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In Vitro Incorporation of Formate-^{14}C, Glycerol-1,3-^{14}C, Choline-1,2-^{14}C, Serine-1-^{14}C, and Ethanolamine-1,2-^{14}C into the Major Phospholipids of Human Peripheral Arteries* (34000)

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Results of investigations into the synthesis of phospholipids from acetate-^{14}C and phosphate-^{32}P precursors in rat and rabbit aortas have been summarized in a previous publication (1). Synthesis of total phospholipids by the human arterial wall has been demonstrated *in vivo* from phosphate-^{32}P (2) and *in vitro* from acetate-^{14}C (3, 4), but thus far the incorporation of other precursors into the individual phospholipid classes of human arteries has not been studied.

Materials and Methods. Above the knee amputated legs were obtained immediately after surgery, and the distal femoral artery, and popliteal and tibial arteries were dissected free. Twenty specimens were obtained, all of which were from male patients, ages 60–80 and all of which showed advanced occlusive atherosclerotic changes. The arteries were freed of adventitia, placed in an isotonic Krebs–Ringer solution, and after addition of 20 μC of either formate-^{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 specific activity of 1.03 mC/mM) to each, the flasks were incubated for 4 hr in an air atmosphere at 37.5° in a Dubnoff metabolic shaking incubator. A total of four incubations 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 was determined as previously described (5).

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 (5, 6). Because of the small amounts and impurity of the phosphatidyl inositol fraction, this phospholipid was not included in the present study. After elution of the phospholipid fractions from the silica gel (6), aliquots were analyzed for phosphorous content (7). Other aliquots were assayed for radioactivity using a PPO–POPOP in toulene scintillator and a Packard model 3314 liquid scintillation spectrometer (5).

Results and Discussion. The amounts of the individual phospholipids (mg/g of dry wt of artery ± SD) were as follows: lysophosphatidyl choline, 0.4 ± 0.1; sphingomyelin, 2.6 ± 0.8; phosphatidyl choline, 3.4 ± 0.7; phosphatidyl serine, 1.1 ± 0.3; and phosphatidyl ethanolamine, 1.8 ± 0.4. Incubation with the different labeled precursors did not significantly affect the amounts of any of the phospholipid classes. Sphingomyelin was high in amount but showed the lowest degree of incorporation with all the precursor compounds.

The $^{14}CO_2$ evolved (dpm/mg of dry wt of artery) was as follows: formate-^{14}C, 1730 ± 370; glycerol-1, 3-^{14}C, 860 ± 115; choline-1, 2-^{14}C, 122 ± 25; serine-1-^{14}C, 145 ± 38; and ethanolamine-1, 2-^{14}C, 36 ± 12.

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TABLE I. Specific Activities of Major Human Peripheral Arterial Phospholipids after Incubation with ^{14}C Labeled Precursors.[a]

Phospholipid	Formate-^{14}C	Glycerol-1,3-^{14}C	Choline-1,2-^{14}C	Serine-1-^{14}C	Ethanolamine-1,2-^{14}C
Lysophosphatidyl choline	1320 ± 155	950 ± 103	1530 ± 162	803 ± 110	1050 ± 149
Sphingomyelin	205 ± 30	180 ± 48	785 ± 95	114 ± 33	332 ± 65
Phosphatidyl choline	1890 ± 360	1220 ± 158	22,700 ±3230	744 ± 98	9180 ±1020
Phosphatidyl serine	4610 ± 590	1560 ± 229	1910 ± 257	6690 ± 860	3010 ± 531
Phosphatidyl ethanolamine	1970 ± 164	1190 ± 130	1620 ± 177	545 ± 74	12,480 ±2380

[a] dpm/mg of phospholipid ± SD.

The specific activities of the major human arterial phospholipids after incubation with the different ^{14}C labeled precursors are indicated in Table I.

The relative degrees of incorporation of the labeled precursors into the phospholipids were choline>ethanolamine>serine>formate >glycerol. The relatively greater incorporation of the choline and ethanolamine may have been due to the more selective utilization of these compounds for phospholipid synthesis. Serine is utilized for protein synthesis and by other pathways; glycerol and formate, as well as being incorporated into other compounds, were oxidized more rapidly to CO_2.

Formate-^{14}C appeared to be incorporated primarily into phosphatidyl serine, most likely by fixation of the one carbon fragment to glycine as has been demonstrated in other tissues (8, 9).

