In vitro Incorporation of Acetate-l-C^{14} into Sphingomyelin, Phosphatidyl Choline and Phosphatidyl Ethanolamine of Rabbit Testes.* (32409)

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Early studies, utilizing elaidic acid as a tracer showed almost no uptake of this fatty acid by the rat testes as compared to other tissues(1). A more recent investigation, however, showed a preferential incorporation of arachidonic acid-1-C^{14} by rat testicular phospholipids(2). Some of the radioactivity was also recovered in 20 carbon polyunsaturated fatty acids other than arachidonic.

Although the total lipid, total phospholipid, and fatty acid composition of the testes of several species has been investigated(3-5) there have been few studies of the metabolism of the individual phospholipids in the testes. The purpose of the present experiments was, therefore, to investigate the detailed fatty acid composition and relative incorporation of acetate-1-C^{14} into the individual phospholipid fatty acids.

Materials and methods. Six male 2-months-old New Zealand white rabbits, fed a stock commercial diet (Purina) since weaning, were anesthetized by I.V. injection of Sodium Pentobarbital. The excised testes from each rabbit were decapsulated, sliced at approximately 0.5 mm intervals and placed in 10 ml of Tyrode's solution(6). After addition of 50 μc sodium acetate-1-C^{14} (Nuclear-Chicago, specific activity 29.0 mc/mM) to each, the flasks were incubated for 4 hours in an air atmosphere at 37.5°C in a Dubnoff metabolic shaking incubator. The flasks were then placed on ice, and after cooling to 0°C the contents were centrifuged, washed twice with 20 ml 0.9% NaCl for 20 minutes each time and recentrifuged. The tissues were then refrigerated overnight in 50 ml of 0.155 M unlabeled sodium acetate. The next day, after one additional washing with 0.9% NaCl and centrifugation, the testes lipids were extracted in a ground glass mortar and pestle with 2 successive 50 ml portions of methylal/methanol, 4:1. The combined extracts were evaporated to dryness in a rotary vacuum evaporator and under N_2, redissolved in chloroform, filtered, and evaporated down to 0.5 ml. Silica gel H (Brinkmann) plates 0.5 mm thick were prepared by the method of Skipski(7). After drying and heating at 110° for one hour, the plates were placed in the developing mixture and the solvents allowed to rise to the top of the plates, in order to remove impurities. The plates were then reactivated for one hour at 110° just prior to use. The total testes lipids were applied as a band across the plate and the phospholipids separated using an ascending developing mixture of chloroform:methanol:acetic acid:water 25:15:4:2. The sphingomyelin, phosphatidyl choline and phosphatidylethanolamine bands were collected with a vacuum zone extractor. Elution of the phospholipids from the silica gel was accomplished as previously described(7). Aliquots of the eluates from each fraction were analyzed for phosphorus content(8). Other aliquots were assayed for radioactivity using a PPO-POPOP in toluene scintillator and a Packard Model 3314 liquid scintillation spectrometer. Quenching was monitored by the channels ratio technique, and all counts corrected to 100% efficiency.

The remainder of the fractions were then hydrolyzed and methylated(14) and the fatty acid composition of each sphingomyelin, phosphatidyl choline and phosphatidyl ethanolamine fraction was determined by gas-liquid chromatography in a Barber-Colman Model 10 Gas Chromatograph using diethylene glycol succinate on Gaschrom P, 70-80 mesh, at 187°C with an argon pressure of 24 lb. The fatty acids were identified by comparison of retention times to those of standards (obtained from the National Insti-

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TABLE I. Amounts and Degree of Incorporation of Acetate-1-C^{14} into Rabbit Testes Phospholipids.*

	Sphingomyelin	Phosphatidyl choline	Phosphatidyl ethanolamine
mg phospholipid/g dry wt testes	6.18 ± 1.22	25.77 ± 5.71	7.96 ± 1.40
Specific activity (DPM/mg phospholipid)	22,840 ± 6,350	92,100 ± 19,360	66,537 ± 10,720
Amount of acetate-1-C^{14} incorporated (mμmoles/g dry wt testes/4 hr)	2.19 ± .61	36.87 ± 7.70	8.23 ± 1.32

* Data expressed as mean ± standard deviation.

