Excretion and Tissue Distribution of [^{14}C]Monensin in Chickens

Alvin L. Donoho,* Richard J. Herberg, Larry L. Zornes, and Robert L. Van Duyn

[^{14}C]Monensin was fed to broiler chickens at a level of 120 ppm, and edible tissues were assayed for radioactivity (RA). At a practical zero withdrawal (6 h), liver contained approximately 0.5 ppm of total RA, kidney, skin, and fat were below 0.2 ppm, and muscle was below 0.03 ppm. After a 3-day withdrawal, liver contained 0.15 ppm and other tissues contained 0.05 ppm or less. Parent monensin in fat and liver was below 0.05 ppm within 1 day off treatment. Only a small fraction of the liver RA was monensin even at zero withdrawal. The most abundant chicken liver metabolites were the same as those observed previously in cattle and rats. In a balance–excretion study [^{14}C]monensin was excreted rapidly and nearly quantitatively (mean of 94% in three chickens) in the urine and feces.

Monensin, a polyether monocarboxylic acid, is the principal factor of the antibiotic complex produced by a strain of *Streptomyces cinnamonensis* (Haney and Hoehn, 1968). The sodium salt of monensin is useful for prevention of coccidiosis in chickens (Shumard and Callender, 1968) and for increasing feed efficiency in cattle (Raun et al., 1976). Donoho and Kline (1968) used thin-layer bioautography to measure monensin in tissues of treated chickens. At zero withdrawal, fat contained 0.05–0.1 ppm of monensin, and muscle, liver, and kidney contained no detectable residue of monensin (less than 0.025 ppm). After 1-day withdrawal, fat contained no detectable residue. These results have recently been confirmed and extended by Okada et al. (1980), demonstrating that chickens fed the highest recommended level of monensin have no detectable residue of monensin (less than 0.0125 ppm) in edible tissues after 2 days of withdrawal from monensin treatment. Herberg and Van Duyn (1969) reported results of excretion and tissue distribution of radioactivity after dosing chickens with [^{3}H]monensin. These results were not definitive for describing monensin-derived residues in tissues because of tritium exchange from the [^{3}H]monensin. Data from [^{14}C]monensin excretion and tissue residue studies in cattle have been reported (Herberg et al., 1978), and metabolism of [^{14}C]monensin in the steer and rat has been described (Donoho et al., 1978). Corresponding studies with [^{14}C]monensin in chickens have not been published.

The work described in this report was undertaken to determine the excretion pattern, tissue distribution, and decline of residues of sodium [^{14}C]monensin in chickens. For convenience, throughout the rest of this paper the term monensin will be used to indicate sodium monensin.

EXPERIMENTAL SECTION

Labeled Compound. [^{14}C]Monensin was prepared by fermentation as described by Day et al. (1973) using [^{14}C]propionate as the substrate. This synthesis labeled the monensin molecule in seven positions as shown in Figure 1. Radiochemical purity was 98% for experiment TR-1, 99% for TR-2 and TR-3, and >97% for the balance studies.

Animal Feeding and Dosing. A balance-excretion experiment was conducted in which three broiler chickens approximately 8 weeks old were housed individually in metabolism cages and fed a standard broiler grower–finisher ration. Beginning 2 weeks before the labeled dose was given, the chickens received a ration containing 120 ppm of unlabeled monensin. The chickens were given a control ration on the day of [^{14}C]monensin dosing. Feeding of the unlabeled monensin was resumed the day following the labeled dose. The [^{14}C]monensin was given orally as a single dose contained in a gelatin capsule. Chicken no. 1 received 7.36 mg of [^{14}]monensin (0.0181 μCi/mg), and chicken no. 2 and chicken no. 3 received 9.99 and 8.04 mg, respectively, of [^{14}C]monensin (0.0266 μCi/mg).