Glycerol-1, 3-^{14}C was more uniformly incorporated into all the phospholipid classes, but at a relatively low level.

Although choline 1,2-^{14}C was incorporated primarily into phosphatidyl choline, smaller amounts also appeared in the other choline-containing phospholipids (lysophosphatidyl choline and sphingomyelin), and also into phosphatidyl serine and phosphatidyl ethanolamine; the latter probably occurred by catabolism of choline to glycine, with subsequent synthesis of serine and ethanolamine.

Serine-1-^{14}C appeared to be incorporated largely into phosphatidyl serine. Although serine can be converted to ethanolamine in other tissues (10), this occurs by decarboxylation of carbon 1, so that significant labeling of phosphatidyl ethanolamine would not be expected in this experiment.

Incorporation of ethanolamine 1, 2-^{14}C occurred primarily into phosphatidyl ethanolamine, with smaller but significant amounts incorporated into the choline-containing phospholipids, particularly phosphatidyl choline. This suggests that the enzyme system for the transmethylation of phosphatidyl ethanolamine to phosphatidyl choline is active in human arteries.

The patterns of incorporation of precursors were generally similar to those found on incubation of these compounds with rabbit aortas (1), suggesting that phospholipid synthesis and catabolism by human and rabbit aortas probably occurs by the same pathways and at comparable rates.

Summary. The relative degrees of *in vitro* incorporation of ^{14}C labeled precursors into surgical specimens of human femoral, tibial, and popliteal arteries were choline>ethanolamine>serine>formate>glycerol. Formate appeared to be incorporated primarily into phosphatidyl serine, probably by fixation to glycine. In addition to *de novo* synthesis, the pathway of transmethylation of phosphatidyl ethanolamine to phosphatidyl choline seemed to be operative in these arterial preparations.

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Relationship of *in Vivo* Gamete Aging and Exogenous Hormones to Early Embryo Development in Rabbits* (34001)

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Noyes and Thibault (1) concluded that many factors influence the functional life of spermatozoa in the female genital tract. In the rabbit, capacitation of spermatozoa must take place before fertilization can occur (2). Rates of capacitation and sperm survival vary, depending on the location of sperm in the female genital tract (3, 4) and upon various hormonal states (1, 3, 5, 6). Fertilizing capacity of spermatozoa in the mated doe diminishes after 22 hr *in utero* (7).

Adams (8) obtained a high fertilization rate following administration of hormones to induce superovulation. Kennelly and Foote (9) found no differences in the fertilization rate of superovulated and nonsuperovulated does inseminated immediately after giving ovulating hormone. Tesh (10) reported a decrease in the fertilization rate in superovulated does with *in vivo* aged spermatozoa, and he suggested that oocytes produced by superovulation are in some way deficient.

It has been reported that the rabbit secondary oocyte has a fertilizable life span of 3.5–8 hr (11–13). Ovulation is delayed in does treated with follicle-stimulating preparations (9, 15). This delay explains the apparent lag in development of embryos obtained from FSH-primed does compared with nonprimed does examined at the same time interval after LH injection (15). The present investigation was conducted to study the critical parameters of aging spermatozoa and secondary oocytes in FSH-primed and control (nonprimed) does using initial cleavage *in vivo* and development during culture as test criteria.

Materials and Methods. The experimental design was a 2 × 4 arrangement with control and FSH-primed groups each subdivided into four subgroups inseminated either 10 hr before LH injection, at the time of LH injection, or 5 or 10 hr after LH injection. These times are designated as +10, 0, —5, and —10 Thus, the sperm were allowed to age in the female as much as 10 hr longer than normal to 10 hr less than normal. Since the late inseminations were expected to coincide with many of the ovulations (14), ovulated oocytes were expected to age at least 4–6 hr (2) during the period required for sperm capacitation before possible fertilization.

Forty-eight uniparous does were assigned to the eight groups, giving six does per group. The FSH-primed does received FSH subcutaneously 2 times daily for 3 days (0.5 mg of FSH/injection) followed by an intravenous injection with LH the fourth morning (2.5 mg of Armour PLH). Controls received only the LH. The time of LH injection was controlled over a 20-hr period so that semen collected from one fertile male could be used promptly and simultaneously to inseminate all eight does comprising one complete 2 × 4 replicate. The same semen donors were used throughout the experiment. Semen was diluted with physiological saline, containing penicillin G and streptomycin sulfate, so as

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