PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 239–243 (1979)

Digestion and Absorption of Radioactive Elastins in Rats (40527)

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Elastin, a normal component of connective tissue, is found in various food products of our diet. However, there seems to be no study as to whether or not elastin is digested or absorbed. Recently, the degradation of [^{14}C]elastin in the peritoneal cavity of rats has been studied in our laboratories (1). The radioactive elastin was apparently degraded with most of the radioactive breakdown products appearing in the urine. The purpose of this study was to examine whether, following oral administration, rats could digest and/or absorb elastin. This information is of potential importance because; (i) desmosine-containing peptides are known to appear in the urine following catabolic breakdown of endogenous elastins and, (ii) the measurement of these urinary desmosines may be useful as an index reflecting the presence and/or extent of elastic fiber breakdown in various pathological conditions, e.g., pulmonary emphysema (2). Because the validity of this latter hypothesis depends on the assumption that endogenously catabolized elastins alone contribute to urinary desmosine excretion, we have studied the relative contribution of exogenous (dietary) sources to the urinary excretion of desmosines.

Exactly measured doses of [^{14}C]- and [^{3}H]elastin and elastase-predigested [^{14}C]- and [^{3}H]elastin as well as [^{3}H]desmosine were orally administered to rats and the excretion of radioactivity in urine and feces was determined.

Materials and methods. Two or three male Sprague–Dawley rats, weighing 380–400 g, were used for each of the following experiments: oral administration of [^{14}C]elastin, elastase-predigested [^{14}C]elastin, [^{3}H]elastin, elastase-predigested [^{3}H]elastin, and [^{3}H]desmosine as well as intraperitoneal injection of [^{3}H]desmosine. For measuring radioactivity, ^{14}C- and ^{3}H-labeled samples were quantitatively converted to carbon dioxide and water in a sample oxidizer (Model 306, Packard Instrument Co., Downers Grove, Ill.), respectively. Then, the radioactivities were measured in a liquid scintillation counter, corrected by the corresponding internal standard, and expressed as dpm. Alkaline-insoluble elastin was prepared from fresh bovine neck ligament according to the method previously described (3). The purified elastin was ground by a Wiley mill to particle size less than 38 μm by passing through a 400-mesh sieve.

[^{14}C]Elastin. [^{14}C]Elastin was prepared by methylation of the ground elastin with [^{14}C]-formaldehyde according to the method described previously (1). The preparation was washed with 0.9% NaCl solution seven times and then two times with water. Finally, [^{14}C]elastin was resuspended in water and lyophilized. Specific activity of [^{14}C]elastin was 2.8×10^5 dpm/mg.

[^{3}H]Elastin. [^{3}H]Elastin was prepared by using the ground elastin according to the method of Lent *et al.* (4). [^{3}H]Elastin was washed with water and centrifuged. The washing processes were repeated 15 times to remove background radioactivity. At the end of the washings, released background ^{3}H was less than 0.5% of the [^{3}H]elastin. The preparation of [^{3}H]elastin was then lyophilized. Specific activity of [^{3}H]elastin was 3.4×10^6 dpm/mg.

[^{3}H]Desmosine. A crude desmosine-containing fraction was prepared by fractionating a HCl-hydrolysate of bovine ligament elastin on a preparative column of Aminex A-5 resin, 7.5 × 15.5 cm (Bio-Rad Lab) according to the procedure described previously (5). Further purification was carried out by passing the desmosine-containing fractions, ca. 1–1.5 mg, on the analytical column of Beckman UR-30 resin and eluting according to the method described previously (6). The latter isolation process was repeated until 6–8 mg of pure desmosine was obtained. The fractions containing desmosine were com-

0037-9727/79/070239-05$02.00/0

bined and desalted by passing through a column (2 × 10 cm) of cellulose-phosphate (H+ form), the column was washed with 100 ml of water four times for removing salts, and desmosine was eluted with 5 ml of 2 *N* NH_4OH. Ammonia was removed by evaporating to dryness *in vacuo*. The preparation was then acidified with HCl and evaporated to dryness.

