High-Fat Diets and Fecal Level of Reductase and Colon Mucosal Level of Ornithine Decarboxylase, β-Glucuronidase, 5′-Nucleotidase, ATPase, and Esterase in Mice[1]

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ABSTRACT—In one experiment Swiss mice were maintained on a 16 or 23% fat diet (laboratory chow with added fat, principally corn oil) or on laboratory chow alone (5.5% fat). In another experiment C57BL/1 mice were given a 23% fat diet (as above) or a low-fat diet (67% laboratory chow, 1.9% corn oil, and 31% starch; 5.5% fat). Colon mucosal samples were analyzed for several enzyme activities. In Swiss mice the analyses revealed the following: 1) Ouabain-insensitive ATPase was unaltered in male mice, but it rose significantly in females fed a high-fat diet (this effect was seen when a resuspended high-speed pellet was analyzed but not seen with the initial homogenate); 2) 5′-nucleotidase activity showed a significant stepwise increase with dietary fat; 3) nonspecific esterase activity tended to rise with a high-fat diet (not significant); 4) β-glucuronidase levels were not altered by diet fat; and 5) ornithine decarboxylase levels were not altered by diet fat. In C57BL/1 mice analyses were done on ouabain-insensitive ATPase, 5′-nucleotidase, nonspecific esterase, and β-glucuronidase, but no diet effects were seen. Fecal reductase activity was measured with the use of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride hydrate). A high-fat diet did not affect the activity in C57BL/1 mice, but it caused a significant rise in Swiss mice.—JNCI 1984; 72:679-684.

Cancer of the colon is one of the most common cancers in Westernized countries. Its epidemiology strongly points to environmental factors as being almost entirely responsible (*1*) and to diet in particular (*2*). A high intake of dietary fat is a prime suspect, but evidence on this is still unclear (*2-5*).

The most plausible route by which the diet could determine colon cancer incidence is by its effect on fecal composition, e.g., the level of initiators and promoters of carcinogenesis (*6, 7*). The interactions between diet, fecal flora, fecal enzyme levels, and the chemical composition of feces are extremely complex (*2, 8*). However, within this general area many clues may be found concerning the etiology of the disease. For this reason we investigated fecal reductase activity with the use of INT, a synthetic electron acceptor, to explore the possibility that a high-fat diet influences this crucial area of metabolism.

Diet also affects the colon mucosa, which is, of course, to be expected if diet determines cancer risk in that organ. We believe studies of diet-induced changes in colon mucosal biochemistry to be highly relevant to this problem. For instance, Wargovich and Felkner (*9*) recently reported that dietary corn oil raised the activity in rat colon microsomes for the conversion of DMH (CAS: 540-73-8) to a mutagen.

It is well established that the tissue level of ODC is closely associated with the process of carcinogenesis (*10, 11*). Apparently, this is also true for the rat colon. In the colon, ODC is induced by bile acids (*12, 13*), some of which are thought to be promoters of colon carcinogenesis (*2*), and also by complete carcinogens (*13-15*). We now report our observations concerning the effect of a high-fat diet on the level of ODC in mouse colon mucosa.

We also studied enzymes associated with the plasma membrane and endoplasmic reticulum to explore the possibility that a high-fat diet enhances colon cancer risk partly by affecting membrane function. Another enzyme studied was β-glucuronidase. This enzyme may hydrolyze glucuronide conjugates of toxins excreted by the liver and may therefore reactivate carcinogens. For instance, in rat colon mucosa the enzyme is induced by an injection of DMH (*16*), a carcinogen that is excreted in the bile after metabolism to a glucuronide derivative (*17*).

MATERIALS AND METHODS

Diets and environmental conditions.—The formulation of diets is shown in table 1. Mice were fed ad libitum. They were housed in a temperature-controlled room with a 12-hour light-dark cycle.

Mice.—Swiss and C57BL/1 mice were used. Details concerning the mice are indicated in tables 2-6. All mice were from colonies maintained in our animal house.

Preparation of samples.—Mice were killed by cervical dislocation and were analyzed individually. For each of tables 2-5, mice were killed over 11-19 days in batches of usually 2 mice per diet group on each occasion. The feces was collected from the entire length of the colon and rectum. It was cooled on ice and mixed, and a sample was homogenized in a Potter-Elvejhem homogenizer (30 mg/ml of 100 m*M* sodium phosphate, pH 7.0). The homogenate was centrifuged

ABBREVIATIONS USED: DMH=1,2-dimethylhydrazine; INT=2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride hydrate; ODC=ornithine decarboxylase; P_i=inorganic phosphate; TCA=trichloroacetic acid.

