increased synthesis of very low density lipoproteins by the liver after partial hepatectomy.

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Phospholipid precursors of prostaglandins

Several unesterified prostaglandin derivates are produced by sheep vesicular gland homogenates *in vitro*[1–3]. Prolonged synthesis of these derivates *in vivo* would tend to deplete the available substrates and draw upon precursors of the free fatty acids since the level of unesterified fatty acids is generally low in tissues. As one possibility, the large reservoir of polyunsaturated acids bound in ester form might undergo oxidative cyclization to prostaglandin either prior to or following hydrolysis. To investigate whether the cyclization reaction could occur directly with the phospholipids, 1-palmitoyl-2-{[2′-^{14}C]eicosatrienoyl)glycero-3-phosphorylcholine was prepared and incubated with enzyme preparations from sheep vesicular glands. Although unesterified prostaglandins were formed from the phospholipid, no appreciable amount of radioactive prostaglandin was found esterified to phospholipid. These results sug-

gest that prostaglandin may be most rapidly formed if hydrolysis precedes the cyclizing reaction.

The fatty acids available in the form of phospholipids in sheep vesicular gland were determined by gas chromatography. Phosphatides extracted from the tissue were eluted separately from small columns of silicic acid and converted to methyl esters by methanolysis in the presence of sodium methoxide. Gas–liquid chromatography on EGSS-X gave values of equivalent chain lengths for the esters that were in close agreement with those obtained with DEGS (ref. 4). Tentative assignments were made after comparing equivalent chain length values of 9 saturated and polyunsaturated reference esters in the manner described by HOFSTETTER AND HOLMAN[4]. The compositions obtained in this way are presented in Table I.

TABLE I

FATTY ACID COMPOSITION OF VESICULAR GLAND PHOSPHOLIPIDS

Figures represent male %.

Fatty acid	*Phosphoglyceride fraction*	
	Choline	*Ethanolamine*
16:0	26.3	12.6
18:0	14.7	17.4
18:1 (n-9)	25.3	21.6
18:2 (n-6)	5.6	5.9
20:1 (n-9) + 18:3 (n-3)	0.8	0.9
20:2 (n-9) + 18:4 (n-3)	1.8	3.5
20:3 (n-6)	17.5	21.8
20:4 (n-6)	1.5	3.1
20:5 (n-3)	1.9	2.4
22:3 (n-9) + (n-6)	1.7	3.5
22:4 (n-6)	0.2	0.3
22:5 (n-6)	0	0.1
22:5 (n-3)	1.4	2.9
22:6 (n-3)	1.4	2.5

The high level of 20:3 and the low level of 20:4 are in marked contrast to the high level of 20:4 found in liver lecithins[5]. If phospholipid acids are the natural precursors of the prostaglandins *in vivo*, prostaglandin E_1 would be expected to be produced in greater abundance since its precursor is 20:3. A similar consideration[6] was applied to the prevalence in renal medulla of prostaglandin E_2 which is derived from the abundant acid, 20:4. The high relative amount of 20:3 in vesicular gland phosphatides was also reflected in a similar composition of the unesterified acids of the tissue. Non-esterified 20:3 was, however, found in amounts (about 10 nmoles/g) that were only 0.2–1.0% of that in phospholipids. We observed that the small amount of unesterified 20:3 in homogenates decreased under incubation conditions that led to prostaglandin E_1 synthesis.

Radioactive eicosatrienoate was synthesized as previously described[3,7].

The CoA thiol ester was prepared from the acid chloride by a modification[8] of the procedure described by SEUBERT[9]. 1-Palmitoyl-2-([2′-^{14}C]eicosatrienoyl)-glycero-3-phosphorylcholine was then synthesized from 1-palmitoylglycero-3-phosphorylcholine[10] and [2-^{14}C]eicosatrienoyl-CoA by the action of microsomal acyltransferase preparation from pig liver. The radioactive product which was purified by silicic acid chromatography contained $2 \cdot 10^5$ counts/min per μmole.

