In Vitro Incorporation of ^{14}C Labeled Precursors into Swine Coronary Arterial Phospholipids

Experimental atherosclerosis in the rabbit is associated with an increased rate of synthesis of phospholipids, particularly phosphatidyl choline, in the aortic wall[1-4]. Different types of phospholipids have been shown to vary in their capacity to stabilize cholesterol emulsions[5]. It is possible that differences in content or synthesis of the various types of phospholipids in the arterial wall could be a factor in the differing susceptibilities of some types of arteries or some species to atherosclerosis. This possibility was explored in the present investigation by studying the coronary arteries of swine in comparison to the pulmonary and other arteries and human peripheral arteries of previous studies[4-6].

Materials and methods. Hearts and portions of the aorta and pulmonary arteries were obtained within 30 min of slaughter from 6-month-old, male, grain fed Hampshire swine. The intima-media portions of the coronary and other arteries were placed in an isotonic Krebs-Ringer-glucose solution at pH 7.4, and after addition of 20 μc of either formate-^{14}C, acetate-1-^{14}C, glycerol-1-3-^{14}C, choline-1-2-^{14}C, serine-1-^{14}C or ethanolamine-1, 2-^{14}C (Nuclear-Chicago, adjusted to specify activity of 1.03 mc/mM) to each, the flasks were incubated for 4 h in an air atmosphere at 37.5°C in a Dubnoff metabolic shaking incubator. A total of 4 incubations for each artery were done with each ^{14}C labeled precursor. Carbon dioxide was collected in a center well containing 20% NaOH, and the $^{14}CO_2$ radioactivity determined as previously described[4].

Washing the tissues free of radioactive precursor, extraction of the lipids, and separation of the phospholipids by thin layer chromatography were all done as previously described[4]. After elution of the phospholipid fractions from the silica gel[7] aliquots were analyzed for phosphorous content[8]. Other aliquots were assayed for radioactivity using a mixture of 2, 5 Diphenyloxazole and 1, 4-*bis*-2-(5-Phenyloxazolyl)-Benzene in toluene scintillator and a Packard Model 3314 Liquid Scintillation spectrometer[4].

Results and discussion. The amounts of the individual major phospholipids in the coronary arteries in mg/g dry weight of arteries ± S.D. were as follows: sphingomyelin 0.7 ± 0.2, phosphatidyl choline 2.8 ± 0.6, phosphatidyl serine 0.8 ± 0.3 and phosphatidyl ethanolamine 1.3 ± 0.4. The amounts of these phospholipids were not significantly different in the coronary or pulmonary arteries or in the aorta; incubation with the different labeled precursors also had no effect upon the amounts of any of these phospholipid classes.

The $^{14}CO_2$ evolved in dpm/mg dry weight of artery for the coronary and pulmonary arteries after incubation with each of the ^{14}C labeled precursors is indicated in Table I. Oxidation of carbon 1 of serine to $C^{14}O_2$ was greater than that for the labeled carbon atoms of the other amino-alcohol substrates utilized. The amounts of $^{14}CO_2$ evolved from the aorta were significantly lower than those from the pulmonary and coronary arteries.

The specific activities of the major coronary arterial phospholipids after incubation with the different ^{14}C labeled precursors are indicated in Table II. The nitrogenous bases appeared to be incorporated to a much greater degree than formate, acetate or glycerol. This was probably due to the more selective utilization of the bases for phospholipid synthesis, and also to the greater oxidation of formate and acetate to CO_2. Spingomyelin showed a relatively low degree of incorporation with all the precursors, including the choline-^{14}C. Phosphatidyl serine was most highly labeled when incubated with the serine-^{14}C or formate-^{14}C, incorporation of the latter occurring probably by fixation of the one carbon fragment to glycine to form serine.

Phosphatidyl choline was significantly labeled after incubation with choline-^{14}C and with ethanolamine-^{14}C,

Table I. $^{14}CO_2$ evolved during incubations of swine arteries with ^{14}C-labeled substrates[a]

Substrate	Coronary	Pulmonary	Aorta
Formate	1,150 ± 203	1,390 ± 313	842 ± 252
Acetate	2,540 ± 224	2,960 ± 306	1,410 ± 151
Glycerol	109 ± 20	141 ± 25	78 ± 19
Choline	26 ± 6	42 ± 11	10 ± 3
Serine	177 ± 28	235 ± 37	81 ± 15
Ethanolamine	22 ± 5	34 ± 10	6 ± 2

[a] dpm $^{14}CO_2$/mg dry weight of artery.

