Low-level mixtures of the toxic components of fungus-infected fescue, along with environmental stress conditions, may be a more insidious detriment to animals than "toxic levels" of the individual compounds. It is unknown at present what the combined effects of 1-halostachine, harmane, and norharmane (found in tall fescue) (Davis and Camp, 1983) and the ergot alkaloids produced by *E. typhina* in the grass (Porter et al., 1981; Plattner and Yates, 1984) are to the animal. The monoamine oxidase activity of the β-carbolines could conceivably potentiate both the pressor activity of halostachine (Davis and Camp, 1983) and the activity of the ergot alkaloids (i.e., ergovaline). Then, too, the combined activities of the loline and perloline alkaloids produced by the grass must be considered (Robbins et al., 1972; Jackson et al., 1984).

The results of this study indicate that monitoring prolactin, catecholamine levels, and catecholamine metabolites in rats is potentially a useful bioassay for toxic fungus-infected fescue. Additionally, we have developed insights into possible mechanisms of fescue toxicity. Future studies involving both the fractionation and synergistic effects of these compounds at levels at which they exist in vivo should provide more insight toward solving the problems associated with this important forage grass.

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Registry No. Prolactin, 9002-62-4; dihydroxyphenylacetic acid, 102-32-9; homovanillic acid, 306-08-1; norepinephrine, 51-41-2; dopamine, 51-61-6; 5-hydroxy-3-indoleacetic acid, 54-16-0; serotonin, 50-67-9.

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Membrane-Degrading Enzymes in the Tubers of Various Cultivars of *Solanum tuberosum*

Robert A. Moreau

Tubers of 41 potato cultivars were surveyed for phospholipase and *p*-nitrophenyl palmitate hydrolase activities. Phospholipase levels ranged from 2.2 to 29.6 μmol min^{-1} (g of fresh weight)$^{-1}$ and *p*-nitrophenyl palmitate hydrolase ranged from 0.7 to 16.3 μmol min^{-1} (g of fresh weight)$^{-1}$. There was no apparent correlation between the two enzymatic activities among the cultivars tested, thus indicating that the popular *p*-nitrophenyl palmitate hydrolase assay is not an accurate assessment of overall lipolytic activity in potato tubers. A European cultivar, Désirée, which had previously been singled out because of its low levels of lipolytic activity, was found to contain as much phospholipase and *p*-nitrophenyl palmitate hydrolase activities as many of the other cultivars.

Galliard (1970) was the first to report that homogenization of potato tubers resulted in a rapid enzymatic breakdown of endogenous phospholipids and galactolipids. Even at 0 °C, 26% of the lipids were hydrolyzed after 10 min. At least three different acyl hydrolases have been identified in potato tubers (Galliard, 1971; Hasson and Laties, 1976a; Hirayama et al., 1975; Shepard and Pitt, 1976). One European cultivar, Désirée, was reported to

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contain much lower levels of acyl hydrolase than other known cultivars (Galliard and Matthew, 1973). When isolating organelles or membranes from potato tubers, it has therefore been suggested that Désirée should be the cultivar of choice.

In this report 40 common North American cultivars were surveyed for lipolytic enzyme activity and compared with Désirée tubers from two U.S. Department of Agriculture collections. This was done to determine whether any commercially available cultivars had acyl hydrolase activity that was as low or lower than that of Désirée. All samples were tested for their capacity to degrade both a natural membrane lipid (phosphatidylcholine) and a popular synthetic substrate (*p*-nitrophenyl palmitate).

EXPERIMENTAL SECTION

Materials. Potato tubers (*Solanum tuberosum*) of each cultivar were harvested in Maine in Sept 1982 and generously provided by R. Webb, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD. In addition, separate samples of Désirée tubers were kindly provided by R. Ross, U.S. Department of Agriculture, Agricultural Research Service, Sturgeon Bay, WI. All tubers were stored at 4 °C for 6 months.

Phosphatidylcholine (dipalmitoyl-^{14}C) was obtained from New England Nuclear. All other reagents were obtained from Sigma.

Enzyme Preparation. Cores (1-cm diameter) were taken from tubers with a brass cork borer and rinsed in distilled water. Samples (4 g) were cut from the cortex and homogenized in a semimicro Waring Blendor in 40 mL of 0.1 M potassium phosphate buffer (pH 7.0) and 2 mM sodium metabisulfite at 0 °C. The homogenate was filtered through two layers of cheesecloth and centrifuged at 20000*g* for 30 min. The supernatant was collected and enzyme activities were measured within 4 h. Both enzyme activities were stable at 0 °C for at least 4 h.