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TABLE 1.—*Composition of diets*

Ingredients	Diet, % composition by weight			
	Control	F16[a]	F23[a]	F6[a]
Chow[b]	100	89	81	67
Corn oil[c]		10.7	18.5	1.9
Oleic acid[d]		0.16	0.28	
Starch[e]				31
Fat content[f]	5.5	16	23	5.5
Nutrient/calories[g]	100	75	61	61

[a] The diets were prepared approximately once per week by mixing the ingredients with water until a soft consistency was obtained. They were stored at 4°C.
[b] Rodent Laboratory Chow (Ralston Purina Co., St. Louis, Mo.).
[c] "Mazola" (Best Foods, San Juan, Puerto Rico).
[d] From Fisher Scientific Co., Fair Lawn, N.J.
[e] Wheat Starch (United States Biochemical Corp., Cleveland, Ohio).
[f] Calculated on a dry weight basis.
[g] Relative content of vitamins, minerals, protein, and fiber per 100 calories.

(1,500×g for 2 min at 4°C). The supernatant was adjusted to 0.1% Triton X-100 (to release bacterial enzymes) and was centrifuged again (24,000×g for 11 min at 4°C). The final supernatant was used for analysis.

Simultaneously with the above, the colon and rectum were removed and cut open longitudinally. The tissue was washed in cold running water. It was then rinsed in homogenization medium, which was, unless stated otherwise, 20 m*M* Tris-HCl (pH 7.4) containing sucrose (10% wt/vol). It was placed on a glass plate on ice, lumen side up, and the mucosal layer was scraped off with microscope slides. The mucosa was homogenized with 6 ml homogenization medium in a glass-Teflon Potter-Elvejhem homogenizer.

Part of the homogenate was centrifuged (130,000×g for 44 min at 4°C), and the pellet was resuspended by homogenization as above. The resuspended pellet contained all of the particulate matter of the homogenate. All samples were stored in an ice bath, and assays were completed within 11 hours of sample preparation.

INT reductase.—Reductase activity of the fecal supernatant sample was measured with the use of INT as electron acceptor. INT can accept electrons from $FADH_2$ but not from NAD(P)H. Incubations were in a final volume of 0.57 ml and contained 0.2 ml supernatant sample (or homogenization medium for controls), 2.9 m*M* INT (Aldrich Chemical Co., Milwaukee, Wis.), and 170 m*M* glycine-NaOH (pH 9.5). After the mixture was incubated for 30 minutes at 25°C, the reaction was stopped with 3.2 ml stopping mixture, which consisted of ethyl acetate-ethanol (96%)-TCA (10%) (20:13:2 by vol) (*18*). The extinctions were then measured at 490 nm by means of a Beckman model 25 spectrophotometer.

Na^+, K^+-ATPase.—The method was adapted from Lewis et al. (*19*). Ouabain-sensitive hydrolysis of ATP was measured in the presence of Na^+, K^+, and Mg^{2+} ions. A preincubation was done for 10 minutes at 0°C in a volume of 0.2 ml containing 0 or 1.5 m*M* ouabain and 0.1 ml sample, after which the reaction was initiated by the addition of 0.3 ml buffer-substrate solution [8.3 m*M* ATP, 200 m*M* NaCl, 33 m*M* KCl, 12.5 m*M* $MgCl_2$, and 50 m*M* Tris-HCl (pH 7.5)]. Incubation was for 20 minutes at 37°C and was terminated by addition of 0.5 ml TCA (20% wt/vol). the P_i concentration was then determined according to Baginski et al. (*20*). During this procedure the tubes were kept at 0-4°C to retard acid hydrolysis of ATP.

Ouabain-insensitive ATPase.—The assay was as above except that all incubations included ouabain. Control incubations contained homogenization medium in place of enzyme sample (resuspended pellet or initial homogenate as indicated).

5′-Nucleotidase.—The assay procedure was as described by Murer et al. (*21*). The incubation volume of 0.5 ml contained 4 m*M* $MgCl_2$, 4 m*M* 5′- or 3′-AMP, 50 m*M* Tris-HCl (pH 7.4), and 0.25 ml resuspended pellet. After the mixture was incubated at 37°C for 1 hour, the reaction was stopped and P_i determined as above. 5′-Nucleotidase corresponds to 5′-AMP hydrolysis minus 3′-AMP hydrolysis.