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Table II. Specific activities of major swine coronary arterial phospholipids after incubation with ^{14}C-labeled substrates[a]

Substrate	Sphingomyelin	Phosphatidyl choline	Phosphatidyl serine	Phosphatidyl ethanolamine
Formate	401 ± 158	188 ± 38	1,760 ± 414	750 ± 62
Acetate	198 ± 35	780 ± 175	469 ± 79	392 ± 81
Glycerol	492 ± 86	1,540 ± 312	1,160 ± 256	1,210 ± 178
Choline	2,281 ± 385	64,600 ± 4,753	1,547 ± 295	1,417 ± 299
Serine	992 ± 227	563 ± 134	30,900 ± 4,854	1,430 ± 176
Ethanolamine	2,258 ± 456	4,706 ± 701	518 ± 130	52,900 ± 3,450

[a] DPM/mg phospholipid ± S.D.

indicating that both the de-novo and the transmethylation pathways are active in the swine arteries.

The patterns of incorporation in the aorta and pulmonary arteries were not significantly different from those of the coronary arteries; the degrees and distribution of radioactivity incorporation were similar to those found on incubation of the same precursors with rabbit aortas[4] or human peripheral arteries[6]. There is, therefore, no evidence from these experiments to suggest that the different susceptibilities to atherosclerosis of the coronary arteries and aorta vs. the pulmonary artery are attributable to differences in phospholipid metabolism of these arteries[9].

Zusammenfassung. Der Grad des Einbaus von ^{14}C-markierten Prekursoren in die Arterienphospholipide des Schweins war folgender: Cholin > Aethanolin > Serin > Glycerin > Format > Azetat. Bei Bebrütung von Arterien mit ^{14}C-Cholin oder ^{14}C-Aethanolamin trat ein deutlich erhöhter Einbau von ^{14}C in das Phosphatidylcholin auf.

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Intestinal Absorption of D-Xylose by Germfree Rats

It is now recognized that the morphology of the intestine is determined in important ways by the presence of the normal intestinal microflora. Recent understanding of these effects has come from studies of germfree animals which were shown to have less mucosal surface area[1] and reduced mucosal cell turnover rate[2–4] when compared to conventional control animals. Differences also have been observed in the morphology of the reticuloendothelial elements of the intestinal mucosa[2,5]. Despite these morphological observations, a surprisingly small amount of information is available concerning intestinal function in germfree animals. We have begun a series of investigations[6] to determine the effects of microorganisms on intestinal absorption and this report summarizes observations on the absorption of D-xylose by germfree and conventionalized rats.

Materials and methods. Fischer strain rats were obtained germfree from the Charles River Breeding Laboratories, Willmington, Massachusetts immediately after weaning. They were divided randomly into 2 groups of equal numbers of male and female rats and were transferred to separate Trexler-type, flexible plastic isolators[7]. One group was 'conventionalized' by a procedure previously described[6] and served as the control group. The other group of rats remained germfree. Both groups received sterilized diet L-356[8], sterilized distilled water ad libitum, and a vitamin supplement. They were otherwise maintained under identical environmental conditions, the single exception being the presence of the microflora in the conventional rats. Germfree status was determined biweekly with standard microbiologic techniques[6].

At the age of 6.5 months, 250 mg of D-xylose in 0.5 ml of water was administered to each rat by gastric tube. 6 h following administration of the test solution, the rats were killed with chloroform and the gastrointestinal tracts removed. The contents of the stomach, small intestine, and large intestine were collected separately by washing with isotonic saline solution. The total amount of D-xylose remaining in each section of the gastrointestinal tract was determined using the method of Roe and Rice[9]. The percentage of D-xylose absorbed was calculated by the procedure of Makela et al.[10] which accounts for differences in gastric emptying.

Results and discussion. The results are presented in the Table. In germfree rats, 11.3% of the D-xylose remained in the stomach at the end of the test period compared to 3.8% in the stomachs of conventionalized rats. This difference was not significant at the 0.05 level but was similar to observations made previously which indicate a decreased rate of gastric emptying in germfree rats[6,11]. There was no difference between germfree and conventionalized groups in the amount of D-xylose which remained in the small intestine but significantly more was recovered in the cecum and colon of germfree rats ($P < 0.025$). The net absorption was 74.3% in the germfree rats and 87.8% in the conventionalized rats, a significant difference ($P < 0.025$).

These observations are different from those previously reported by Heneghan[12] in which it was concluded that xylose is absorbed more rapidly in germfree rats. Our data are not comparable to his because he measured absorption during the first hour following administration, a time when both germfree and control animals had absorbed less than half of the administered dose. There may have been other differences which are not immediately apparent, e.g., in the composition of the intestinal microflora of the control animals.

Our observations suggest that normal intestinal microorganisms have a significant effect on the intestinal absorption of D-xylose but the explanation is not completely clear. We do not believe the decreased absorption in germfree rats was related to intestinal motility. Abrams

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