Phospholipase Assay. Phospholipase activity was routinely measured by using [^{14}C]phosphatidylcholine (PC). The substrate was prepared by sonicating labeled PC (dipalmitoyl-*1*-^{14}C) and unlabeled PC in 0.4% Triton X-100 in a bath sonicator (Bransonic 12, Branson Inc., Shelton, CT) until the emulsion became crystal clear. The standard reaction mixture (1 mL) contained 1 mM PC-dipalmitoyl (50 000 dpm), 0.2% Triton X-100, 100 mM Tricine–NaOH (pH 8.5), 1 mM $CaCl_2$, and 2–5μL of enzyme. Two different enzyme concentrations were tested for each cultivar. The reaction vials were incubated in a shaking water bath (140 rpm) at 30 °C for 30 min. The enzymatic reactions were simultaneously stopped and extracted by adding 50 μL of acetic acid, 7 mL of 3:2 hexane–2-propanol (v/v), and 5 mL of 6.7% Na_2SO_4. The lipids were removed in the top phase and dried under a stream of N_2. Fifty micrograms each of oleic acid and PC (soybean) was added as carriers, and the lipid samples were spotted on 250-μm silica gel G TLC plates and developed in 70:30:1.5 hexane–diethyl ether–acetic acid (v/v/v). The free fatty acid and PC spots were visualized with I_2. The radioactivity in the two regions was measured with a Berthold LB 283 linear analyzer. The percent conversion of [^{14}C]PC to ^{14}C free fatty acid was used to calculate enzyme activity based on the initial amount of substrate (1 μmol).

Esterase Activity. The activity of *p*-nitrophenyl palmitate hydrolase (PNP hydrolase) was measured spectrophotometrically as described by Galliard and Matthew (1973). The reaction mixture (1 mL) contained 0.40 mM PNP, 0.2% Triton X-100, 50 mM potassium phosphate (pH 8.0), and 2–20 μL of enzyme. The change in absorbance at 405 nm was measured continuously for 3–4 min with a Beckman Model 35 spectrophotometer. Three different enzyme concentrations were tested for each cultivar.

Protein Assay. Samples of tuber supernatants were mixed with an equal volume of 10% trichloroacetic acid and incubated at 0 °C for 18 h. The mixture was then centrifuged at 5000*g* for 10 min. The pellet was resuspended in 0.1 N NaOH, and protein was assayed by the Lowry–Markwell procedure (Markwell et al., 1978).

RESULTS

Preliminary experiments were conducted in order to determine the optimal conditions for assaying phospholipase activity. By use of a homogenate from Katahdin tubers, maximum phospholipase activity was obtained with 1 mM PC, 0.2% Triton X-100, 100 mM Tricine–NaOH (pH 8.5), and 1 mM $CaCl_2$. Because these conditions were very similar to those of two previous studies (Hasson and Laties, 1976b; Hirayama et al., 1975), phospholipase activity in all cultivars was measured under these conditions.

Among the 41 cultivars that were obtained from Maine, total phospholipase activity ranged from 2.20 to 29.60 μmol min^{-1} (g of fresh weight)$^{-1}$ and the specific activity ranged from 0.94 to 6.43 μmol min^{-1} (mg of protein)$^{-1}$ (Table I). PNP hydrolase activity ranged from 0.66 to 16.32 μmol min^{-1} (g of fresh weight)$^{-1}$. There was no apparent correlation between PNP hydrolase and phospholipase activities. Because the PNP hydrolase and phospholipase activity in Désirée tubers was much higher than previously reported (Galliard and Matthew, 1973), a second batch of Désirée tubers was obtained from Sturgeon Bay, WI. The Désirée tubers from the Wisconsin collection had phospholipase and PNP hydrolase activities that were very similar to those in the tubers from the Maine collection.

The concentration of soluble protein ranged from 2.05 to 4.92 mg/g of fresh weight. In general, the cultivars with the lowest total phospholipase activity also had the lowest soluble protein levels, and the higher phospholipase cultivars had higher soluble protein levels.

DISCUSSION

The purpose of this study was to compare the levels of membrane-degrading enzymes in the tubers of commercially available cultivars with those of Désirée. These results indicate that, at least for the North American scientist, there is no advantage in choosing Désirée tubers over any of the North American cultivars. Among the cultivars that were surveyed, Russett Burbank had the lowest phospholipase levels. Because it has the lowest phospholipase content and because it is the most popular North American cultivar, Russett Burbank should probably be the cultivar of choice when membrane destruction needs to be minimized. However, even a tuber homogenate of Russett Burbank has the potential ability (at pH 8.5 and 30 °C) to degrade all of its membrane lipids in less than 1 min [based on an average lipid content of 1 mg/g of fresh weight as measured by Galliard (1973)]. It is therefore obvious that cultivar selection is only the first of several precautions that need to be followed to minimize membrane breakdown. Working at 0 vs. 25 °C is helpful, but it only reduces membrane hydrolysis by about 60% (Galliard, 1970; Hasson and Laties, 1976b). Freshly harvested tubers (Wardale, 1980) or tubers stored at 3 vs. 7 °C (Walcott et al., 1982) may be preferable because they contain less PNP hydrolase activity. Some organelles such as mitochondria can be sedimented fast enough to minimize membrane destruction (Hasson and Laties, 1976b). However, other subcellular fractions such as plasma membranes, endoplasmic reticulum, and Golgi vesicles