The labeling of desmosine was carried out by New England Nuclear, Boston, Massachusetts. The purified desmosine, 6.1 mg, was dissolved in 0.3 ml of glacial acetic acid and labeled with tritium by a catalyst (25 mg of Pt)-exchanging reaction with 10 Ci of tritiated water. An aliquot of [^{3}H]desmosine, 0.8 μmole, was further purified by passing through the analytical column of UR-30 resin. Aliquots of the chromatographically pure [^{3}H]desmosine were used for determination of radioactivity and the amount of desmosine. The specific activity was found to be 47.7×10^8 dpm/μmole of desmosine.

Oral and intraperitoneal administration of [^{3}H]desmosine. The [^{3}H]desmosine was administered to rats orally with 3 ml of saline containing 0.057 μmole of desmosine and 2.72×10^8 dpm, as well as injected intraperitoneally with 0.5 ml of saline containing 0.038 μmole of desmosine and 1.81×10^8 dpm. Radioactivity in fecal and urinary samples was determined.

Oral administration of the radioactive elastins. The ^{14}C- and ^{3}H-labeling of elastin seemingly produces no appreciable change in the nature of the protein, since the enzymatic susceptibility of [^{14}C]elastin (7) and [^{3}H]elastin (8) showed no significant difference from that of unlabeled elastin. A suspension of radioactive elastin, 35–45 mg in 5 ml of saline, was orally administered to rats by a syringe attached to a stomach tube. The radioactive elastin remaining in the syringe was transferred with water on a Millipore filter (pore diameter 0.45 μm) and the remaining [^{14}C]- or [^{3}H]elastin was combusted in the sample oxidizer. Radioactivity was determined, and the amount of remaining radioactive elastin was calculated. During the experiments, the rats were fed ground Purina standard rat chow *ad libitum*. Each rat was placed in a plastic metabolism cage (Maryland Plastics, N.Y.) in which urine and feces could be collected separately. The samples were collected twice daily for a total of 3 days. Fecal samples in the [^{14}C]elastin experiment were dried, weighed, and ground. ^{14}C was measured in aliquots of urinary and fecal samples after combustion in the sample oxidizer, and radioactivity was measured. At the end of the experiment, the rats were sacrificed, and the whole carcass of each rat was hydrolyzed by boiling with concentrated HCl. Radioactivity was measured in the fractions of the HCl-hydrolysate, HCl-insoluble, and crude fat according to the method described previously (1). Expiratory $^{14}CO_2$ from a rat, which had been orally administered with 31 mg of the [^{14}C]elastin suspension, was also measured for a period of 70 hr. The rat was placed in a vacuum desiccator equipped with continuous air flow; outcoming air was passed through a series of three CO_2 traps, each containing 100 ml of Carbo-Sorb II (Packard Instrument Co.). The amount of radioactivity recovered as $^{14}CO_2$ was measured in aliquots (1 ml) of Carbo-Sorb II by the liquid scintillation counter.

For the [^{3}H]elastin experiment, fecal samples were homogenized with 30 ml of water in a Virtis homogenizer. Aliquots of the homogenized fecal and urinary samples were used for determining radioactivity as described above.

Oral administration of the elastase-predigested radioactive elastins. The predigested radioactive elastins were prepared by solubilizing [^{14}C]- or [^{3}H]elastin with elastase, respectively. Elastin, 40 mg per 100 Sachar units of porcine pancreatic elastase (ESFF, Worthington Biochem. Co., Freehold, N.J.) in 5 ml of 0.1 *M* phosphate buffer at pH 8.8, was incubated at 37° for 4 hr. The soluble radioactive elastin was filtered through a Millipore filter (pore diameter 0.2 μm) and then administered orally to rats as described above.

