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# EFFECTS OF DIETARY PECTIN AND CELLULOSE ON HEPATIC AND INTESTINAL MIXED-FUNCTION OXIDATIONS AND HEPATIC 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE IN THE RAT

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**Abstract**—The aim of this study was to determine if feeding dietary fiber (cellulose or pectin) to male rats could influence hepatic and intestinal mixed-function oxidation. We simultaneously compared hepatic drug-oxidizing activity with the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme for cholesterol biosynthesis. Three groups of six animals were fed a purified diet containing by weight either 10.4% cellulose or 10.4% pectin, or a standard cereal-based diet containing 4.5% crude fiber; the caloric contributions by carbohydrate, protein and fat in the three diets were similar. In the cellulose-fed rats, the hepatic microsomal cytochrome P-450 content and the activities of ethylmorphine *N*-demethylase and aniline hydroxylase were significantly lower when compared with those of rats fed pectin or the cereal-based diet. The hepatic microsomal cytochrome P-450 content and the activities of ethylmorphine *N*-demethylase and aniline hydroxylase were similar in the pectin-fed and cereal diet-fed rats. Hepatic HMG-CoA reductase activity, hepatic microsomal cytochrome $b_5$ content, and intestinal benzo[*a*]pyrene hydroxylase activity were comparably lower in rats fed the purified diet with either dietary fiber when compared to those fed the cereal diet. It is concluded that dietary pectin and cellulose exert distinctly different influences on the hepatic microsomal mixed-function oxidase system for drug metabolism, but not on liver cholesterol synthesis or intestinal benzo[*a*]pyrene hydroxylation, suggesting that different physiological mechanisms control these enzyme systems.

Dietary fiber has a number of substantial metabolic effects including alterations in cholesterol and bile acid metabolism [1–3], glucose homeostasis, and insulin responses [4]. Moreover, there is epidemiological evidence suggesting that dietary fiber can alter susceptibility to gastrointestinal diseases such as diverticulosis and colon cancer [5]. Experiments in rats and mice fed semipurified or stock diets have suggested that dietary fiber is capable of altering the metabolism of foreign chemicals such as sodium cyclamate and the antioxidant 2, 5-di-t-butylhydroquinone [6].

Previous studies in rats have demonstrated that feeding a purified diet results in lower activity of the mixed-function oxidase system in the liver [7], intestinal mucosa [8] and lung [9], in comparison with animals fed cereal-based diets. Such observations are potentially important because the microsomal mixed-function oxidase system, for which multiple cytochrome P-450 and P-448 species serve as terminal oxidases, metabolizes a variety of drugs and foreign chemicals as well as endogenous compounds such as steroids and fatty acids [10]. Attempts to define components found in cereal-based diets but not in purified diets that induce greater mixed function oxidase activity are inconclusive. The present study was designed to determine whether cytochrome P-450 and associated activities of the mixed-function oxidase system in the liver and intestine of the rat are influenced by feeding either cellulose or pectin as components of a purified diet and, also, to examine the specificity of any dietary-induced change in hepatic drug oxidation relative to the activity of hepatic microsomal 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (mevalonate: NADP oxidoreductase, EC 1.1.1.34), the rate-controlling enzyme for cholesterol biosynthesis [11]. It is known that cholesterol metabolism is influenced by dietary fiber intake and also that hepatic cholesterol and drug metabolism are regulated in a non-coordinated manner by certain drugs and surgical procedures [12, 13]. It was therefore of interest to compare the effects of dietary fiber, as a nutritional supplement, on these two microsomal enzyme systems.

## MATERIALS AND METHODS

*Isotopes.* *R,S*-[4-$^3$H]mevalonic acid (250 mCi/mmole) was purchased from Amersham/Searle (Arlington Heights, IL) and D,L-3-hydroxy-3-methyl-[3-$^{14}$C]glutaryl-CoA (26.5 mCi/mmole) from the New England Nuclear Corp. (Boston, MA). The

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isotopes were shown to be 95–98% pure by thin-layer chromatography and were used without further purification.