β-Glucuronidase.—Samples of initial homogenate were assayed by a procedure adapted from Reddy et al. (*22*). The incubation volume (0.5 ml) contained 0.25 ml sample (or homogenization medium for controls), 0.05% Triton X-100, 1 m*M* phenolphthalein glucuronide, and 80 m*M* sodium acetate (pH 4.5). After the mixture was incubated for 3 hours at 37°C, the incubation was terminated by addition of 2.25 ml stopping mixture (56 m*M* glycine-NaOH in 1.1% TCA), giving a final pH of approximately 10.4. The tubes were then centrifuged (1,500×g for 10 min), and the extinctions were measured at 550 nm.

Esterase (naphthyl acetate hydrolase).—Nonspecific esterase was assayed by the measurement of 1-naphthol production from 1-naphthyl acetate in samples of initial homogenate (*23*). Incubations were for 30 minutes at 37°C in 30 m*M* barbitone sodium-HCl buffer (pH 7.4), which contained 0.45 m*M* 1-naphthyl acetate.

ODC.—ODC was measured by the estimation of the [^{3}H]putrescine production from [^{3}H]ornithine on the basis of the procedure of Djurhuus (*24*). Colon mucosal homogenate was prepared as described above except that the homogenization medium was 50 m*M* sodium phosphate (pH 7.2), containing 0.1 m*M* pyridoxal 5-phosphate and 0.1 m*M* EDTA. Supernatant was used for analysis after centrifugation (90,000×g for 10 min at 4°C).

In a volume of 112 μl was contained 87 μl supernatant sample (or homogenization medium for controls) plus 0.55 m*M* 1,4-dithiothreitol, 0.22 m*M* pyridoxal 5-phosphate, and 56 m*M* sodium phosphate (pH 7.2). After equilibration for 10 minutes at 37°C, 11 μl of 1 m*M* L-[5-^{3}H]ornithine (14 Ci/mol) (Schwarz/Mann, Spring Valley, N.Y.) was added. After incubation for 30 minutes at 37°C, the tubes were cooled to 0°C and 90 μl incubation mixture was transferred to P81 paper (Whatman Inc., Clifton, N.J.). The unre-

TABLE 2.—*Effect of diet on colon mucosal enzyme activities of female Swiss mice*

Group	Diet period 1[a]	Diet period 2[b]	No. of mice	Ouabain-insensitive ATPase[c]		5'-Nucleotidase[c,e]	Esterase[c]	β-Glucuronidase[c]
				Resuspended pellet[d]	Homogenate			
1	Control	Control	8	7.90±1.73	6.71±1.85	0.384±0.139	74.4±15.5	0.775±0.355
2	Control	F16	8	9.45±5.27	7.10±1.88	0.510±0.221	94.9±35.4	0.614±0.276
3	F16	F16	8	13.01±4.83	6.98±1.80	0.579±0.284	81.2±15.4	0.650±0.271
4	F16	F23	7	13.16±5.23	7.38±1.35	0.855±0.453	95.7±43.8	0.807±0.263

[a] Diet consumed by mice for 7.5 mo after weaning. The same diet had been fed to the parents of the mice since weaning. Control and F16 diets contain 5.5 and 16% fat, respectively. *See* table 1.
[b] Diet consumed for 5 mo before sacrifice commencing at end of period 1. Control, F16, and F23 diets contain 5.5, 16, and 23% fat, respectively. *See* table 1.
[c] Values are means ± SD. Units are μmol/hr/mg protein. Results show no significant difference unless indicated.
[d] Groups significantly different: 1 vs. 3 and 1 vs. 4 (both $P<.02$); 1 vs. 3, 4 pooled ($P<.01$); 1 vs. 2–4 pooled ($P<.05$). No significant trend was found with the use of one-way analysis of variance.
[e] Groups significantly different: 1 vs. 4 ($P<.02$); 2, 3 pooled vs. 4 ($P<.05$). A significant trend is indicated ($P<.05$) by one-way analysis of variance.

acted ornithine was removed with 0.09 *M* ammonia solution (*24*). [^{3}H]Putrescine was then eluted with 1.7 ml of 2 *M* NaOH. A 1.12-ml aliquot was neutralized with 0.385 ml HCl and counted in 2.15 ml Scintiverse (Fisher Scientific Co., Fair Lawn, N.J.) by means of a Beckman LS-3133T scintillation counter.

