN, AND M. T. CLANDININ. Persistence of changes in intestinal transport following feeding of semisynthetic diets containing high or low ratios of polyunsaturated to saturated fatty acids. *Lipids* 22: 22–27, 1987.
23. THOMSON, A. B. R., M. KEELAN, M. T. CLANDININ, AND K. WALKER. Dietary fat selectively alters transport properties of rat jejunum. *J. Clin. Invest.* 77: 279–288, 1986.
24. THOMSON, A. B. R., M. KEELAN, M. T. CLANDININ, AND K. WALKER. A high linoleic acid diet diminishes the enhanced intestinal uptake of sugars in diabetic rats. *Am. J. Physiol.* 252 (*Gastrointest. Liver Physiol.* 15): G262–G271, 1987.
25. THOMSON, A. B. R., AND R. RAJOTTE. Effect of dietary modifications on the enhanced uptake of cholesterol in diabetic rats. *Am. J. Clin. Nutr.* 37: 244–252, 1983.
26. THOMSON, A. B. R., AND R. RAJOTTE. Effect of dietary modifications on the enhanced uptake of glucose, fatty acids and alcohols in diabetic rats. *Am. J. Clin. Nutr.* 38: 394–403, 1983.
27. WESTERGAARD, H., AND J. M. DIETSCHY. Delineation of the dimensions and permeability coefficients of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. *J. Clin. Invest.* 54: 718–732, 1974.