Table I. Levels of Phospholipase and *p*-Nitrophenyl Palmitate Hydrolase in Potato Tubers[a]

cultivar	phospholipase, μmol min^{-1} (g of fresh wt)$^{-1}$	phospholipase, μmol min^{-1} mg^{-1}	PNP hydrolase, μmol min^{-1} (g of fresh wt)$^{-1}$	protein, mg (g of fresh wt)$^{-1}$
Russett Burbank	2.20	0.94	2.31	2.35
Hudson	2.63	1.18	0.80	2.23
Green Mountain	3.84	1.87	0.66	2.05
Red La Sada	3.86	1.17	5.57	3.30
Lemki	4.01	1.51	2.14	2.66
Cobbler	4.27	1.37	3.59	3.12
Désirée (Wisconsin)	4.62	1.26	2.42	3.67
Désirée (Maine)	5.69	1.93	1.77	2.95
Bounty	5.87	2.41	1.59	2.44
Red Pontiac	6.27	1.97	3.62	3.19
Centennial	6.44	2.45	2.50	2.63
Butte	6.85	1.77	3.73	3.87
White Rose	7.64	3.07	1.74	2.49
Houma	7.95	2.25	3.14	3.54
Abnaki	8.01	2.86	3.06	2.80
Crystal	8.52	2.38	4.29	3.58
Bison	9.31	2.76	2.70	3.37
Campbell-12	9.44	2.83	8.34	3.34
Saco	10.09	3.01	7.97	3.35
Campbell-13	10.15	2.48	3.20	4.09
La Rouge	10.21	2.27	12.21	4.49
Buckskin	10.45	3.49	4.19	2.99
Katahdin	10.88	3.60	1.75	3.02
Superior	12.64	3.39	10.70	3.73
Dakchip	12.86	3.38	10.36	3.81
Kennebec	13.06	3.80	7.23	3.44
Wausseon	13.27	3.51	2.68	3.78
Bel Rus	13.36	3.51	6.74	3.81
Sebago	13.48	4.29	8.16	3.14
Norgold Russett	13.70	4.44	16.32	3.08
Alaska Red	13.74	3.56	2.75	3.86
Campbell-11	15.16	3.85	3.93	3.94
Norchip	15.32	4.17	5.85	3.67
Atlantic	16.93	3.59	4.05	4.72
Monoma	17.08	4.80	6.79	3.56
Ontario	17.36	4.47	6.49	3.88
Denali	20.25	4.98	4.03	4.07
Belchip	20.47	4.26	3.93	4.80
Pungo	22.62	5.76	11.38	3.93
La Chipper	23.91	5.45	6.09	4.39
Cherokee	25.85	5.25	8.02	4.92
Highlat	29.60	6.43	9.34	4.60

[a] Data represent the averages of two or three separate experiments (separate tuber samples, homogenizations, and enzyme assays) for each cultivar. The deviations from the mean were less than 30% for all cultivars.

require longer centrifugation times and are therefore nearly impossible to isolate from potato tubers without extensive membrane degradation. Another alternative is to add exogenous inhibitors to the homogenizing medium. Inhibitors such as bovine serum albumin (BSA) (Hasson and Laties, 1976b), diisopropyl fluorophosphate (Galliard, 1971; Matsuda and Hirayama, 1979), and dibucaine (Pun et al., 1980) have been reported to be effective.

PNP hydrolase activity in the Désirée tubers of this study was about 20 times higher than that reported by Galliard and Matthew (1973) or Wardale (1980). The reason for this discrepancy is not clear. Perhaps differences in cultural practices or nutrient availability in Great Britain and in the United States of America may account for this observation. Because this clone was introduced into the United States directly from Great Britain, the genotype should be unchanged. The levels of PNP hydrolase and phospholipase in the 40 other cultivars are consistent with those previously reported (Galliard and Matthew, 1973; Hasson and Laties, 1976b; Matsuda and Hirayama, 1979). The level of phospholipase activity in Désirée tubers has not previously been published; however, a recent report (Theologis and Laties, 1980) demonstrated similar rates of phospholipid breakdown in tuber slices of Désirée and Russett Burbank (12% vs. 19% loss of lipid phosphorus per 2 h, respectively).