*Coenzymes and substrates.* D,L-3-Hydroxy-3-methylglutaryl-coenzyme A was purchased from P-L Biochemicals (Milwaukee, WI); NADP, NADH, glucose-6-phosphate dehydrogenase (from torula yeast), glucose-6-phosphate sodium salt, and benzo[*a*]pyrene from the Sigma Chemical Co. (St. Louis, MO); aniline hydrochloride from the Eastman Kodak Co. (Rochester, NY); and ethylmorphine hydrochloride (Dionin) from E. Merck Laboratories (Elmsford, NY).

*Animals.* Male Sprague–Dawley rats weighing 190–200 g (Holtzman, Madison, WI) were housed individually in a light cycled room (12 hr/cycle) to decrease coprophagy [14, 15]. The animals were randomly assigned to three groups of six rats and each group was fed *ad lib.* one of the three diets. After 5 weeks the rats were decapitated in mid-light cycle, blood was collected in Vacutainer tubes containing EDTA as the anticoagulant (Becton–Dickinson Co., Rutherford, NJ) and the liver and small intestine were removed and placed on ice.

*Diet compositions.* The three diets were prepared by Teklad Test Diets (Madison, WI). The two purified diets contained by weight 58.5% sucrose, 21.6% casein, 5.0% lard, 3.5% AIN-76 mineral mix (Teklad Cat. No. 76514), 1.0% vitamin mix (No. 40060) and either 10.4% cellulose (No. 160390) or 10.4% pectin (calculated caloric density = 3.65 cal/g). The cellulose (Solka Floc, Brown Co., Berlin, NH) contained 90% wood alpha cellulose and 10% nonglucose hemicellulose (analyzed by the Brown Co.). The citrus pectin (ICN Pharmaceuticals, Cleveland, OH) had a molecular weight of 150,000–300,000 and contained 6.7% methoxyl groups (analyzed by ICN Pharmaceuticals).

The cereal-based diet was the NIH-07 open formula rat and mouse ration [16] and provided by weight 64.5% crude carbohydrate, 23.5% crude protein, 5.0% crude fat, and 4.5% crude fiber (calculated caloric density = 3.97 cal/g). This diet is satisfactory for normal growth rate, reproduction, and lactation of both rats and mice and is similar in macronutrient composition to commonly used closed-formula rations for rodents.

*Plasma chemistry.* Plasma cholesterol [17] and triglyceride concentrations [18] were measured using a Technicon Auto Analyzer, model II (Technicon Instruments Corp., Tarrytown, NY). Plasma glucose concentrations were measured with a Technicon SMA 12/60 autoanalyzer modified for animal blood samples [19].

*Microsome preparation.* Hepatic microsomes were prepared at 0–4° using the procedure of Shapiro and Rodwell [20]. Briefly, livers were minced with scissors and homogenized in a total volume of 40 ml of buffer containing 30 mM EDTA, 70 mM NaCl, and 10 mM 2-mercaptoethanol, pH 6.0, using a Potter–Elvehjem Teflon–glass homogenizer. Intact cells, nuclei, and mitochondria were removed by two successive centrifugations at 17,300 *g* for 15 min. Microsomes were collected by a final centrifugation at 65,000 *g* for 1 hr, resuspended in buffer (0.5 ml/g liver) containing 50 mM $KH_2PO_4$, 30 mM EDTA, 70 mM KCl, and 1.0 mM dithiothreitol, pH 7.4 [21], and frozen in liquid nitrogen. Microsomes retained full HMG-CoA reductase activity [22] and mixed-function oxidase activity [23] for at least several months when prepared and stored in this manner.

All assays were performed within 4 weeks of storage. For the assays below, liver microsomes were rapidly defrosted in a 37° waterbath. Protein concentration was measured by the procedure of Lowry *et al.* [24].

*HMG-CoA reductase.* Hepatic microsomal HMG-CoA reductase activity was measured as described previously [21, 25, 26]. Radioactivity was measured with external quench correction using a Tri-Carb model 3390 liquid scintillation spectrometer, equipped with an absolute activity analyzer model 544 (Packard Instrument Co., Downers Grove, IL).