Three separate tissue residue experiments were conducted in which broiler-type chickens were fed rations containing 120 ppm of [^{14}C]monensin, the highest recommended use level, to simulate actual use conditions. The rations were prepared by pipetting a chloroform or dichloromethane solution of [^{14}C]monensin onto an aliquot of feed, allowing the solvent to evaporate, and then blending that aliquot into the remainder of the ration. Hubbard × White Mountain cross broiler chickens were reared from 1 day of age on unmedicated ration. Feeding of medicated ration was initiated at approximately 7 weeks of age so the chickens would be approximately 8 weeks old at sacrifice. Muscle, liver, kidney, fat, and skin with attached subcutaneous fat were assayed for total radioactivity. Tissues from contemporary control chickens were assayed in each experiment. Information on dosing and withdrawal periods is given for each experiment under results and discussion.

Sample Preparation and Analysis. Excreta samples were collected daily in the balance–excretion experiments and were blended with enough water to give a uniform homogenate. Tissues from the residue experiments were ground and frozen until assayed. Excreta, muscle, liver, and kidney samples were assayed in triplicate or quadruplicate by combustion and liquid scintillation counting (LSC) of the trapped $^{14}CO_2$ as described by Herberg et al. (1978).

Fat and skin samples were assayed either by combustion analysis or by direct solubilization. Fat samples for solubilization (1 g) were weighed into counting vials and warmed at 50 °C for a few minutes. A 15-mL aliquot of toluene cocktail (0.5% PPO and 0.01% POPOP) was then added, and the sample was counted. Skin samples for solubilization (0.5 g) were placed in counting vials with 3 mL of NCS tissue solubilizer (Amersham/Searle, Arlington Heights, IL). The samples were held overnight at 50–55 °C, then 15 mL of toluene cocktail was added, and the samples were allowed to stand for 4–6 h prior to counting.

Monensin concentrations were determined in selected tissues by thin-layer bioautography essentially as described by Donoho and Kline (1968). Quantitation of positive experimental samples and sensitivity of detection for negative samples were determined from the response of graded levels of standard added to control tissues and assayed on the same TLC plate. This automatically com-

Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana 46140.

0021-8561/82/1430-0909$01.25/0

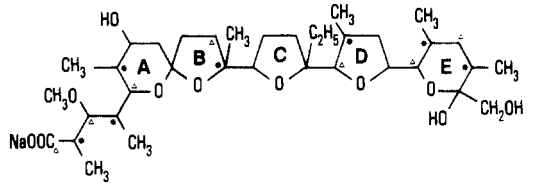

Figure 1. Structure of monensin sodium showing positions of labeling when propionate-*1*-^{14}C (△) or propionate-*2*-^{14}C (*) was used as the substrate. Experiment TR-1 used (△); experiments TR-2 and TR-3 used (*).

Table I. Daily Recovery of Radioactivity in Excreta of Chickens Given Single Oral Doses of [^{14}C]Monensin

days after dosing	radioact[a] as % of dose		
	chicken 1	chicken 2	chicken 3
1	60.8	74.6	83.1
2	7.8	9.2	4.1
3	8.3	0.5	3.8
4	2.8	1.0	2.1
5	5.7		1.7
6-12	16.1		
totals	101.5	85.3	94.8

[a] Doses were 0.133, 0.266, and 0.214 μCi for chickens 1, 2, and 3, respectively. Assays were conducted in triplicate, and values are net above the predose mean.

pensated for percent recovery of monensin through the assay procedure.

RESULTS AND DISCUSSION

Balance–Excretion Studies. The rate and extent of excretion of [^{14}C]monensin by chickens are shown in Table I. The recovery of ^{14}C averaged 94% for the three chickens. Urine and feces were not separated in these studies so the route of excretion was not determined. Similar studies with cattle (Herberg et al., 1978) and rats (Donoho et al., 1978) in which separate urine and feces data were derived demonstrated that [^{14}C]monensin radioactivity was excreted almost entirely in the feces. A similar route of excretion was suggested in chickens from the [^{3}H]monensin studies of Herberg and Van Duyn. Chickens that were surgically prepared to afford separate collection of urine and feces excreted 99% of the recovered ^{3}H in the feces.

