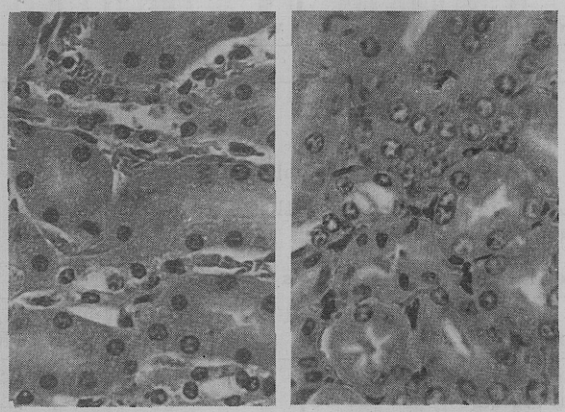

Figure 1. Right, nephrocytomegalia in mice kidney (400X). Left, the kidney of control animals (400X).

media were able to utilize equally as well lysinoalanine when added in a lysine equivalent amount.

The ability of *T. pyriformis* W to utilize lysinoalanine instead of L-lysine diminishes its value as a test for protein quality in processed foods containing lysinoalanine. The growth response of mice, which have the same essential amino acid requirements as rats and humans (Bauer and Berg, 1943), may indicate the inability of the latter two species to significantly utilize lysinoalanine when challenged with a lysine limited protein.

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Interaction of Wheat Bran with Nitrosamines and with Amines during Nitrosation

Nitrosamines are adsorbed by wheat bran, and the extent of binding is apparently related to the structure of the nitrosamine. The rate of formation of *N*-nitrosodipropylamine is increased when the nitrosation of dipropylamine is carried out in the presence of bran.

The intake of fiber from wheat foods in the Western diet has undergone a substantial decline during the past century (Heller and Hackler, 1978; Southgate and Bingham, 1978), and this decline has been associated, epidemiologically, with some of the major digestive diseases, including bowel cancer (Walker and Burkitt, 1976; Trowell, 1976).

Wheat bran appears to effect the rate of absorption and metabolism of dietary fat, carbohydrate, and protein, and to alter sterol metabolism and mineral balance (Kimura, 1977; Cummings, 1978). Dietary fiber can also counteract the effects of a wide range of toxic compounds (Ershoff, 1976) and can, in some cases, accelerate the metabolism of these substances (Chadwick et al., 1978). Wheat bran has recently been claimed to protect rats against colonic cancer induced by cycasin analogues (Fleiser et al., 1978).

A common inference drawn from such experiments is that these beneficial properties of fiber can be associated with a bulking effect on feces and a consequent reduction of mucosal exposure to carcinogens and cocarcinogens in the intestinal lumen (Burkitt et al., 1972). Not all observations, however, are consistent with the dilution/contact hypotheses; interactions on the surface of fiber particles, for example, may be involved.

It therefore appears necessary to explore some of the physicochemical properties of fibrous materials which might be associated with these various biochemical and physiological phenomena and thereby affect the digestive and absorptive functions.

Several experiments have now shown that carcinogenic nitrosamines will form readily, under physiological conditions, from amines and nitrite (Mysliwy et al., 1974; Tannenbaum et al., 1978a). The large intestine, in addition, contains relatively high levels of nitrite (Tannenbaum et al., 1978b), and nitrosatable amines can enter the intestinal tract via a number of routes including diet. These observations together suggest that nitrosamines may be responsible in part for the initiation of intestinal cancer,

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Table I. Binding of Nitrosamines to Bran

compd[a]	concentration, μmol/mL[b] control	bran	% control
NDPA	0.54 ± 0.01	0.51 ± 0.02	94
NDBA	0.70 ± 0.02	0.47 ± 0.02	67
NP	0.52 ± 0.02	0.49 ± 0.01	94
NM	0.61 ± 0.02	0.57 ± 0.01	93

[a] NDPA = *N*-nitrosodipropylamine, NDBA = *N*-nitrosodibutylamine, NP = *N*-nitrosopyrrolidine, NM = *N*-nitrosomorpholine. [b] Mean ± SE (n = 9).

and we have consequently begun an investigation of possible interactions of fiber with preformed nitrosamines and with the reactions of amines and nitrite to form nitrosamines.