*Ethylmorphine* N-*demethylase.* A micro-assay for microsomal ethylmorphine *N*-demethylase activity was developed [26] by a modification of published procedures [27–29]. All steps of the assay were done at 0–4° unless otherwise noted. A volume of microsomal suspension containing 0.4 to 0.5 mg protein was diluted to 350 μl with 0.1 M potassium phosphate buffer, pH 7.4, containing 15 mM semicarbazide hydrochloride. Fifty microliters of 50 mM $MgCl_2$ in 1.15% KCl was added, followed by 55 μl of cofactor solution (10 mM NADP, 100 mM glucose-6-phosphate, and 1.0 I.U. of glucose-6-phosphate dehydrogenase in 1.15% KCl). Fifty microliters of 100 mM ethylmorphine hydrochloride in 1.15% KCl was added, and the reaction was initiated by incubation at 37° in a water bath. After 15 min of incubation, the reaction was stopped by adding 200 μl of 1.24 M $ZnSO_4$ solution, followed by 200 μl of saturated $Ba(OH)_2$ solution [30]. The samples were mixed after each of these additions, centrifuged to remove the precipitate, and then 500 μl of the supernatant fraction was removed for analysis. To this portion was added 300 μl of double-strength Nash's reagent containing 4.0 M ammonium acetate, 0.05 M acetic acid, and 0.02 M acetylacetone [30, 31]. The samples were heated at 60° for 15 min to develop the color. The optical density was read at 412 nm with a Beckman DU spectrophotometer; formaldehyde formed in the reaction was calculated using a molar extinction coefficient of 8000 $cm^{-1}$ [31]. Enzyme activity was expressed as μmoles formaldehyde $\cdot hr^{-1} \cdot$ (mg microsomal protein)$^{-1}$. Blank assays contained 50 μl of 1.15% KCl instead of the ethylmorphine substrate solution. Duplicate assays were run for each sample and the coefficient of variation was 5–10 per cent. Enzyme activity was demonstrated to be linear over a range of 0 to 0.5 mg microsomal protein and for incubation times of 0–20 min.

*Aniline hydroxylase.* Microsomal aniline hydroxylase activity was measured by a procedure similar to that of Imai *et al* [32] but modified for small samples [26]. Buffers, cofactor concentrations, and volumes used were the same as for the ethylmorphine *N*-demethylase assay, except that semicarbazide was omitted. The substrate solution was 50 μl of 50 mM aniline hydrochloride in 1.15% KCl [28]. The reaction was initiated by incubation at 37° in a water bath and was terminated after 20 min by adding

200 μl of 20% trichloroacetic acid. The samples were centrifuged and 350 μl of each supernatant fraction was removed. To this aliquot 350 μl of 1 M $Na_2CO_3$ was added, followed by 350 μl of 0.5 N NaOH–2% phenol. After standing for 30 min at room temperature, the resulting blue color was measured at 630 nm. Enzyme activity was expressed as nmoles *p*-aminophenol formed·$hr^{-1}$·(mg microsomal protein)$^{-1}$; the *p*-aminophenol formed was calculated using an extinction coefficient of 0.373 for a 1 μg/ml solution [33]. Blank assays contained 50 μl of 1.15% KCl instead of the aniline substrate solution. Duplicate assays were run for each sample and the coefficient of variation was 5–10 per cent. Aniline hydroxylation was linear from 0 to 30 min incubation time and over a range of 0 to 0.5 mg microsomal protein.

*Microsomal cytochromes.* Cytochrome P-450 and $b_5$ contents were measured by the procedure of Omura and Sato [34] using an Aminco DW-2 spectrophotometer (American Instrument Co., Savage, MD).