Tissue Radioactivity Concentrations. The first experiment, TR-1, was conducted to determine what dosing interval with [^{14}C]monensin was required in chickens to obtain steady-state residues. The ration contained [^{14}C]monensin (0.202 μCi/mg) at a concentration of 109 ppm as measured by extraction and LSC. Chickens were fasted for 6 h before sacrifice to simulate a practical zero withdrawal interval. Results of this experiment are presented in Table II. The data demonstrate that a 4-day dosing period was adequate to approximate maximum tissue radioactivity. None of the tissues from the 6-day treatment group contained statistically higher residues than the 4-day treatment group (Student's t test, $P < 0.05$). Only the fat residues were statistically different, and the 6-day residues were lower than those from the 4-day treatment group. There was no indication of a difference in residues between males and females.

The second tissue residue study, TR-2, was conducted to determine the zero-time tissue radioactivity in [^{14}C]-monensin-treated chickens and to give liver and fat samples for fractionation and monensin assay. Three chickens were fed a ration containing [^{14}C]monensin (0.361 μCi/mg, measured radioactivity 111 ppm) for 5 days. These chickens were then removed from treatment and sacrificed immediately. A pool of each tissue from all three chickens was prepared, and the pools were assayed. Net radioactivity concentrations were as follows: muscle, 0.051, fat, 0.227, liver, 0.825, kidney, 0.223, and skin, 0.287 ppm of monensin equivalents. These concentrations were slightly higher but still of the same order of magnitude as experiment TR-1 in which the withdrawal time was 6 h.

The third tissue residue study, TR-3, was conducted to determine the decline rate of radioactivity in chicken tissues after withdrawal of [^{14}C]monensin. Chickens were fed a ration containing [^{14}C]monensin (0.361 μCi/mg, measured radioactivity 112 ppm) for a period of 5 days. One male and one female were sacrificed at a practical zero withdrawal as described above. The remaining chickens were transferred to unmedicated ration and then were sacrificed at various withdrawal periods. Results of this study are presented in Table III. After the recommended withdrawal period of 3 days, the mean liver residue was 0.15 ppm and muscle contained essentially no net radioactivity.

In conjunction with tissue residue experiments TR-1 and TR-3, recovery data were derived from control tissues fortified with [^{14}C]monensin and assayed in a manner identical with that of the experimental samples. Recovery

Table II. Radioactivity Levels in Edible Tissues from Experiment TR-1 in Which Chickens Were Fed [^{14}C]Monensin for either Four or Six Days and Sacrificed at a Practical Zero Withdrawal Time (Six Hours Off Treatment)

[^{14}C]-monensin dosing interval, days	chicken no., sex	net radioact, ppm of monensin equiv[a]				
		muscle	fat	liver	kidney	skin
4	3090, M	0.015	0.140	0.856	0.185	0.026
	3045, F	0.011	0.126	0.425	0.088	0.076
	3102, M	0.004	0.121	0.574	0.106	0.137
	3123, F	none[b]	0.122	0.363	0.089	0.117
	3148, F	none	0.171	0.557	0.118	0.103
	mean ± SD:	<0.010	0.136 ± 0.021	0.555 ± 0.190	0.117 ± 0.040	0.092 ± 0.043
6	3146, F	none	0.099	0.395	0.087	0.088
	3094, M	0.021	0.091	0.597	0.147	0.103
	3126, F	0.015	0.071	0.442	0.167	0.058
	3085, M	0.008	0.061	0.394	0.105	0.063
	3127, F	0.025	0.077	0.385	0.089	0.099
	3082, M	0.015	0.013	0.389	0.120	0.031
	mean ± SD:	0.014 ± 0.009	0.069 ± 0.031	0.434 ± 0.083	0.119 ± 0.032	0.074 ± 0.028

[a] Mean of four assays for muscle and three assays for other tissues. [b] Not statistically different from control (Student's t test, $P < 0.05$).