There was no apparent correlation between phospholipase activity and PNP hydrolase activity in the 41 cultivars. This is consistent with the results of Shepard and Pitt (1976), who were able to separate phospholipase activity and PNP hydrolase activity by isoelectric focusing. These results indicate that screening potato tubers with the convenient PNP hydrolase assay used in the previous survey (Galliard and Matthew, 1973) is not an accurate assessment of overall lipolytic activity.

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Registry No. PNP-hydrolase, 9001-62-1; phospholipase, 9013-93-8.

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Attachment of *N*-Acetyl-L-methionine into Whole Soybeans and the Nutritional Consequences for the Rat

Günter Matheis, Lourminia C. Sen, Andrew J. Clifford, and John R. Whitaker*

The reactive *N*-hydroxysuccinimide ester of *N*-acetyl-L-methionine was used to attach *N*-acetyl-L-methionine (NAM) into whole soybeans. The modified soybeans contained 70% more methionine than the original soybeans. The added methionine was presumably present in the modified soybeans as NAM covalently attached to protein amino groups or other nucleophilic groups or tightly adsorbed by some noncovalent linkage since it was not removed by exhaustive dialysis. Rat feeding experiments using 10% protein diets demonstrated that NAM in the modified soybeans was biologically available, although to a lesser extent than free DL-methionine added to unmodified soybeans. The unadjusted protein efficiency ratio (PER) value of modified soybeans was 1.75 ± 0.10 compared to 1.19 ± 0.08 of control soybeans. When control soybeans were supplemented with free DL-methionine to equal the level of total methionine of modified soybeans, the PER value was increased to 2.29 ± 0.05. Part of the *N*,*N*-dimethylformamide (DMF) used to keep the *N*-hydroxysuccinimide ester of NAM soluble during the infusion procedure remained in the modified soybeans after dialysis. DMF caused a small transitory decreased growth rate of the rats during the initial 4 days.

The nutritive value of a food protein depends largely upon its ability to supply nutritionally essential amino acids that are absorbed by the gastrointestinal tract. Proteins from plants, single cells, and other less conventional sources are often of limited nutritional value because of their low content of one or more essential amino acids. As a consequence, a number of important studies have been done on food proteins to improve their nutritional value.

Supplementing foods with free essential amino acids is a common method, but it has some disadvantages (Puigserver et al., 1982). Among the disadvantages are possible losses during processing and cooking, Strecker degradation products that affect food flavor and color, and differences in the absorption rates and levels of amino acids present in proteins from that of free amino acids. The potential for improving protein quality by genetic means is promising, and seed protein quality has been improved by genetic and breeding practices (Mertz et al., 1964; Munck, 1972; Johnson and Mattern, 1978). Supplementation of cereal proteins with oilseed proteins (Sarwar et al., 1978) has also attracted considerable attention.

A variety of chemical and enzymatic methods have been applied to food proteins, or suggested for use, to improve their functional and nutritional properties (Feeney and Whitaker, 1977, 1982). Enzymatic protein degradation and resynthesis (plastein reaction) has been used, among other purposes, to incorporate essential amino acids into proteins (Fujimaki et al., 1977; Monti and Jost, 1979). Chemical methods available for covalent attachment of amino acids into proteins, via isopeptide bonds, include methods to modify carboxyl and amino groups of proteins. Limiting essential amino acids have been covalently attached to soy protein (Voutsinas and Nakai, 1979) and wheat gluten (Li-Chan et al., 1979) by enzymatic methods. A variety of amino acids have been incorporated into β-lactoglobulin (Puigserver et al., 1982) and casein (Puigserver et al., 1978, 1979a-c, 1982) by chemical methods.

Except for limiting amounts of methionine and cysteine, soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) are excellent sources of food protein (Jaffé, 1949; Kellor, 1974). Recently, the nutritional improvement of whole common bean seeds by methionine infusion has been reported (Antunes et al., 1979). However, during soaking (prior to cooking) about one-third of the infused methionine diffused out into the soaking water. Therefore, it seems desirable to bind the infused methionine strongly to the bean constituents to avoid losses during processing and cooking.

The purpose of the present work was to study the feasibility of infusing whole soybeans with the *N*-hydroxysuccinimide ester of NAM in order to covalently attach NAM to the amino groups of soybean protein and possibly other soybean constituents by the active ester method (Anderson et al., 1964). The nutritional value of soybeans modified in this way was studied in rats.

MATERIALS AND METHODS

Materials. Soybeans (No. 3585, Asgrow Seed Co., Sacramento, CA) were obtained from Professor B. S. Luh, Department of Food Science and Technology, University

Department of Food Science and Technology (G.M., L.C.S., and J.R.W.) and Department of Nutrition (A.J.C.), University of California, Davis, California 95616.

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