Initial experiments have been carried out to measure the extent of binding of a representative set of nitrosamines to a standard bran and to assess the effect of bran on the rate of formation of nitrosamines from amines and nitrite.

EXPERIMENTAL SECTION

Chemicals. *N*-Nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopyrrolidine (NP), *N*-nitrosomorpholine (NM), dipropylamine, morpholine, pyrrolidine, and sodium nitrite were commercial reagent grade materials and were used without further purification. The amines and nitrosamines were examined by gas chromatography with a nitrosamine-specific detector. No *N*-nitrosamine impurities were present at the levels involved in these experiments.

Citrate–phosphate buffers were prepared as described by McIlvane (1921).

The wheat bran, standardized and extensively analyzed for various components such as crude fiber and minerals, was obtained from the American Association of Cereal Chemists, St. Paul, MN.

Analyses. All analyses were performed on a Varian Model 204 gas chromatograph equipped with detection by thermal energy analysis (TEA; Fine et al., 1973). Columns were stainless steel packed with Carbowax 20M/TPA/KOH, with OV-17, or with SP1000. Helium was the carrier gas.

Binding Studies. These experiments were carried out with buffered solutions (citrate–phosphate; pH ~7) containing NDPA, NDBA, NP, and NM and were run as nine sets of experiment/control pairs. The controls were 10-mL aliquots of the nitrosamine solution and the experimental mixtures were 10-mL aliquots of the same solution but containing 200 mg of the standard bran. The solutions were initially incubated at 37 °C for 2 h or 24 h and then analyzed by direct injection into the gas chromatograph. There were no significant differences between the 2 h and the 24 h experiments, and there was no difference when the bran was presoaked before the nitrosamines were added. Subsequent experiments were consequently done without presoaking and for only 2 h.

Nitrosation Reactions. As above, these experiments were carried out as paired sets (n = 6) of experiment and control. Dipropylamine, morpholine, and pyrrolidine were dissolved in buffer (~10 μL each in 50 mL) and the pH was adjusted to ~6 with dilute HCl. Five milliliters of aqueous $NaNO_2$ (200 mg in 50 mL of buffer) was added to 200 mg of standard bran, the pH was adjusted to ~6, and then 5 mL of the amine solution was added either to the bran-containing solutions or to 5 mL of nitrite-buffer alone. The resulting mixtures were incubated at 37 °C for 2 h and then extracted with methylene chloride. The methylene chloride extracts were then analyzed for nitrosamines using GC–TEA.

Table II. Effect of Bran on Nitrosation of Amines

compd[a]	amount formed,[b] μmol/mL[c] control	bran	% control
NDPA	0.08 ± 0.02	0.3 ± 0.04	375
NP	ND	ND	
NM	1.5 ± 0.04	1.5 ± 0.1	100

[a] NDPA = *N*-nitrosodipropylamine, NP = *N*-nitrosopyrrolidine, NM = *N*-nitrosomorpholine. [b] Reaction time = 2 h. [c] Mean ± SE (n = 6). [d] ND = none detected (c < 0.01 μmol/mL).

RESULTS AND DISCUSSION

The results of these experiments are summarized in Tables I and II. In the binding experiments, the recovery of NDBA from the solutions containing bran was significantly lower than from the control solutions. The recovery of the other nitrosamines was also lower than from the controls but these differences, although statistically significant (paired t tests; 95% confidence level), were not nearly as striking as with NDBA.

The hexane–water partition coefficient for NDBA is much larger than those for the other compounds (Druckrey et al., 1967), and this suggests that the binding to bran may involve a nonspecific hydrophobic interaction similar to that reported for bile acids and fiber (Eastwood and Hamilton, 1968; Kritchevsky and Story, 1974).

An effect of bran on the amine nitrosation reaction was apparent only in the case of NDPA. No NP was detected in either system, and similar large amounts of NM were formed in both systems. The formation of NDPA was significantly enhanced by the bran (paired t tests; 95% confidence level).

These results indicate that dietary fiber can indeed interact with nitrosamines both during and after their formation and suggest that the extent of these interactions may depend on the structures of the amines and nitrosamines. The binding interactions appear to be related to partition coefficients. Structure–activity relationships in the nitrosamine series indicate that the more hydrophobic compounds may be less carcinogenic (Wishnok et al., 1978; Wishnok and Archer, 1976); if the intestine is in fact protected from exposure to nitrosamines by the binding of these compounds to fiber, then this protection may be less effective with the more potent carcinogens.