*Benzo[a]pyrene hydroxylase in small intestinal mucosa.* The small intestine was removed and irrigated promptly with ice-cold 0.9% NaCl. The mucosa of the proximal 15 cm was scraped off with a scalpel at 0–4°. The recovered mucosa was weighed, a volume of ice-cold 0.05 M Tris buffer (pH 7.4) equal to three times the mucosal weight was added, and the mixture was then homogenized using a tight-fitting Potter–Elvehjem Teflon–glass homogenizer. This was centrifuged at 9000 *g* for 15 min at 4° and the supernatant fraction was used for the assay.

Benzo[*a*]pyrene hydroxylase activity was measured by a procedure similar to that of Nebert and Gelboin [35] but modified for small samples. In a darkened room, 50 μl of the mucosal supernatant fraction was combined with 55 μl of a cofactor–substrate solution containing 0.10 μmole NADPH, 0.3 μmole $MgCl_2$, and 9.9 nmoles benzo[*a*]pyrene. The tubes were incubated for 5 min in a 37° shaking water bath and the reaction was terminated by adding 100 μl of ice-cold acetone. After 325 μl of *n*-hexane was added, the tubes were vigorously agitated on a Vortex type mixer for 30 sec and then centrifuged to separate the phases. Two hundred microliters of the hexane layer (top) was removed and added to a second tube containing 1.00 ml of 1.0 N NaOH; these samples were mixed vigorously and then centrifuged. 3-Hydroxybenzo[*a*]pyrene present in the alkali phase was measured with a Hitachi FPF-2A recording spectrophotofluorimeter, using an activation wavelength of 396 nm and an emission wavelength of 522 nm. The 3-hydroxybenzo[*a*]pyrene product was specifically identified in each sample by recording its fluorescence emission spectrum, which corresponded to that of a 3-hydroxybenzo[*a*]pyrene standard (peak at 522 nm). The fluorescence peak for water (i.e. Lamon peak) was used to calibrate the spectrophotofluorimeter. Specific activities were expressed as nmoles 3-hydroxybenzo[*a*]pyrene·$hr^{-1}$·(mg supernatant protein)$^{-1}$. Assays were performed in duplicate and compared to a blank in which acetone was added prior to incubation. The coefficient of variation of the duplicate assays was 5–10 per cent. The activity was demonstrated to be linear from 0 to 20 min incubation time and over a range of 0 to 0.68 mg supernatant protein.

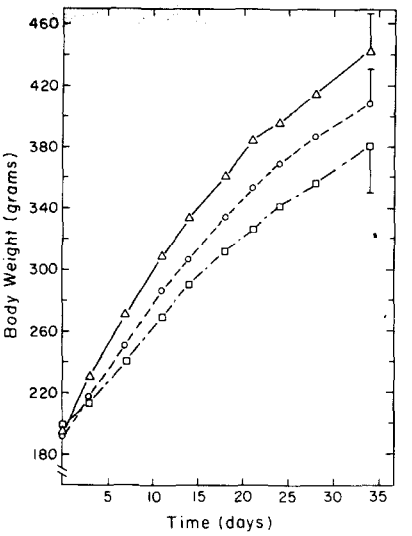


Fig. 1. Effect of three diets on body growth rates in rats. Mean body weights are shown for three groups of six rats each fed diets that were cereal-based (△——△), purified with cellulose (○---○), and purified with pectin (□—·—□). The bars with the final body weights are 1 S.D.

*Statistics.* Statistical analyses were carried out using a Hewlett–Packard 97 Calculator programmed for Student's *t*-test [36] as supplied in the Hewlett–Packard Stat Pac 1. All results are expressed as means ± 1 S.D.

## RESULTS

All three groups of rats gained weight rapidly as shown in Fig. 1, although at slightly different rates. Differences in average weight gain (g/34 days) were statistically significant only for the group fed the cereal-based diet as compared with the rats fed the pectin-containing diet. Although starvation [37] or a markedly reduced food intake [26] can decrease microsomal mixed-function oxidase activities for various substrates in the livers of male rats, it seems unlikely that the small differences in weight gain observed were a major metabolic influence in this study because all animals gained weight in a rapid and satisfactory fashion.