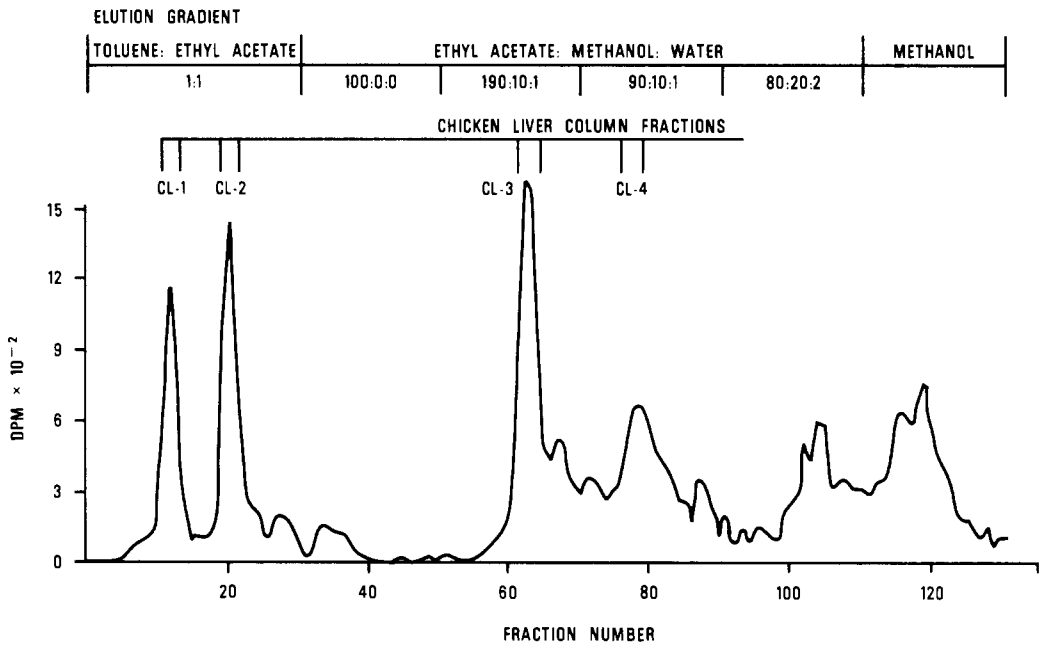

Figure 2. Gradient elution silica gel (Quantum LP-1) column chromatography of liver extract from [14C]monensin-dosed chickens. Elution was initiated with toluene and proceeded with the gradient shown.

Table III. Radioactivity Levels in Edible Tissues from Experiment TR-3 in Which Chickens Were Fed [14C]Monensin for Five Days and Then Sacrificed at Various Withdrawal Times

withdrawal time, days	chicken no., sex	net radioact, ppm of monensin equiv[a] muscle	fat	liver	kidney	skin
practical 0	4426, M	0.019	0.073	0.518	0.157	0.091
	4430, F	0.028	0.071	0.543	0.205	0.148
1	4422, M	0.012	0.113	0.343	0.093	0.065
	4431, F	0.010	0.032	0.200	0.064	0.030
2	4432, F	none[b]	0.043	0.249	0.050	0.047
	4435, F	none	0.078	0.196	0.044	0.064
	4421, M	0.012	0.077	0.221	0.077	0.062
3	4423, M	none	0.050	0.148	0.038	0.037
	4424, M	0.010	0.078	0.137	0.042	0.066
	4425, M	none	0.067	0.207	0.060	0.054
	4433, F	none	0.032	0.106	0.039	0.022
	4436, F	none	0.022	0.147	0.045	0.022
5	4428, M	none	0.081	0.107	0.034	0.045
	4429, M	none	0.040	0.136	0.048	0.025
	4434, F	none	0.029	0.076	0.024	0.027

[a] Mean of four assays. [b] Not statistically different from control (Student's t test, $P < 0.05$).

and quantitation of added [14C]monensin were good to excellent (88 to 125%) in both experiments at recovery levels of 0.1 ppm and above. The lower limit of reliable detection was in the range of 0.025–0.05 ppm as determined from the samples fortified at those levels.