Within the set of nitrosamines tested, there does not appear to be a structurally systematic effect of fiber on the rate of nitrosamine formation.

We plan to extend our investigation to a large sample of amines and nitrosamines and to other types of fiber in order to clarify the details of these interactions.

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Biopotency of Vitamin A in Fortified Flour after Accelerated Storage

The biopotency and availability of the stabilized vitamin A remaining in a fortified flour after storage under accelerated conditions (40 or 45 °C) were studied by estimating relative biopotency by a maleic anhydride procedure and by comparing growth and body storage of vitamin A in rats fed diets containing the stored or fresh flours. The quantity of vitamin A remaining in the stored flour, as determined colorimetrically, had practically the same biopotency as when originally added and was fully available to rats as a source of vitamin A for growth and body storage.

In 1974 the Food and Nutrition Board of the National Academy of Sciences proposed a new fortification policy for cereal-grain products to replace that adopted in 1943 (*Federal Register*, 1943) by adding vitamin A, pyridoxine, folic acid, calcium, magnesium, and zinc and increasing the amount of iron (National Academy of Sciences, 1974). Before a decision could be made on implementation of the proposal it was necessary to prepare and test the fortified products under appropriate conditions of manufacture, storage, and use.

When fortified flours, which contained 0.28 mg of retinol equivalents/100 g along with the other added nutrients, were stored under accelerated conditions at 40 °C, they lost about 30% of the stabilized vitamin A in 3 months (Parrish et al., 1978) and 45% in 6 months. But there was a question whether the remaining vitamin A, as determined colorimetrically (Association of Official Analytical Chemists, 1975), was bioactive and available to meet the vitamin A requirements or whether it had undergone isomerization or degradation so that the analytical values overestimated the actual vitamin A activity (Parrish, 1977).

This is a report on relative biopotency and bioavailability of stabilized vitamin A in stored fortified flour as measured by two different methods: (1) estimation of relative biopotency by the maleic anhydride procedure (Ames and Lehman, 1960) and (2) growth of rats and storage of vitamin A by those rats when fed diets containing the fortified flour. Although there are reports on losses of vitamin A in other stored fortified flours, as determined by standard analytical procedures, nothing was found on the biopotency of the stabilized vitamin A remaining in the flour.

EXPERIMENTAL SECTION

Fortified bread flour, 9.9% protein, was stored for 6 months at 40 °C and then placed in a freezer until used for the feeding trial. Vitamin and mineral additions (Table I), plus amounts naturally in the flour, brought contents of those nutrients to the proposed fortification levels, with overages of 5–20% to provide for normal manufacturing and storage losses (American Bakers Association Inter-Industry Committee, 1976).

Table I. Vitamin-Mineral Additions to Flour (g/100 kg)[a]

thiamin mononitrate	0.565
riboflavin	0.396
niacinamide	4.62
pyridoxine hydrochloride	0.44
folic acid	0.057
vitamin A palmitate, 250-SD	4.4
tricalcium phosphate	0.66
reduced iron	2.52
calcium sulfate	665
magnesium oxide	44
zinc oxide	2.1

[a] All ingredients listed, except the last three, were added as a premix in a starch base, 40% of premix weight.

The original proposed fortification for vitamin A of 0.48 g/100 g was determined to be too high and was changed by the panel to 0.28 mg/100 g of retinol equivalents (Hepburn, 1976); that level was used in this study. Iron fortification was not increased to 8.81 mg/100 g because approval of an increase seemed unlikely (*Federal Register*, 1977). Vitamin A was added as stabilized vitamin A palmitate, 250-SD (Hoffman-LaRoche). Because of overages in the vitamin A product, premix, and flour, vitamin A content by analysis (Association of Official Analytical Chemists, 1975) was about 25% higher than the proposed level. All vitamin A data in this report are based on analytical values.

To provide sufficient vitamin A for a good test of estimated biopotency by the maleic anhydride procedure (Ames and Lehman, 1960), vitamin A palmitate, 250-SD,

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