Relative liver weights and microsomal protein contents were not significantly different in the three groups of rats (Table 1). On the other hand, average cecal weights (which included cecal contents) were clearly influenced by the type of diet consumed. As shown in Table 1, the pectin diet-fed rats had a 3.5-fold, and the cereal diet-fed rats a 2.0-fold, greater mean cecum weight when compared with rats fed the cellulose containing diet.

Average plasma glucose concentrations were not significantly different in the three groups. The average cholesterol level was significantly lower in the rats fed the cereal-based diet when compared with

Table 1. Comparison of physiological values in rats fed a cereal-based diet or a purified diet containing cellulose or pectin*

| Value | Purified diet w/cellulose (A) | Purified diet w/pectin (B) | P (A vs B) | Cereal-based diet (C) | P (A vs C) |
|---|---|---|---|---|---|
| Liver wt. (% of body wt) | 4.62 ± 0.41 | 4.75 ± 0.52 | NS† | 4.26 ± 0.21 | NS |
| Hepatic microsomal protein (mg/g) | 12.87 ± 1.26 | 13.14 ± 1.43 | NS | 13.53 ± 1.07 | NS |
| Cecum wt (g) | 3.58 ± 0.46 | 12.70 ± 3.08‡ | < 0.001 | 7.24 ± 1.25‡ | < 0.001 |
| Plasma glucose (mg/dl) | 165 ± 34 | 158 ± 22 | NS | 155 ± 16 | NS |
| Plasma cholesterol (mg/dl) | 92 ± 17 | 76 ± 17 | NS | 74 ± 7 | < 0.05 |
| Plasma triglycerides (mg/dl) | 349 ± 145 | 337 ± 179 | NS | 187 ± 59 | < 0.05 |

* Groups of six animals were fed each diet for 5 weeks. Diet compositions are given in Materials and Methods. Results are expressed as mean ± 1 S.D.
† Not significant ($P > 0.05$).
‡ Rats fed diet B differ from rats fed diet C with $P < 0.01$.

the cellulose-fed rats, but the mean cholesterol concentration in the pectin-fed rats was not significantly different from the other groups. Average plasma triglyceride concentrations were considerably lower in rats fed the cereal diet when compared with either of the purified diets, although only the comparison between cellulose- and cereal diet-fed rats showed a statistically significant difference.

Mean values for ethylmorphine *N*-demethylase, aniline hydroxylase, and cytochrome P-450 in liver microsomes were lower in cellulose-fed rats than in rats fed the pectin diet or the cereal-based diet (Table 2). The mean values for cytochrome P-450 and associated drug-oxidizing activities were not significantly different in rats fed the purified diet containing pectin in comparison with those consuming the cereal-based diet. Mean values for HMG-CoA reductase and cytochrome $b_5$ were similar in livers from rats fed pectin or cellulose, and these values were lower in both groups fed dietary fiber in a purified diet than in the cereal diet-fed group. These findings demonstrate that dietary fiber, and in particular the substitution of pectin for cellulose, can alter hepatic mixed-function oxidase activities in male rats without changing the activity of HMG-CoA reductase, the rate-limiting enzyme for cholesterol biosynthesis.

Intestinal benzo[*a*]pyrene hydroxylase activity was measured in the small intestinal mucosa from the three groups of rats (Table 3). Average benzo[*a*]pyrene hydroxylase activities were very similar in the two groups of rats fed the purified diet containing either cellulose or pectin, and in both of these groups this enzyme activity was markedly lower than in the intestines of rats fed the cereal-based diet.

## DISCUSSION

The present study has shown that hepatic microsomal mixed-function oxidase activity, when assessed in terms of cytochrome P-450 content or activities of ethylmorphine *N*-demethylase or aniline hydroxylase, was substantially lower in rats fed a purified diet containing 10.4% cellulose than in rats fed a purified diet containing 10.4% pectin. When compared to the cereal diet-fed rats, the rats fed the purified diet with 10.4% cellulose had significantly lower hepatic mixed-function oxidase system acivity, while the rats fed the purified diet with 10.4% pectin had similar activity. Thus, cellulose and pectin appear to have substantially different effects on the cytochrome P-450 system in the rat liver.