Residues of Parent Monensin in Tissues. Microbiological assays for monensin activity were conducted on selected fat samples to determine the proportion of total radioactivity that could be accounted for as monensin. The pooled fat sample from chickens sacrificed at zero withdrawal (experiment TR-2) was assayed in triplicate, giving a monensin assay of 0.16 ppm. The total radioactivity in that sample was 0.227 ppm. Therefore, approximately 70% of the radioactivity was monensin. The five fat samples from the 4-day dosing group in experiment TR-1 (Table II) were also assayed, and the results were as follows: 0.046, 0.052, 0.078, and 0.116 ppm for chickens 3045, 3102, 3123, and 3148, respectively. There was none detectable in fat from chicken 3090. These assays were single determinations due to limited sample size so the results are more approximate than those from the replicated assay cited above. Single assays were also performed on the practical zero time, 1-day, and 2-day fat samples from experiment TR-3 (Table III). The results from these assays gave no detectable monensin residue in any of the samples (sensitivity of 0.05 ppm or better).

Liver samples were also assayed microbiologically or by chromatographic fractionation of radioactivity to determine the approximate monensin concentration. The pooled liver sample from the zero-time treatment (experiment TR-2) was subjected to fractionation as described in the following section. Results are shown in Figure 2. The radioactivity in the monensin fraction was less than 7% of the total liver radioactivity, giving a monensin concentration of 0.06 ppm or less. A similar fractionation of radioactivity was performed on a pool of all 11 livers from experiment TR-1. The radioactivity in the monensin fraction was less than 3% of the total in that sample, giving a monensin concentration of 0.015 ppm or less. Monensin assays were performed by bioautography on liver samples from the practical zero withdrawal and the 1-day withdrawal chickens in experiment TR-3. A pool of the two livers was assayed for each withdrawal time. No monensin residue was found in either sample (detection level of 0.05 ppm).

Results from monensin assays on the liver and fat samples demonstrate that fat contained a higher monensin residue than liver even though liver contained the highest concentration of radioactivity. For both fat and liver the monensin concentration appeared to decline rapidly after cessation of monensin treatment. Within 1-day withdrawal no monensin was detected in either tissue (detection level of 0.05 ppm).

The data from these [14C]monensin studies are in complete agreement with published reports on microbiological assay for monensin in tissues of chickens fed unlabeled monensin. Donoho and Kline (1968) found 0.05–0.1 ppm of monensin in the fat of chickens fed monensin for 8 weeks and sacrificed at zero withdrawal. No monensin was found in muscle, liver, or kidney at zero time or in fat at 1-day withdrawal (detection level of approximately 0.025 ppm). Okada et al. (1980) found 0.110 ppm of monensin

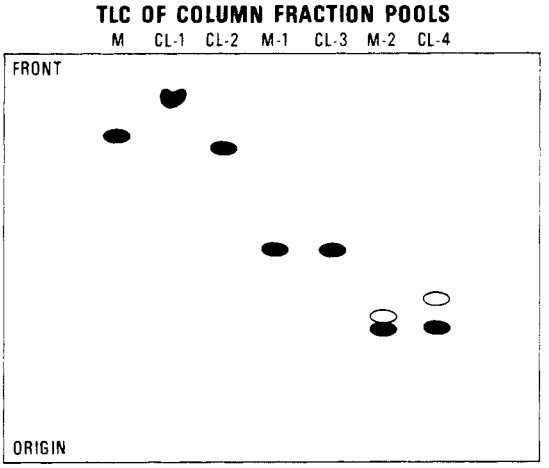

Figure 3. Tracing of TLC autoradiogram from chromatography of chicken liver (CL) column fractions on Whatman LK5 silica gel plates developed in ethyl acetate–diethyl ether–3A alcohol–water–diethylamine, 100:100:3:3.5:4. Standards of monensin (M) and metabolites M-1 and M-2 (containing a trace of M-3) were applied for comparison. Dark zones (●) and light zones (○) of radioactivity are indicated.

