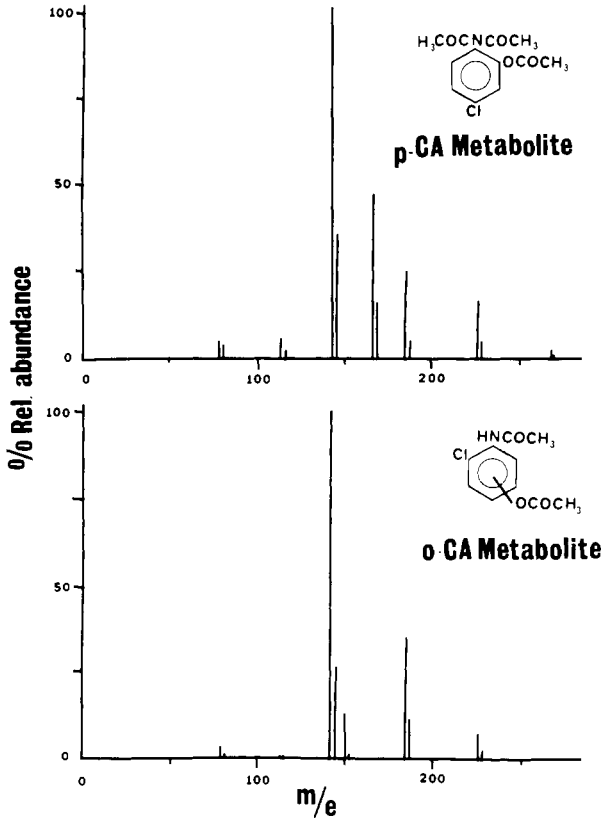

Figure 6. Mass spectral fragmentation pattern of acylated 4-chloro- and 2-chloroaniline metabolites.

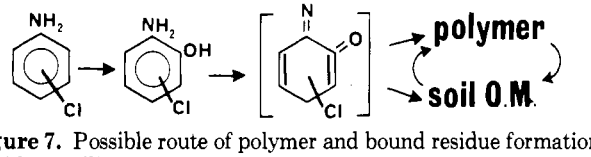

Figure 7. Possible route of polymer and bound residue formation of chloroanilines.

ronmental significance of that reaction (Figure 7). *o*-Aminophenols are relatively unstable molecules in that they can form quinonide structures which are very reactive in condensation and polymerization reactions and with the organic materials in soils. This is evidenced by the fact that there is a steady loss of parent *o*-aminophenol with time after extraction, and formation of more polar, most likely polymerized products, when analyzed by TLC. Interactions of aminophenols with soil organic matter would account for some of the soil-bound residues observed from aniline-based pesticides.

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Growth Response of Mice and *Tetrahymena Pyriformis* to Lysinoalanine-Supplemented Wheat Gluten

Tetrahymena pyriformis, a microorganism suggested for determinations of protein nutritional value, is capable of utilizing lysinoalanine instead of L-lysine. Similarly, L-lysine-dependent mutants of *Escherichia coli*, *Bacillus subtilis*, and *Aspergillus niger* can grow on media where L-lysine was replaced with lysinoalanine. In contrast, the poor growth response of mice indicated their inability to significantly utilize lysinoalanine when challenged with a wheat gluten–lysinoalanine diet.

Recent interest in lysinoalanine was spurred by the controversial detection of kidney lesions, called nephrocytomegalia, in rats fed a diet containing this amino acid (O'Donovan, 1976; Struthers et al., 1978). Lysinoalanine [N^{ϵ}-(DL-2-amino-2-carboxyethyl)-L-lysine] appears in proteins subjected to specific conditions of heating (Sternberg et al., 1975) or alkali treatment (Bohak, 1964; Patchornick and Sokolowski, 1964), associated with a decrease of cystinyl, lysyl, and/or seryl residues by β elimination and condensation reactions (Bohak, 1964; Nashef et al., 1977).

Alkali treatment of casein, peanut meal, and soya protein caused a decrease of protein nutritional quality as determined by net protein utilization (NPU) in rats (deGroot and Slump, 1969); however, no direct correlation was made between the NPU decrease and appearance of

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lysinoalanine in the protein. Alkali treatment of proteins may induce other changes beside the formation of lysinoalanine, such as, loss of amino acids, racemizations and intra- or intercatenary cross-links, contributing to the observed decrease of nutritional value (Provansal et al., 1975).

There is no published work on the effect of added lysinoalanine on the nutritional value of a protein in test systems. In this communication we are reporting the growth response of *Tetrahymena pyriformis* and mice to diets containing wheat gluten and lysinoalanine. We decided to investigate *T. pyriformis* because this protozoan has been suggested for evaluating protein quality in humans (Hackler, 1977). Mice were selected in preference to rats because they are less sensitive to the nephrotoxic effect elicited by dietary lysinoalanine. In both instances we wanted to see if the nutritional value of wheat gluten will be affected by lysinoalanine additions and if the test organisms are capable of distinguishing between the essential amino acid lysine and its possible reaction product, lysinoalanine. Finally, wheat gluten seemed an attractive protein because it is isolated by a mild procedure unlikely to affect any of its amino acids and has a low lysine content which makes it convenient to be used for amino acid supplementation studies.

MATERIALS AND METHODS

Tetrahymena pyriformis W from the American Type Culture Collection No. 10542 was propagated on the media of Stott et al. (1963) with the modification and technique of Landers (1975). The number of cells counted in each sample with a hemocytometer counter chamber were expressed as relative nutritive value (RNV):

$$\text{RNV} = \frac{\text{count at 0.2 mg of N/mL for test protein}}{\text{count at 0.2 mg of N/mL for casein}} \times 100$$

The RNV was then correlated with the protein efficiency ratio (PER) by running the *T. pyriformis* assay on protein samples of known PER determined by the rat assay and plotting a standard curve of RNV vs. PER. The standard curve was used to express the PER values reported here using the RNV obtained by the *T. pyriformis* assay.