Table 2. Comparison of hepatic microsomal enzyme activities in rats fed a cereal diet or a purified diet containing cellulose or pectin*

| Value | Purified diet w/cellulose (A) | Purified diet w/pectin (B) | P (A vs B) | Cereal-based diet (C) | P (A vs C) |
|---|---|---|---|---|---|
| Ethylmorphine *N*-demethylase ($\mu$moles·hr$^{-1}$·mg$^{-1}$) | 0.423 ± 0.073 | 0.568 ± 0.088 | < 0.02 | 0.552 ± 0.065 | < 0.02 |
| Aniline hydroxylase (nmoles·hr$^{-1}$·mg$^{-1}$) | 10.23 ± 2.09 | 24.64 ± 7.57 | < 0.001 | 24.92 ± 6.80 | < 0.001 |
| Cytochrome P-450 (nmoles/mg) | 0.376 ± 0.030 | 0.456 ± 0.056 | < 0.02 | 0.530 ± 0.081 | < 0.01 |
| Cytochrome $b_5$ (nmoles/mg) | 0.147 ± 0.021 | 0.170 ± 0.029† | NS‡ | 0.246 ± 0.029† | < 0.001 |
| HMG-CoA reductase (pmoles·min$^{-1}$·mg$^{-1}$) | 31.9 ± 18.2 | 32.1 ± 10.1† | NS | 56.1 ± 9.0† | < 0.05 |

* Composition of diets is given in Materials and Methods. Groups of six rats were fed each diet for 5 weeks.
† Rats fed diet B differ from rats fed diet C with $P < 0.01$. ‡ Not significant.

Table 3. Average benzo[*a*]pyrene hydroxylase activity in the small intestinal mucosa of rats fed a purified diet containing either cellulose or pectin or a cereal-based diet*

| Diet | Benzo[*a*]pyrene hydroxylase (nmoles·mg$^{-1}$·hr$^{-1}$) | | |
|---|---|---|---|
| (A) Purified with cellulose | 0.13 ± 0.12 | NS† | P < 0.001 |
| (B) Purified with pectin | 0.11 ± 0.08 | P < 0.001 | |
| (C) Cereal-based | 1.70 ± 0.55 | | |

* Diet compositions are given in Materials and Methods. Six rats were fed each diet for 5 weeks.
† Not significant.

In comparing results obtained from animals fed the purified diets, which contained either 10.4% cellulose or pectin, with those from animals fed the cereal-based NIH-07 diet, it should be noted that the fiber content of the latter is not directly comparable to that of the two purified diets. The cereal-based diet contained 4.5% "crude fiber", which refers to the weighed residue of the diet after extraction with acid, alkali, alcohol, and ether [38]. Measurement of crude fiber in this manner substantially underestimates total dietary fiber in that it recovers only 50–80 per cent of the cellulose, 10–50 per cent of the lignin, and approximately 20 per cent of the hemicellulose [38, 39]. Pectins especially are readily soluble in hot aqueous solutions [40] and are therefore not quantitated by this method for assessing dietary fiber. Thus, the 4.5% crude fiber content is an underestimate of the actual amount of dietary fiber in the NIH-07 diet; in an attempt to compensate for this, the purified diets were formulated with 10.4% of either cellulose or pectin. Because of these differences in amount and types of dietary fiber, comparisons between the rats fed the purified or cereal-based diets must be interpreted cautiously. On the other hand, the two purified diets were identical except for the source of dietary fiber, and comparisons between the rats fed these diets are valid.

Our results demonstrate that the increase in hepatic mixed-function oxidase activity that occurred when pectin was substituted for cellulose in the purified diet was specific to some degree for the cytochrome P-450 system since the activity of another hepatic microsomal enzyme, HMG-CoA reductase, was unchanged. In our study these two major enzymatic systems of the endoplasmic reticulum were not altered in a parallel manner by dietary fiber. This is perhaps not surprising since HMG-CoA reductase is regulated largely by the enterohepatic circulation of cholesterol and bile salts [11], which have not been shown to affect substantially drug metabolism by cytochrome P-450 [13].