Analysis of urinary samples for desmosines. The remaining urinary samples were combined and filtered. Then 5-ml aliquots of the samples from the [^{3}H]elastin experiments were placed in membrane tubes (Spectrapor membrane weight cutoff 3500, Spectrum Medical Industries, Los Angeles, Calif.). The samples were dialyzed against running water for 24 hr at room temperature and the radio-

activity of the dialyzable and nondialyzable fractions measured. The urinary sample was then hydrolyzed and analyzed for desmosines according to the method of Goldstein and Starcher (2), except that after paper chromatography the eluate was analyzed using a Beckman UR-30 resin column as described previously (6).

Results and discussion. Studies of elastin digestion and absorption. As shown in Tables IA and II the majority (59.3–71.5%) of orally administered radioactive elastins remained in the feces while 3.2–3.5% of the [^{14}C]elastins and 24.8–27.8% of the [^{3}H]elastins were excreted in the urine.

The effects of elastase predigestion are also presented in these tables. In the predigested [^{14}C]elastin experiments 3.5% of the radioactivity appeared in the urine and 64.4% in the feces versus 3.2 and 66.8% in the corresponding fractions when intact [^{14}C]elastin was given (Table IA). The data (Table II) for the administered predigested [^{3}H]elastin show that 24.8% of the radioactivity appeared in the urine and 71.5% in the feces versus 27.8% and 59.3% in the corresponding fractions when intact [^{3}H]elastin was given. Therefore, the predigestion of the elastins failed to enhance absorption, with similar results in both the [^{14}C]- and [^{3}H]elastin experiments. This is significant because the radioactive elastins were completely solubilized by the enzyme prior to the oral administration and were then presumably further hydrolyzed in the gastrointestinal tract of the rats by pepsin (9) as well as pancreatic elastase (10).

Although the percentage of radioactivity excreted in the feces was similar for both labels, considerably greater amounts of ^{3}H appeared in the urine (24.8–27.8%) as compared to the ^{14}C label (3.2–3.5%). We attribute a significant portion of this reduction in urinary ^{14}C excretion to losses of the label via expired gas as $^{14}CO_2$. We have previously shown that this occurs following the intraperitoneal injection of [^{14}C]elastin (1) and the data presented in Table IB show that 15.8% of the administered radioactivity was found in expired CO_2 following oral administration of [^{14}C]elastin. Loss of ^{14}C via the expired gas would also help reconcile the lower percentage recovery for ^{14}C which averaged 69% versus a 92% recovery for the ^{3}H label. The reason for the appearance of ^{14}C in expired gas probably relates to the fact that [^{14}C]-elastin was labeled as *N*-methyl groups probably at ϵ-amino groups of lysine moieties as well as N-terminal groups of the protein

TABLE IA. EXCRETION OF ^{14}C IN URINE AND FECES AFTER ORAL ADMINISTRATION OF [^{14}C]ELASTIN[a]

	[^{14}C]Elastin[b]		Elastase-predigested [^{14}C]elastin[c]	
Hours after administration	Urine (%)	Feces (%)	Urine (%)	Feces (%)
0–22	2.3	57.1	2.2	63.1
22–30	0.2	4.6	0.4	0.6
30–46	0.4	4.8	0.4	0.4
46–54	0.1	0.1	0.2	0.1
54–70	0.2	0.2	0.3	0.2
Total in urine or feces	3.2 ±0.6[d]	66.8 ±0.6[d]	3.5 ±0.4[d]	64.4 ±0.8[d]
Whole carcass[e]	3.0 ± 0.2[d]			

[a] [^{14}C]Elastin was administered orally by a stomach tube to three rats. The excreted ^{14}C was expressed as percentage of total administered [^{14}C]elastin.

[b] Each rat received a suspension of [^{14}C]elastin (35–40 mg in 5 ml of saline, 2.8×10^5 dpm/mg). The data are the average from three rats.

[c] Elastase-predigested [^{14}C]elastin was prepared by incubating 40 mg of [^{14}C]elastin with 100 units of elastase at 37° for 4 hr. The predigested [^{14}C]elastin (equivalent to 40 mg of [^{14}C]elastin) was filtered through a Millipore (0.2-μm) filter and administered to each rat.