Mice, all male, Charles River CF-1, 18–21 days old, were kept at 70–72 °F and 50–55% humidity. Groups of ten mice were kept in common and fed a diet of 10% protein, 70% sucrose, 8% cottonseed oil, 5% sodium chloride, 1% alpha-cel fiber from Nutritional Biochemicals Company, 1% vitamin mix (AOAC-39.166), and 5% added water. After 21 days the total weight gain of one group and total protein intake were expressed as the protein efficiency ratio (PER):

$$\text{PER} = \frac{\text{wt gain of 10 mice after 21 days}}{\text{protein intake in the same time interval}}$$

Weighing of the mice was done as a group and determination of the food intake was made after careful separation of debris from spilled food. The sucrose amount in the diet containing wheat gluten was reduced to 67.5% to compensate for the carbohydrate content in the gluten. Casein was ANRC reference from Humko-Sheffield. Vital wheat gluten "Viking-1" from Paniplus Co. contained 80% protein and 20% carbohydrate. L-Lysine hydrochloride, from Mallinkrodt, was added as 4.96% vs. the protein which corresponds to 4% lysine. Lysinoalanine dihydrochloride was synthesized in the laboratory (Okuda and Zahn, 1965), added as 8.35% to the protein content of the diet which corresponds to 4% in terms of the lysine moiety. Lysinoalanine contained less than 0.3% lysine as determined by automated amino acid analysis.

Table I. PER Determinations with *T. pyriformis*

protein	PER
casein	2.50
wheat gluten	0.65
wheat gluten + lysine	1.62
wheat gluten + lysinoalanine[a]	1.19

[a] Lysine impurity in the synthesized lysinolanine is less than 0.3%.

Table II. PER Determinations with Mice

protein	group wt grain, g	feed consumed,[a] g	PER
casein	92	1493	0.62
wheat gluten	86	1657	0.52
wheat gluten + lysine	128	1502	0.85
wheat gluten + lysinoalanine	51	1654	0.31

[a] Feed contains 10% of protein.

RESULTS AND DISCUSSION

The PER determinations with *T. pyriformis* presented in Table I gave a value of 0.65 for wheat gluten which is in agreement with data determined by the rat bioassay. Thus Takeuchi and Muramatsu (1971) reported a PER of 0.8 ± 0.1 for a protein concentration in the diet of 9.2% while Jansen (1962) found a PER of 0.5. When 3.4% lysine monohydrochloride was added to the diet in Jensen's experiment, the PER increased to 1.68, favorably comparing to PER 1.62 found in our tests with *T. pyriformis*. Growth of mice also benefited when wheat gluten was supplemented with lysine monohydrochloride as reflected by an increase from 0.52 to 0.85 (Table II).

The response, however, to lysinoalanine addition diverged in the two examined species. The PER of mice dropped to 0.31, below the value of 0.52 given by unsupplemented wheat gluten, though feed consumption was identical. A toxic effect of lysinoalanine manifested by nephrocytomegalia could be detected on microscopic examination of kidney sections. Mice are known to be much less sensitive than rats to lysinoalanine added to their diet. Feron et al. (1978) reported that 100 ppm lysinoalanine in the diet causes, after 4 weeks of feeding, slight to moderate nephrocytomegalia in male and female rats, whereas Swiss mice showed a minimal degree of the same renal change only after being fed for 4–8 weeks 10 000 ppm lysinoalanine. Our level of feeding was 8350 ppm lysinoalanine dihydrochloride, and nephrocytomegalic changes were obvious after 3 weeks (Figure 1).

In contrast to mice, *T. pyriformis* can utilize, at least in part, lysinoalanine instead of lysine. The number of organisms on gluten media expressed as the PER was 0.65, increasing to 1.19 when lysinoalanine was supplemented to the gluten as compared to an increase of the PER to 1.62 obtained by addition of L-lysine to the gluten. The ability of *T. pyriformis* to respond to lysine additions to defatted ground wheat by improved multiplying was reported (Stott et al., 1963) and is due to the fact that L-lysine is an essential amino acid (Kidder and Dewey, 1951) for its development. Conceivably *T. pyriformis* is able to metabolize at least one of the isomers present in the synthetic LL, DL-lysinoalanine either into L-lysine or to a product of the L-lysine pathway. This property was found by us in other microorganisms as well. *Escherichia coli* J5, *Bacillus subtilis* 168, and *Aspergillus niger* 1794.38 mutants requiring lysine for their growth on synthetic

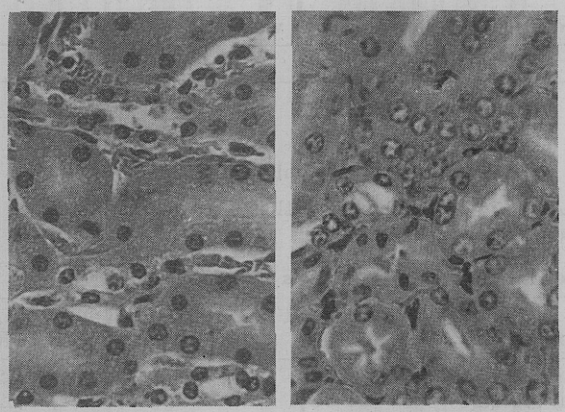

Figure 1. Right, nephrocytomegalia in mice kidney (400X). Left, the kidney of control animals (400X).

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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