in fat and 0.039 ppm in liver of chickens fed 120 ppm of monensin for 9 weeks and sacrificed at zero withdrawal. At 1-day withdrawal fat contained 0.017 ppm of monensin and other tissues were negative (detection level of 0.0125 ppm).

A recent publication, Fahim et al. (1980), reports monensin concentrations far in excess of those found in the current [^{14}C]monensin studies and the microbiological assay data. For example, Fahim et al. reported that muscle from chickens fed 110 ppm of monensin for 7 days and sacrificed at zero time contained 2 ppm of monensin. This concentration is 40 times higher than the total radioactivity in muscle in the current study. Likewise, they reported 4.6 ppm of monensin in liver at zero withdrawal, a level approximately 100 times the actual monensin concentration in the current ^{14}C studies. The probable reason for this discrepancy is that the measurement system used by Fahim et al. was a nonspecific measurement subject to interference by extraneous materials unrelated to monensin.

Fractionation of Radioactivity in Liver Samples. A 100-g aliquot of the pooled liver sample from experiment TR-2 was extracted with methanol. The extract was diluted with aqueous NaCl, and the radioactivity was partitioned into CCl_4–$CHCl_3$. The sample was then subjected to HPLC on a silica gel column and visualization by TLC–autoradiography as described for feces samples by Donoho et al. (1978). Twenty-five percent of the radioactivity was not extractable, and an additional 10% remained in the aqueous methanol. Chromatography of the CCl_4–$CHCl_3$ fraction that contained approximately two-thirds of the liver radioactivity is described in Figure 2. Peaks of radioactivity identified as fraction pools CL-1, CL-2, CL-3 and CL-4 eluted from the column at the retention volumes of metabolite M-6, monensin, metabolite M-1, and metabolite M-2, respectively. Proposed structures for these metabolites were reported by Donoho et al. (1978). These four column fraction pools were applied to silica gel TLC plates along with monensin, M-1, and M-2 standards. A tracing of the autoradiogram is shown in Figure 3. These results demonstrate that the most abundant monesin metabolites in chicken liver were the same as those found in cattle liver and rat and cattle feces. No metabolite M-6 standard was available for TLC comparison with the CL-1 fraction. However, the metabolite

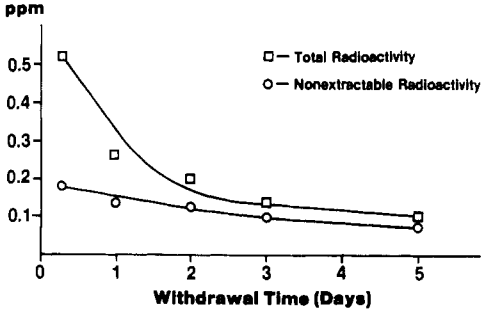

Figure 4. Plot of total radioactivity and nonextractable radioactivity in liver from [^{14}C]monensin-treated chickens at various withdrawal times.

in the CL-1 fraction had chromatographic characteristics identical with historical M-6 data in both the LC and TLC systems and is presumed to be M-6.

In addition to the work mentioned above, pooled liver samples from chickens at each of the five withdrawal periods in experiment TR-3 were assayed to determine the relative proportions of extractable and nonextractable radioactivity. A 15-g sample was extracted with two 150-mL aliquots of methanol by blending and vacuum filtration. The methanol extracts were assayed by LSC, and the spent tissue residue was dried and assayed by combustion analysis.