The absence of a difference in hepatic HMG-CoA reductase activity in rats fed the pectin and cellulose diets is opposite to the results of Reiser *et al.* [41] but consistent with the findings of Kelley and Tsai [42]. Differences in the chemical properties of the pectin or cellulose employed in this and previous studies [41, 42] are a possible explanation for these discrepancies, inasmuch as Wells and Ershoff [43, 44] showed that the response of plasma cholesterol levels to pectin feeding in rats is influenced by the methoxyl content of added dietary pectin and, possibly, by the molecular weight and viscosity of the pectin preparations. The previous studies concerning the effects of dietary fibers on hepatic cholesterol synthesis [41, 42] did not provide sufficient information concerning the chemical composition of their dietary cellulose and pectin preparations to determine if this is a reasonable explanation for the differing results. Whether differences in chemical or physical properties of different types of pectin or cellulose may also affect the mixed-function oxidase system for drug metabolism is not yet known.

Previous studies have shown that a number of food components can influence drug oxidations in animals [45] and in humans [46, 47]. Although dietary fiber is known to influence drug absorption [48], its effects on drug oxidation rates, to our knowledge, have not been examined previously. The present studies show that two different types of dietary fiber can have distinct influences on hepatic drug metabolism, and they suggest that such effects should be considered when designing diets for investigations of drug metabolism in laboratory animals. Moreover, our results have broader implications, in that the microsomal mixed-function oxidase system can both activate and inactivate many exogenous chemicals as well as endogenous compounds, including steroid hormones, and the balance between these processes is an important determinant of the ultimate action of such chemicals in the host [49]. Therefore, our studies suggest that a change in dietary fiber content, e.g. substituting pectin for cellulose as the fiber source, has the potential to alter host response to endogenous compounds and to environmental carcinogens and toxins.