The radioactive lecithin was shown to be homogeneous by thin-layer chromatography on silica gel H plates using chloroform–methanol–water (65:35:5, by vol.). A small amount of the radioactivity (2–4%) appeared on the control thin-layer chromatographic plates as free acid even after repeated purifications of the lecithin and is most likely produced when evaporating and applying the washed lipid sample to the plate.

Sheep vesicular glands were homogenized in 3 vol. of phosphate buffer[11] and centrifuged at 8000 × *g* for 15 min. The supernatant fraction, which contained microsomes, was used for several incubations. In some cases, this preparation was further centrifuged at 100000 × *g* for 1 h to obtain a microsomal fraction which was used both with and without supplemental additions of the 100000 × *g* supernatant. The radioactive phospholipid (6000 counts/min) was incubated at 37° for varying periods of time and then all lipids were extracted with chloroform–methanol (2:1, v/v). The total lipid extract was applied to thin-layer plates containing silica gel G and developed with a solvent system modified from that recommended by GRÉEN AND SAMUELSSON[12]. The plate was first developed to 15 cm with benzene–dioxane–acetic acid (80:18:2, by vol.) and then after drying, to 4 cm with diethyl ether. The lipids were located by spraying 1% iodine in methanol over the plate. After the iodine evaporated, the regions were scraped into scintillation vials, suspended in aqueous dioxane[13] and counted in a scintillation counter.

When the radioactive phospholipid was incubated with the 8000 × *g* supernatant of a vesicular gland homogenate, unesterified prostaglandin E_1 was slowly produced. The amount of prostaglandin E_1 was increased by adding a 100000 × *g* supernatant fraction from rat liver or sheep vesicular gland homogenates. The results in Table II show that added supernatant increased the total free acids produced from the precursor lipid. Supernatant alone, which contains no cyclizing activity, led to an increase of radioactivity in only non-esterified 20:3. The radioactive

TABLE II

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF TOTAL RADIOACTIVE LIPIDS FROM INCUBATION MIXTURE

Enzyme preparations in Expt. A were the 8000 × *g* supernatant (VG) from sheep vesicular gland and a 100000 × *g* supernatant (LS) from rat liver. The control incubation was stopped immediately with chloroform–methanol, whereas the other tubes were incubated at 37° for 75 min. Enzyme preparations in Expt. B were microsomes (VGM) prepared from sheep vesicular gland and the 100000 × *g* supernatant (LS) from rat liver. The incubations were carried out at 37° for 1 h. Figures represent counts/min per fraction.

Band designation	*Expt. A*			*Expt. B*		
	Control	*VG+LS*	*LS*	*Control*	*VGM+LS*	*LS*
Solvent front	170	20	40	60	30	50
Normal acids	20	150	630	10	160	910
	10	50	10	10	140	0
Hydroxy acids	0	90	20	80	260	10
	0	30	10	10	4	10
Prostaglandins	10	390	20	50	570	30
	20	20	10	130	290	90
Phospholipids (Origin)	5140	3870	4600	3900	2940	3610

phospholipid remaining at the origin of the chromatographic plates was eluted with methanol and hydrolyzed at 37° for 45 min with 0.5 M NaOH in 75% methanol in water. The saponification mixture was acidified with acetic acid and treated with ether. The extracted acids derived from the phospholipid were chromatographed on silica gel G in benzene–dioxane–acetic acid (82:13:2, by vol.). 93% of the radioactivity (±3%) chromatographed with unreacted 20:3 and no other major component was detected. Significantly, the same result was obtained if the radioactive phospholipid was incubated with only buffer, or allowed to react with the vesicular gland preparation for less than 30 sec. These results, therefore, suggest that no newly synthesized prostaglandin is esterified to the phospholipid. The radioactive prostaglandins produced in incubation mixtures containing added supernatant fractions seem most probably to have been formed from the liberated 20:3 rather than from the esterified form. Similar results were obtained independently by VONKEMAN AND VAN DORP[14].

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