Results from these assays are presented in Figure 4. Total radioactivity declined over the course of the withdrawal period, but the extractable radioactivity declined more rapidly than the nonextractable radioactivity. Approximately two-thirds was extractable at zero time, but by 3 days of withdrawal less than one-third was extractable. This pattern of extractable and nonextractable liver residues is qualitatively the same as that found in preliminary studies when [^{14}C]monensin was administered to chickens by gavage (Donoho and Amundson, 1978). It is probable that the nonextractable liver radioactivity is ^{14}C that has been extensively metabolized and incorporated into natural tissue components. The same pattern of slowly depleting nonextractable radioactivity was observed in chickens which were dosed with [^{14}C]glucose and sacrificed at various withdrawal times (Donoho and Amundson, 1978). Therefore, ^{14}C radioactivity that has entered the normal metabolic pathways does give rise to a compartment of radioactivity that behaves in this manner. These results suggest that the residues of monensin and related metabolites have declined to mean concentrations of 0.050 ppm or less by 3 days of withdrawal even in liver, the tissue with the highest radioactivity levels.

ACKNOWLEDGMENT

Appreciation is expressed to R. L. Hamill for synthesis of [^{14}C]monensin and to G. W. Bewley, M. D. Copeland, L. M. Hartman, A. J. Lane, and D. E. Ruggles for technical assistance.

LITERATURE CITED

Day, L. E.; Chamberlin, J. W.; Gordee, E. Z.; Chen, S.; Gorman, M.; Hamill, R. L.; Ness, T.; Weeks, R. E.; Stroshane, R. *Antimicrob. Agents Chemother.* **1973**, *4*, 410.

Donoho, A.; Amundson, M. "Animal Health Products, Design and Evaluation"; Amercian Pharmaceutical Association: Washington, DC, 1978; p 67.

Donoho, A. L.; Kline, R. M. "Antimicrobial Agents and Chemotherapy—1967"; American Society for Microbiology: Ann Arbor, MI, 1968; p 763.

Donoho, A. L.; Manthey, J. A.; Occolowitz, J. L.; Zornes, L. L. *J. Agric. Food Chem.* **1978**, *26*, 1090.

Fahim, M.; del Valle, G.; Lattanzio, F.; Pressman, B. *Pharmacologist* **1980**, *22*, 286.

Haney, M. E., Jr.; Hoehn, M. M. "Antimicrobial Agents and Chemotherapy—1967"; American Society for Microbiology: Ann Arbor, MI, 1968; p 349.
Herberg, R.; Manthey, J.; Richardson, L.; Cooley, C.; Donoho, A. *J. Agric. Food Chem.* **1978**, *26*, 1087.
Herberg, R.; Van Duyn, R. *J. Agric. Food Chem.* **1969**, *17*, 853.
Okada, J.; Higuchi, I.; Kondo, S. *Shokuhin Eiseigaku Zasshi* **1980**, *21*, 177.
Raun, A. P.; Cooley, C. O.; Potter, E. L.; Rathmacher, R. P.; Richardson, L. R. *J. Anim. Sci.* **1976**, *43*, 670.
Shumard, R. F.; Callender, M. E. "Antimicrobial Agents and Chemotherpy—1967"; American Society for Microbiology: Ann Arbor, MI, 1968; p 369.

Received for review March 3, 1981. Revised manuscript received June 23, 1981. Accepted May 11, 1982.

Milk Casein: Inhibitor of Lipoxygenase-Catalyzed Lipid Peroxidation

Simo Laakso* and Esa-Matti Lilius

Various milk products inhibited lipoxygenase-catalyzed lipid peroxidation in a model system consisting of linoleic acid, one of the two purified soybean isozymes, and the inhibitor to be tested. The inhibition by milk was not dependent on its fat content, pasteurization, dialyzation, or heating. The inhibitory effect was associated with the isoelectrically precipitated casein fraction. This effect was verified with a commercial casein product. However, on the basis of kinetic grounds neither did the absorption of substrate to casein nor did calcium cause the inhibition. Luminometric measurements showed that casein inhibition was accompanied by a loss of chemiluminescence emission. This suggests that casein either has radical trapping properties or changes the mode of attack of the enzyme so that such radicals are not formed. The inhibition was not limited to the purified enzymes but a 50% reduction of oxygenation in various plant homogenates was achieved by 0.06–3.2% (w/w) casein supplementation.