[d] Standard error of mean.

[e] Whole carcass was hydrolyzed with 11 *N* HCl. Aliquots were used to determine ^{14}C after combustion in a sample oxidizer.

TABLE IB. EXPIRATORY $^{14}CO_2$ AFTER ORAL ADMINISTRATION OF [^{14}C]ELASTIN[a]

Hours after administration	Percentage of total administered[b]
0–24	13.1
24–48	1.6
48–70	1.1
Total	15.8

[a] [^{14}C]Elastin (31.4 mg, 2.8×10^5 dpm/mg) was suspended in 5 ml of saline, and then administered to a rat by a stomach tube. The rat was placed in a vacuum desiccator equipped with continuous air flow, outcoming air being trapped in a series of three flasks containing Carbo-Sorb II. Radioactivity was measured by the liquid scintillation counter.

[b] An aliquot (1 ml) of each CO_2 trap was used from measuring radioactivity by a liquid scintillation counter. The amount of radioactivity was expressed as percentage of total administered [^{14}C]elastin.

which likely included the moieties of glycine, alanine, serine, valine, leucine, etc. (7). Some of these labeled amino acid moieties would be likely to undergo digestion and absorption and be metabolized to $^{14}CO_2$.

Because both radioactive labels appeared in the urine, we analyzed HCl-hydrolysates of whole urinary samples as well as their concentrated nondialyzable fractions from both the [^{14}C]- and [^{3}H]elastin experiments for desmosines. No desmosines were detected in these samples. In addition, only a small fraction of the urinary radioactivity from the [^{3}H]elastin experiments (2.5–8.1% of urinary radioactivity or 0.7–2.0% of total administered radioactivity) was present in the nondialyzable fraction, the majority being lost in the dialysate media (Table II).

Interestingly, the digestion and absorption of a highly cross-linked native collagen in rats were also low, 39% of the ingested collagen (11).

TABLE II. EXCRETION OF ^{3}H IN URINE AND FECES AFTER ORAL ADMINISTRATION OF [^{3}H]ELASTIN[a]

Hours after administration	[^{3}H]Elastin[b]		Elastase-predigested [^{3}H]elastin[c]	
	Urine (%)	Feces (%)	Urine (%)	Feces (%)
0–24	18.4	47.3	21.8	52.6
24–36	3.5	5.1	0.8	13.8
36–48	4.8	5.5	1.6	4.7
48–56	0.5	0.8	0.2	0.2
56–70	0.6	0.6	0.4	0.2
Total in urine or feces	27.8	59.3	24.8	71.5
Nondialyzable[d]	0.7		2.0	
Estimated dialyzable[d]	27.1		22.8	

[a] [^{3}H]Elastin was administered orally by a stomach tube to two rats. The excreted ^{3}H was expressed as percentage of total administered [^{3}H]elastin.

[b] Each rat received a suspension of [^{3}H]elastin, 44–46 mg in 5 ml of saline, 3.4×10^{6} dpm/mg. The data are the average of two rats.

[c] Elastase-predigested [^{3}H]elastin was prepared by incubating 60.7 mg of [^{3}H]elastin with 100 units of elastase at 37° for 4 hr. The predigested [^{3}H]elastin (equivalent to 55.08 mg of [^{3}H]elastin) was filtered through a Millipore (0.2 μm) filter and administered to each rat.

[d] The first 48-hr urinary samples were combined, and aliquots (5 ml) were dialyzed against running water for 24 hr. Radioactivity in the nondialyzable fraction was measured.