Protein.—Protein determinations were made by the method of Lowry et al. (*25*).

Statistical analyses.—Statistical significance of differences between diet groups was calculated with the use of the two-tailed Student's *t*-test; *P*-values less than .05 were considered significant. The data from the mice fed a high-fat diet were analyzed both with and without pooling of different groups. Where indicated, data were analyzed by one-way and two-way analyses of variance (both one-tailed).

RESULTS

Studies on Na$^+$,K$^+$-ATPase and 5'-Nucleotidase

Our initial interest was Na$^+$,K$^+$-ATPase. Repeated attempts to accurately analyze this enzyme in mouse colon mucosa samples were unsuccessful. The problem was caused by the high level of ouabain-insensitive ATPase relative to the level of Na$^+$,K$^+$ (ouabain-sensitive)-ATPase. With Swiss mice ouabain-sensitive activity generally represented under 10% of the total ATPase activity. This percentage was found to be slightly higher in C57BL/1 mice.

We tested sodium dodecyl sulfate (*26, 27*), Lubrol PX (*28*), Triton X-100 (*23*), repeated freezing and thawing, an altered incubation time, and the addition of 1,4-dithiothreitol to the homogenization medium, but these modifications proved of no value. Therefore, in our diet studies analyses were directed at ouabain-insensitive ATPase.

5'-Nucleotidase was found to be assayable with far greater sensitivity than was possible for Na$^+$,K$^+$-ATPase because in resuspended pellet samples, as used here, 5'-nucleotidase represents a much higher proportion of the total (5'-AMPase) activity (mean: 54% in C57BL/1 mice and 71% in Swiss mice). Resuspended pellet rather than initial homogenate was used for routine analysis due to the presence of an enzyme activity that hydrolyzes 3'-AMP at a faster rate than 5'-AMP and which is removed in the high-speed supernatant (*29*).

Colon Enzyme Levels and Diet

Ouabain-insensitive ATPase activity in a high-speed pellet of colon mucosa from female Swiss mice rose with a high-fat diet (table 2). This was seen in the 2 groups where high-fat diets had been fed for 2 generations (groups 3 and 4) but not where such a diet had only been fed for a much shorter time period (group 2). It is possible, of course, that this was merely a chance observation. No such response was seen in male Swiss mice (table 3). When the initial homogenate was analyzed for ouabain-insensitive ATPase, no diet effect

TABLE 3.—*Effect of diet on colon mucosal enzyme activities of male Swiss mice*

Diet period 1[a]	Diet period 2[a]	No. of mice	Ouabain-insensitive ATPase[b]		Esterase[b]	β-Glucuronidase[b]
			Resuspended pellet	Homogenate		
Control	Control	11	15.59±5.94	9.26±2.95	37.1±14.5	0.628±0.234
Control	F16	10	16.77±5.62	9.32±2.79	53.4±31.5	0.593±0.162
F16	F16	10	14.91±6.09	9.82±3.10	44.6±19.9	0.735±0.130
F16	F23	9	16.92±5.36	8.96±1.86	45.9±19.2	0.600±0.131

[a] Diet histories as in table 2 except that after weaning diet period 1 lasted 0.5 mo followed by diet period 2, which lasted 8 mo.
[b] Values are means ± SD. Units are μmol/hr/mg protein. Results show no significant difference.

TABLE 4.—*Effect of diet on fecal INT reductase in Swiss mice*

Group	Diet period 1[a]	Diet period 2[a]	INT reductase[b]	
			Female[c]	Male[d]
1	Control	Contol	35.8±18.4	42.3±19.3
2	Control	F16	64.0±36.0	59.7±17.0
3	F16	F16	50.4±29.6	60.2±30.2
4	F16	F23	57.6±26.4	65.1±15.2

[a] Diet histories and numbers of mice as in table 2 (female) and table 3 (male).
[b] Values are means ± SD. Units are nmol/hr/mg feces. (Quantity of feces refers to original feces from which the sample was extracted.)
[c] Results show no significant difference with the use of a *t*-test. Significant trends were found with two-way analysis of variance for both diets ($P<.001$) and batch variation ($P<.0005$); *see* text.
[d] Groups significantly different: 1 vs. 2 ($P<.05$); 1 vs. 4 ($P=.01$); 1 vs. 2–4 pooled ($P<.02$).

was seen in either sex (tables 2, 3). In female Swiss mice mucosal 5′-nucleotidase activity appeared to manifest a dose-response relationship with diet fat (table 2). Thus, in comparison to the activity in mice fed a control diet, the activity was higher by about 42% in mice fed diet F16 (not significant) and by 123% in mice fed diet F23 ($P<.02$). In both male and female Swiss mice mucosal esterase activity was a little higher in mice fed diets F16 and F23 compared with the activity in mice fed the control diet (tables 2, 3). The differences were not significant. No diet-induced alterations were apparent for mucosal β-glucuronidase (tables 2, 3).