tutes of Health and Calbiochem) and by graphic representation of retention times. The areas under the peaks were estimated by triangulation. Only the major fatty acids 16, 16:1, 18, 18:1, 18:2, 20:3(8,11,14), 20:4 and 22:5 have been included in the tabulations. Other fatty acids, identified in amounts either too small or having too long a retention time to measure and collect accurately, were 14, 20:1, 22:4, and 22:6. Aldehydes of 16 and 18 carbon atoms were also identified as minor constituents in most of the fractions. After weighing the total fatty acid methyl esters, the individual methyl esters were collected from the gas-chromatography effluent in anthracene cartridges using a Packard Gas Chromatography Fraction Collector equipped with a tight rubber seal. Average recovery of fatty acids was 88%. Specific activities of each fatty acid methyl ester were calculated as described by Evans and Norcia (9).

The probabilities (p) that apparent differences in the data were due to chance were calculated by the *t* test, and only those differences where $p<0.01$ have been considered significant.

Results and discussion. The amounts of the 3 major phospholipids in the rabbit testes are indicated in Table I. Phosphatidyl choline was present in by far the greatest amount and was higher in proportion to the other phospholipids than has been reported in rats (10,11). Phosphatidyl choline had both the relative and absolute highest utilization of the acetate-1-C^{14}, as indicated by both the specific activity data and the calculated number of mμmoles of the substrate incorporated during the 4-hour incubation period (Table I). Sphingomyelin was the least metabolically active of the 3 phospholipids in terms of acetate incorporation.

The fatty acid compositions of the individual testicular phospholipids are indicated in Table II. Considering the long chain saturated fatty acids, sphingomyelin and phosphatidyl choline had higher percentages of palmitic acid than did phosphatidyl ethanolamine. Stearic acid was lowest in phosphatidyl choline. All the phospholipids contained a high proportion of polyunsaturated fatty acids (sphingomyelin 34%, phosphatidyl choline 41%, phosphatidyl ethanolamine 47%). Linoleic acid was highest in phosphatidyl choline whereas linolenic acid was highest in sphingomyelin. A high pro-

TABLE II. Fatty Acid Composition of Rabbit Testes Phospholipids.*

		Sphingomyelin	Phosphatidyl choline	Phosphatidyl ethanolamine
Fatty acid	16:0	26.5 ± 5.2	25.3 ± 4.5	15.6 ± 2.1
	16:1	2.9 ± .9	2.6 ± 1.1	2.2 ± 2.3
	18:0	19.3 ± 3.1	10.4 ± 1.4	17.7 ± 2.0
	18:1	16.9 ± 3.7	21.3 ± 4.8	17.5 ± 1.8
	18:2	5.0 ± .8	7.3 ± 1.2	4.8 ± 1.0
	18:3	3.8 ± .5	.4 ± .2	1.7 ± 1.0
	20:3†	5.7 ± .5	3.8 ± .8	4.2 ± .6
	20:4	7.8 ± 1.7	15.5 ± 1.6	19.2 ± 1.9
	22:5	12.0 ± 2.1	13.6 ± 1.9	17.3 ± 2.3

* Mean percentages ± standard deviations.
† 8, 11, 14-eicosatrienoic acid.

TABLE III. Specific Activities and Amounts of Acetate-1-C^{14} Incorporated into Individual Rabbit Testes Phospholipids.

		Sphingomyelin	Phosphatidyl choline	Phosphatidyl ethanolamine
Fatty acid	16:0	34,560 ± 4,700*	220,600 ± 13,190	148,400 ± 12,600
		.324 ± .044 †	16.2 ± 1.0	2.07 ± .18
	18:0	13,650 ± 1,150	61,320 ± 16,820	25,700 ± 6,100
		.0935 ± .007	1.85 ± .51	.407 ± .097
	18:1	1,120 ± 384	13,350 ± 2,140	9,630 ± 968
		.007 ± .002	.823 ± .132	.151 ± .015
	20:3	1,700 ± 447	3,720 ± 980	3,100 ± 532
		.003 ± .001	.041 ± .011	.012 ± .002
	20:4	2,080 ± 369	6,890 ± 1,780	4,460 ± 748
		.006 ± .001	.310 ± .080	.077 ± .013
	22:5	1,530 ± 363	3,235 ± 675	1,690 ± 436
		.007 ± .002	.127 ± .027	.026 ± .007

* Mean DPM/mg ± standard deviation.
† Mean mμmoles acetate-1-C^{14} incorporated/g dry wt of testes ± standard deviation.