TABLE III. EXCRETION OF ^{3}H IN URINE AND FECES AFTER ADMINISTRATION OF [^{3}H]DESMOSINE

Hours after administration	Oral administration[a]		Intraperitoneal injection[b]	
	Urine (%)	Feces (%)	Urine (%)	Feces (%)
0–7	1.8	0.1	89.9	0.0
7–22	0.8	48.8	3.1	0.1
22–30	0.2	25.6	0.8	0.8
30–46	0.3	6.4	1.4	1.5
46–54	0.2	3.0	0.2	0.5
54–70	0.2	0.4	1.0	0.3
Total	3.5	84.3	96.4	3.2

[a] [^{3}H]Desmosine was administered orally by a stomach tube to the rats. Three milliliters of saline containing 0.057 μmole of the [^{3}H]desmosine was given for a total dosage of 2.72×10^{8} dpm per rat. Fecal samples were homogenized with water. Aliquots were burned in sample oxidizer and radioactivity was measured. The excreted ^{3}H was expressed as percentage of total administered [^{3}H]desmosine. The data present the average of two rats.

[b] An intraperitoneal injection of 0.038 μmole of [^{3}H]-desmosine (1.81×10^{8} dpm) in 0.5 ml of saline was given to each rat. Fecal samples were homogenized with water. Aliquots were burned in a sample oxidizer and radioactivity was measured. The excreted ^{3}H was expressed as percentage of total administered [^{3}H]desmosine. The data represent the average of two rats.

Absorption of orally administered [^{3}H]desmosine and excretion of intraperitoneally injected [^{3}H]desmosine. [^{3}H]Desmosine, when given orally, was poorly absorbed with 84.3% appearing in the feces (Table III). When injected intraperitoneally, 93.0% of the radioactivity appeared in the urine within 22 hr of administration. Virtually all (96.4%) of the radioactivity was found in the urine as [^{3}H]desmosine. This was demonstrated by cochromatographic analysis of the urinary sample showing that the radioactive fractions coincided with desmosine as confirmed by the ninhydrin reaction of desmosine added to the urinary sample prior to separation. This result is consistent with the data of Goldstein and Starcher (2), who used ^{14}C-labeled desmosines in similar experiments.

Although gastrointestinal digestion and absorption of small amounts of the radiolabeled intact elastin occurred, as evidenced by the appearance of low percentages of radioactivity in the urine, desmosine-containing peptides were not found in the urinary samples. These results could be explained by either, (i) a failure in gastrointestinal digestion hy-

drolyzing elastin to sufficiently small desmosine-containing subunits, or (ii) the inability of the gastrointestinal tract to absorb the desmosine-containing peptides. The results of our experiments with orally administered desmosine indicate that the latter explanation is the most likely operative mechanism, although incomplete digestion may also have occurred since pancreatic elastase hydrolyzes a large portion of elastin to predominately large peptides (12). Despite its relatively low molecular weight (526), the structure of the desmosine molecule, even with its favorable configuration of L-amino acids with α-amino-α-carboxcylic groups, absorption may have been prevented by the steric hinderance imposed by the bulky groups attached to the pyridinium nucleus.

Our data suggest that the measurement of urinary desmosine may be a useful index of the catabolic breakdown of endogenous elastin uncontaminated by the dietary ingestion of desmosine-containing food products. However, caution must be exercised in extrapolating these results to man, as the digestive and absorptive capacity of the human gastrointestinal tract may differ from those of the rat.

Summary. The digestion and absorption of elastin was studied in rats by feeding intact or elastase-predigested elastin labeled with ^{14}C or ^{3}H. Radioactive elastins (59.3–71.5%) remained in feces whether feeding intact or predigested elastins. Of the absorbed products, 3.2–3.5% and 24.8–27.8% appeared in urine for [^{14}C]- and [^{3}H]elastin, respectively. However, desmosine-containing degrative products were not found in the urine. The absorption of [^{3}H]desmosine was also studied following oral and intraperitoneal administration. Given orally, ^{3}H appeared predominately in the feces, but when injected intraperitoneally, ^{3}H was mostly in the urine. Therefore, even if elastin were completely digested, desmosine would not be absorbed.

This study was supported by the Medical Research Service of the Veterans Administration and Grant No. HL16118 from the National Heart and Lung Institute.

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Received December 4, 1978. P.S.E.B.M. 1979, Vol. 161.