In Swiss mice of both sexes high-fat groups had a rise in fecal INT reductase of about 40–80% in comparison to the level of fecal INT reductase in mice fed the control diet (table 4). In female mice but not in males the activity fell in each diet group by roughly 50–68% between the first and last batches (*see* "Preparation of samples," "Materials and Methods"). The cause of this is unknown, but it may be related to the fact that males were studied at a different time of the year than females. As a result, a *t*-test revealed a significant trend in males only. The data for female mice were reanalyzed by two-way analysis of variance, and this analysis indicated significant differences for both diet and batch variation.

TABLE 6.—*Effect of diet on ODC activity of colon mucosa*[a]

Diet period 1[b]	Diet period 2[b]	No. of mice	ODC[c]
Control	Control	6	133.1±48.4
F16	F16	7	120.1±56.8
F16	F23	6	125.4±37.3

[a] Mice are all Swiss females.
[b] Diet histories as in table 2 except that diet period 2 lasted 4.5 mo.
[c] Values are means ± SD. Units are pmol/30 min/mg protein. Results show no significant difference.

The above enzyme activities were also studied in C57BL/1 mice, but no significant effects of a high-fat diet were observed (table 5). In female Swiss mice colon mucosal ODC activity did not respond to diet fat (table 6).

DISCUSSION

The studies on Swiss mice used a different experimental strategy to those on C57BL/1 mice. The parents of most Swiss mice fed a high-fat diet also had been maintained on such a diet. The parents of all C57BL/1 mice, however, were fed only a control diet.

With Swiss mice the control (low-fat) diet differed from the high-fat diets not only in its fat level but also in its (higher) content of nutrients. Inasmuch as diets F16 and F23 were prepared by simple addition of fat to the control diet, this procedure reduced the content of vitamins, minerals, protein, and fiber per 100 calories to 75% (diet F16) or to 61% (diet F23) of the level in the control diet. This experimental design does not test fat as an independent variable, but it does resemble the human situation in Westernized countries where the high fat content will tend to be at the expense of other nutrients.

In the case of C57BL/1 mice, the low-fat diet (diet F6) resembled diet F23 in all respects except its fat and starch contents. These various dietary differences are reflected in the quantity of feces obtained. With C57BL/1 mice similar quantities of feces were present in the 2 diet groups. However, with Swiss mice only

TABLE 5.—*Effect of diet on colon mucosal and fecal enzyme activities of C57BL/1 mice*

Diet[a]	Sex	No. of mice	Mucosal[b]					Fecal[b]
			Ouabain-insensitive ATPase		5′-Nucleotidase	Esterase	β-Glucuronidase	INT reductase
			Resuspended pellet	Homogenate				
F6	♀	7	11.64±3.19	11.65±2.99	0.293±0.086	54.8±19.5	0.201±0.082	58.5±10.2
F23	♀	8	12.11±2.03	12.17±2.83	0.324±0.115	68.5±31.1	0.228±0.079	63.5±12.3
F6	♂	11	12.53±3.36	7.91±0.96	0.318±0.170	74.1±51.1	0.768±0.480	65.6±15.2
F23	♂	10	12.43±2.97	8.15±2.10	0.363±0.186	54.0±19.7	0.835±0.422	76.3±19.9

[a] Mice received a control diet until 4 mo after weaning. The experimental diet was then fed for 7.5 mo. Diets F6 and F23 contain 5.5 and 23% fat, respectively.
[b] Values are means ± SD. Units are μmol/hr/mg protein (mucosal) or nmol/hr/mg feces (INT reductase). Groups of the same sex show no significant difference.

about half as much feces was present with diet F16 and F23 than with the control diet (data not shown). For the above reasons (and also differences in the diet histories of the animals as indicated in tables 2-5), results with the two strains are not directly comparable. It is also noteworthy that the high-fat diets induced obesity in Swiss mice but not in C57BL/1 mice (Temple NJ, El-Khatib SM: Unpublished data).