portion of 8,11,14-eicosatrienoic acid, an intermediate in the synthesis of arachidonic from linoleic acid(12,13), was found in all 3 phospholipids, being highest in sphingomyelin. This fatty acid has also been noted in similar proportions in the total phospholipids of pig and human testes(5,3). Although 8,11,14-eicosatrienoic acid has been noted in comparable amounts in livers of animals fed excess cholesterol with polyunsaturated fat (14), it has not been found in measurable amounts in any tissue other than the testes under normal dietary conditions. This may reflect a less efficient dehydrogenation mechanism in the testes than in the other tissues. Arachidonic and docosapentaenoic acid were both in highest proportions in phosphatidyl ethanolamine. In general, most of the polyunsaturates present were linoleic acid or its derivatives (others were present but in amounts less than 1%). The high susceptibility of the testes to linoleic acid deficiency with the resultant sterility(15) is understandable in view of this predominance of the linoleic acid series of polyunsaturates in the major testicular phospholipids.

The individual fatty acid specific activity data and the mμmoles of radioactive acetate incorporated are listed in Table III. In general, these figures paralleled those for the phospholipids given in Table I in that incorporation into the phosphatidyl choline fatty acids was greater than into phosphatidyl ethanolamine fatty acids which in turn was greater than into sphingomyelin fatty acids. In all 3 phospholipids, palmitic acid showed by far the highest incorporation of the acetate precursor, followed by stearic and oleic acids. These relative degrees of incorporation are similar to those found in the liver(9,16). Lower, but significant amounts of radioactivity were found in 8,11, 14-eicosatrienoic, arachidonic and docosapentaenoic acids. The latter has been demonstrated to be formed from arachidonic in the testes(17). Although the proportions of arachidonic and docosapentaenoic acids were higher in phosphatidyl ethanolamine than in phosphatidyl choline, the specific activities were lower, suggesting a slower rate of turnover of these fatty acids in testicular phosphatidyl ethanolamine than in phosphatidyl choline.

Summary. Rabbit testes were incubated with acetate-1-C^{14}, following which the amounts, radioactivities, fatty acid compositions and fatty acid specific activities of the major phospholipids were determined. Phosphatidyl choline was present in highest amount and incorporated the most acetate-C^{14}. All the phospholipids contained high percentages of polyunsaturated fatty acids, particularly of the linoleic acid series. The rabbit testicular phospholipids were found to contain a higher percentage of 8,11,14-eicosatrienoic acid than is found in other normal

tissues. Palmitic acid incorporated the highest amount of acetate-C^{14} in all the phospholipid classes. Smaller, but significant amounts of this precursor were also utilized in the synthesis of 8,11,14-eicosatrienoic, arachidonic and docosapentaenoic acids by the testes.

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Autoregulation of Glucose Metabolism in the Isolated Perfused Rat Liver.* (32410)

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Between 1850 and 1853, Claude Bernard described glycogen as a temporary storage form of carbohydrate, and the ability of the liver to maintain blood glucose levels in the absence of carbohydrate in the diet(1). Soskin in 1938 refined these observations and demonstrated that the dog liver would take up glucose when perfused with hyperglycemic blood, and would release glucose when the blood sugar was low(2). Searle and Chaikoff, using the technique of measuring glucose concentration and specific activity of C^{14} glucose in the blood(3), found that hyperglycemia inhibited glucose production. These observations have been reconfirmed in both animals and man(4-7).

In the present study autoregulation of glucose production and utilization was investigated using the isolated perfused rat liver. Livers from normal fasted, alloxan diabetic, and adrenalectomized rats were perfused with media containing glucose concentrations varying from 0 to 1000 mg/100 ml. Both glucose production and utilization were determined.

Materials and methods. Albino, male rats of the Wistar strain, obtained from Holtzman Co., weighed from 200-250 g. Animals were maintained on lab chow (Ralston Purina Company, St. Louis, Mo.) and water *ad lib.* Normal rats were fasted 24 hours prior to use.

Rats were made diabetic by the injection of alloxan, (Eastman Organic Chemicals, Rochester, N. Y.), 40 mg/kg I. V.; and maintained on 4 units of protamine zinc insulin (Eli Lilly and Co., Indianapolis, Ind.) for 2 weeks prior to use. Insulin was with-

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