The range of known or potential lipoxygenase inhibitors is limited. This presents a significant problem for the storage and processing of lipid-rich plant materials. Especially in cases where heat treatment is not wanted, cracking or bruising of intact tissue promotes lipoxygenase action and results in peroxidation of unsaturated fatty acids accompanied by flavor alterations and pigment destruction.

Poisons such as cyanide, azide, or *p*-(chloromercuri)-benzoate do not inhibit the enzymes (Holman, 1947). Polyunsaturated fats with trans double bonds do inhibit the enzymes (Mitsuda et al., 1967), but because their effect is competitive with the *cis,cis*-pentadiene fatty acid substrates, they are of less practical value in food processing. Although numerous reports on the inhibitory effect of thiols and divalent cations, especially Ca^{2+}, have emerged, there exists no consistent picture of their application to lipoxygenases. Currently, antioxidants are widely used as lipoxygenase inhibitors. They also inhibit the autoxidation of lipoxygenase substrates. However, a limited number of safe antioxidants remains when dealing with plant materials intended for human consumption. The development of a general lipoxygenase inhibitor with the same role as a flavor-improving additive seems an ambitious plan. A realistic approach toward this goal is the search for naturally occurring inhibitors from living tissues and biological fluids because biological systems have defense mechanisms against the damaging effects of activated forms of oxygen and free radical intermediates generated during lipoxygenase catalysis (Galliard and Chan, 1980). Consequently, the search for compounds presumably having either oxygen scavenger or general radical trapping capabilities is tempting.

Department of Biochemistry, University of Turku, 20500 Turku 50, Finland.

The motivation to study cow's milk as an inhibitor of lipid peroxidation arose during attempts to analyze various biological fluids for the content of unsaturated fatty acids by modifying the luminometric lipoxygenase assay of Lilius and Laakso (1982). In these experiments milk samples were found to inhibit the emission of chemiluminescence, suggesting that they contained a factor able to interfere with the free radical mechanism of enzymic lipid peroxidation. Therefore, in this work casein and other milk proteins were studied for their possible role as "biological inhibitors". This report gives evidence that milk casein has a direct effect on the mechanism of lipoxygenase catalysis. Milk casein stabilized suspensions of various plant seeds including soybean, green peas, wheat, rye, oats, barley, and turnip rape against their lipoxygenase-catalyzed peroxidation of unsaturated lipids.

EXPERIMENTAL SECTION

Materials. Linoleic acid (grade III) was from Fluka AG, Switzerland. Casein was the "vitamin-free" product of Sigma Chemical Co. Powdered skim milk (1% fat) was a commercial product of Kuivamaito Oy, Finland. Pasteurized and homogenized cow's milk with varying fat content (0, 2.9, and 3.5%) were commercial dairy products (Kulmala Oy, Finland). Untreated cow's milk was a mixture of five different collection tanks from various Finnish dairy farms. Dialyzed milk was prepared from 50 mL of pasteurized low-fat milk (2.9% fat) by dialysis at 8 °C for 48 h against 3 L of deionized water followed by two changes of 3 L of 50 mM sodium phosphate buffer, pH 6.5. Isoelectrically precipitated casein was obtained by adjusting the pH of a 50-mL portion of pasteurized low-fat milk (2.9% fat) to 4.6 with 0.5 N HCl and by incubating this mixture at 25 °C for 1 h. The precipitate was collected by centrifugation at 5000*g* and 25 °C for 15 min and washed with 100 mL of 50 mM sodium phosphate buffer, pH 6.5. This washed precipitate was dialyzed

0021-8561/82/1430-0913$01.25/0