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## REFERENCES


1. B. Morgan, M. Heald, S. D. Atkin and J. Green, *Br. J. Nutr.* **32**, 447 (1974).
2. D. Mathe, C. Lutton, J. Rautureau, T. Coste, E. Gouffier, J. J. Sulpice and F. Chevallier, *J. Nutr.* **107**, 446 (1977).
3. D. Kritchevsky, *Am. J. clin. Nutr.* **30**, 979 (1977).
4. D. J. A. Jenkins, A. R. Leeds, M. A. Gassull, B. Cochet and K. G. M. M. Alberti, *Ann. intern. Med.* **86**, 20 (1977).
5. D. P. Burkitt, *Am. J. clin. Nutr.* **31**, s58 (1978).
6. D. Kritchevsky, *Fedn. Proc.* **36**, 1692 (1977).
7. R. R. Brown, J. A. Miller and E. C. Miller, *J. biol. Chem.* **209**, 211 (1954).
8. L. W. Wattenberg, *Cancer* **28**, 99 (1971).
9. L. W. Wattenberg, *Toxic. appl. Pharmac.* **23**, 741 (1972).
10. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
11. V. W. Rodwell, J. L. Nordstrom and J. J. Mitschelen, *Adv. Lipid Res.* **14**, 1 (1976).
12. D. R. Abernethy and D. L. Azarnoff, *Biochem. Pharmac.* **26**, 481 (1977).
13. K. A. Mitropoulos and S. Balasubramaniam, in *Bile Acid Metabolism in Health Disease* (Eds. G. Paumgartner and A. Stiehl), Chap. 3, University Park Press, Baltimore (1977).
14. B. L. Walker, in *Analysis of Lipids and Lipoproteins* (Ed. E. G. Perkins), Chap. 17. American Oil Chemists' Society, Champaign, IL (1975).
15. T. A. Miettinen, A. Proia and D. J. McNamara, *J. Lipid Res.*, **22**, 485 (1981).
16. J. G. Bieri, G. S. Stoewsand, G. M. Briggs, R. W. Phillips, J. C. Woodward and J. J. Knapka, *J. Nutr.* **107**, 1340 (1977).
17. W. D. Block, K. J. Jarrett and J. B. Levine, in *Automation in Analytical Chemistry* (Ed. L. T. Skeggs, Jr.) p. 345. Mediad, New York (1966).
18. G. Kessler and H. Lederer, in *Automation in Analytical Chemistry* (Ed. L. T. Skeggs Jr.), p. 341. Mediad, New York (1966).
19. R. J. Wilkens and A. I. Hurvitz, *J. Am. Anim. Hosp. Ass.* **11**, 29 (1975).
20. D. J. Shapiro and V. W. Rodwell, *J. biol. Chem.* **246**, 3210 (1971).
21. D. J. McNamara, F. W. Quackenbush and V. W. Rodwell, *J. biol. Chem.* **247**, 5805 (1972).
22. J. L. Nordstrom, V. W. Rodwell and J. J. Mitschelen, *J. biol. Chem.* **252**, 8924 (1977).
23. J. M. Tredger and R. S. Chhabra, *Drug Metab. Dispos.* **4**, 451 (1976).
24. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
25. D. J. Shapiro, J. L. Nordstrom, J. J. Mitschelen, V. W. Rodwell and R. T. Schimke, *Biochim. biophys. Acta* **370**, 369 (1974).
26. A. Proia, *Ph.D. Dissertation,* The Rockefeller University, New York (1979).
27. A. P. Alvares and G. J. Mannering, *Molec. Pharmac.* **6**, 206 (1970).
28. C. L. Litterst, E. G. Mimnaugh, R. L. Reagan and T. E. Gram, *Biochem. Pharmac.* **23**, 2391 (1974).
29. M. A. Correia and G. J. Mannering, *Molec. Pharmac.* **9**, 455 (1973).
30. M. W. Anders and G. J. Mannering, *Molec. Pharmac.* **2**, 319 (1966).
31. T. Nash, *Biochem. J.* **55**, 416 (1953).
32. Y. Imai, A. Ito and R. Sato, *J. Biochem., Tokyo* **60**, 417 (1966).
33. B. B. Brodie and J. Axelrod, *J. Pharmac. exp. Ther.* **94**, 22 (1948).
34. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
35. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
36. J. H. Zar, *Biostatistical Analysis,* p. 101. Prentice-Hall, Englewood Cliffs, NJ (1974).
37. R. Kato, *Xenobiotica* **7**, 25 (1977).
38. J. H. Cummings, in *Fiber in Human Nutrition* (Eds. G. A. Spiller and R. J. Amen), Chap. 1. Plenum Press, New York (1976).
39. P. J. Van Soest, *Am. J. clin. Nutr.* **31**, s12 (1978).
40. R. W. Bailey, A. Chessan and J. Monro, *Am. J. clin. Nutr.* **31**, s77 (1978).
41. R. Reiser, G. R. Henderson, B. C. O'Brien and J. Thomas, *J. Nutr.* **107**, 453 (1977).
42. J. J. Kelley and A. C. Tsai, *J. Nutr.* **108**, 630 (1978).
43. A. F. Wells and B. H. Ershoff, *J. Nutr.* **74**, 87 (1961).
44. B. H. Ershoff and A. F. Wells, *Expl. Med. Surg.* **20**, 272 (1962).
45. T. C. Campbell and J. R. Hayes, *Pharmac. Rev.* **26**, 171 (1974).
46. K. E. Anderson, A. H. Conney and A. Kappas, *Clin. Pharmac. Ther.* **26**, 493 (1979).
47. A. Kappas, A. P. Alvares, K. E. Anderson, W. A. Garland, E. J. Pantuck and A. H. Conney, in *Microsomes and Drug Oxidations* (Eds. V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook and A. H. Conney), p. 703. Pergamon Press, Oxford (1977).
48. R. C. Brown, J. Kelleher, B. E. Walker and M. S. Losowsky, *Gut* **18**, A946 (1977).
49. J. A. Miller and E. C. Miller, *Fedn. Proc.* **35**, 1316 (1976).