We were not able to detect any significant alteration in colon mucosal ODC level in female Swiss mice. This finding contrasts with a stimulation of between 3- and 35-fold in rat colon after bile acid application (*12, 13*). Possibly, dietary fat produces a weak enhancement of ODC activity, which, nevertheless, is of biologic importance over a prolonged time period. The assay for ODC in virtually all other laboratories is based on the determination of $^{14}CO_2$ release from [1-^{14}C]ornithine. In reality, some of this activity may be a measure of the combined effects of enzymes other than ODC (*24*). The assay used here measures the conversion of [^{3}H]-ornithine to [^{3}H]putrescine.

In the guinea pig small intestinal mucosa an endoplasmic reticulum localization has been demonstrated for nonspecific esterase (with the use of the same assay procedure as that used here) (*30*). In mouse colon mucosa, however, we found appreciable, but highly variable, quantities in the high-speed supernatant (5.7×10^6 g/min) (data not shown). Its precise localization in this tissue is therefore unclear. In Swiss mice, but not in C57BL/1 mice, a trend was observed for the high-fat groups to show a raised activity of the esterase, but this did not achieve statistical significance.

We failed to observe any changes in mucosal β-glucuronidase activity resulting from a high-fat diet. Reddy et al. (*22*) reported no effect on the β-glucuronidase activity of rat colon mucosa after feeding diets rich in beef protein and beef fat or soybean protein and corn oil (added at the expense of starch). However, these diets did increase the level of the enzyme in the rat small intestinal mucosa.

As part of our studies of changes in colon mucosa membranes induced by a high-fat diet, we attempted to measure the level of Na^+,K^+-ATPase. Unfortunately, this proved impossible to perform accurately, because the proportion of the total ATPase activity that is ouabain-sensitive is about 10%, which is similar to that observed in rat colon mucosa (*31*) but is markedly different from that reported in other tissues. In rat brain, for instance, 40-80% of the total ATPase is ouabain-sensitive [(*32*); Ferchmin P: Personal communication].

With respect to the level of mucosal ouabain-insensitive ATPase, no significant effects of a high-fat diet were seen except in female Swiss mice where the activity was elevated in 2 groups fed a fat-rich diet. This elevated activity was seen with resuspended pellet samples but not with homogenate. The relationship of this elevation to carcinogenesis is unknown as is also whether the apparent sex effect is genuine or spurious. Ouabain-insensitive ATPase is probably mainly due to nonspecific phosphatases in lysosomes and brush borders.

With Swiss mice mucosal 5'-nucleotidase activity manifested a stepwise increase with the level of dietary fat. No dietary effect was observed with C57BL/1 mice. Possibly, this strain difference results from the experimental design as discussed above. If, however, it is genuine, it may reflect changes in the feces or may be related to the fact that a high-fat diet induces obesity in Swiss mice but not in C57BL/1 mice (Temple NJ, El-Khatib SM: Unpublished data).

Swiss mice have a much greater susceptibility to DMH-induced colon tumors than do C57BL/1 mice. This phenomenon has been found both by ourselves with the colonies of mice used here (Temple NJ, El-Khatib SM: Unpublished data) and by Evans et al. (*33*). This finding may be related to the apparently different responses of colon mucosa 5'-nucleotidase activity of the two strains to a high-fat diet.

In another investigation we studied the colon mucosa membranes of Swiss mice by performing density gradient centrifugation (isopyknic banding). Fractions were analyzed for 5'-nucleotidase. The enzyme is apparently divided between the basolateral plasma membrane and the brush border. It was observed that diets F16 and F23 significantly reduce the median density of the brush borders (*29*). Thus diets F16 and F23, which in some respects resemble human diets associated with a high colon cancer incidence, cause changes in the colon mucosa and at least one of its associated enzymes. We consider this area of diet-colon interaction worthy of further investigation.

In Swiss mice, but not in C57BL/1 mice, a high-fat diet resulted in a raised enzyme activity for fecal INT reductase. This possible strain difference is noteworthy in view of the above-mentioned strain difference in susceptibility to colon tumors. We do not know the significance of the altered INT reductase level. Possibly, it is associated with the ratio of anaerobic to aerobic bacteria. Hill et al. (*8*) reported that a high ratio was often found in human populations where colon cancer is common. Fecal INT reductase should be studied in both experimental animals and humans to clarify its possible relationship to colon carcinogenesis.

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