INCORPORATION OF [U-¹⁴C]PALMITIC ACID INTO THE PHOSPHOLIPIDS OF BOVINE SEMEN

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Fatty acids play a major rôle in the metabolism of spermatozoa. The endogenous respiration of these cells depends largely on the oxidation of hydrolysed acyl groups of plasmalogens (Hartree & Mann, 1961); endogenous fatty acids may also be utilized and are readily incorporated into the lipids of spermatozoa (Payne & Masters, 1968; Mills & Scott, 1969). This communication was initiated in order to provide further data on the turnover of individual phospholipids, and constitutes a preliminary report on the rate and specificity of incorporation of palmitic acid into the phosphatides of bovine semen.

Samples (1-ml) of freshly ejaculated semen were incubated under aerobic conditions at 32° C with [U-¹⁴C]palmitic acid (0.5 µC) in Krebs–Ringer phosphate buffer (pH 7.4) containing 1.1 ml of 3% bovine serum albumin (Calbiochem, Los Angeles, California), fructose (0.1 ml, 22 µmol) and 300 U of penicillin. After incubation for periods from 2 min to 2 hr, lipids were extracted with chloroform–methanol (2:1 v/v) according to the method of Folch, Lees & Sloane-Stanley (1957). Phospholipids were separated two dimensionally on thin layer plates (silica gel H. R. Merck, Darmstadt, Germany, 0.5 mm) using the solvent systems described by Owens (1966). Before development in the second dimension, plates were exposed to HCl fumes to hydrolyse plasmalogens. Individual phospholipids were identified by comparison with standard phosphatides (Applied Science Laboratories, State College, Pa., U.S.A.) under various chromatographic conditions. The distribution of the incorporated fatty acid was determined by autoradiography of the thin layer plates and areas of activity were scraped from the plates into vials containing 4 ml of scintillation fluid (0.4%, 2,5-diphenyloxazole, 0.01% 1,4-bis-[2-(5-phenyloxazolyl)]-benzene in toluene). Activity was measured in a Nuclear-Chicago liquid scintillation counter, efficiency of counting being corrected by use of an external standard.

It may be seen from the results (Table 1) that [U-¹⁴C]palmitic acid is actively incorporated into the phospholipids of bovine semen. When the spermatozoa and seminal plasma were separated by centrifugation after incubation, radioactivity was observed to be localized in the sperm lipids.

Of the individual phospholipid components of spermatozoa, phosphatidyl inositol was by far the most rapidly labelled. A high activity was evident within 2 min and continued to increase up to 1 hr after the commencement of incubation. Phosphatidic acid and phosphatidyl choline were also rapidly labelled,

though to lesser degrees. Incorporation of label into phosphatidic acid reached a maximum in 1 hr but the labelling of phosphatidyl choline continued to increase over the time scale investigated. Uptake of palmitic acid by the other phospholipid components was of a lower order of activity. Labelling of phosphatidyl serine was more rapid than that of phosphatidyl choline and minor labelling also occurred in phosphatidyl ethanolamine. Other phosphatides did not show activity. Analysis of the hydrolysed fatty acyl groups of labelled phosphatides by argentation and reversed phase thin layer chromatography (Morris, 1964) demonstrated that palmitic acid entered the various phosphatides without modification (i.e. no chain lengthening, desaturation, etc., was evident).

TABLE 1
INCORPORATION OF [U-¹⁴C]PALMITIC ACID INTO THE PHOSPHOLIPIDS
OF BOVINE SEMEN AT VARIOUS TIME INTERVALS

Phospholipid	Counts/min/10 ⁹ spermatozoa at:				
	2 min	15 min	30 min	60 min	120 min
Phosphatidyl ethanolamine	337	320	288	352	550
Phosphatidyl inositol	2154	12220	18006	22907	20606
Phosphatidyl serine	—	641	1106	2007	1947
Phosphatidyl choline	291	3926	7709	19157	33128
Phosphatidal choline	—	—	399	637	1127
Phosphatidic acid	—	4534	6352	19185	10352

Values are counts/min/10⁹ spermatozoa and are adjusted to 1×10^6 counts/min added at the commencement of incubation.

In regard to these results, it may be noted that phosphatidyl inositol, phosphatidic acid and phosphatidyl serine are minor components of sperm phospholipid, representing approximately 0.6%, 0.8% and 1.0% respectively of the total phospholipids in ejaculated ram spermatozoa (Scott, Voglmayr & Setchell, 1967). As such, they exhibit an extremely high specific activity for incorporation of label when compared to phosphatidyl choline and phosphatidyl ethanolamine which account for some 35% and 20% respectively of the phospholipids of bovine spermatozoa (Pursel & Graham, 1967).

A similar enhanced incorporation of isotope into these acidic phospholipids has been observed in processes requiring movement and activity of cell membranes, and it is of interest to consider the significance of the present findings in this light. Phagocytosis in polymorphonuclear leucocytes, for example, is accompanied by an increase in the turnover of these minor phospholipids as measured by the incorporation of inorganic ³²P (Karnovsky & Wallach, 1961). The major phosphatides, however, were not very active, and showed low specific activities in these studies. Again, acinar cells of the pancreas exhibit a marked increase in phosphatidyl inositol turnover as measured by the incorporation of inorganic ³²P, [1-¹⁴C]glycerol and [2-³H]inositol when stimulated by acetylcholine (Hokin & Hokin, 1958). Similarly, there is a rapid turnover of phosphatidic acid and phosphatidyl inositol when the avian salt gland is stimulated to secrete sodium chloride against a concentration gradient (Hokin & Hokin, 1964).

In view of these facts, then, it seems that the present findings which indicate an extremely active rôle for phosphatidyl inositol in the fatty acid uptake of bovine semen, may provide further insight into the mechanism of sperm motility. Initial studies with ram semen support these observations.

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REFERENCES

- FOLCH, J., LEES, M. & SLOANE-STANLEY, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**, 497.
- HARTREE, E. F. & MANN, T. (1961) Phospholipids in ram semen: metabolism of plasmalogen and fatty acids. *Biochem. J.* **80**, 464.
- HOKIN, L. E. & HOKIN, M. R. (1958) Phosphoinositides and protein secretion in pancreas slices. *J. biol. Chem.* **233**, 805.
- HOKIN, L. E. & HOKIN, M. R. (1964) *Interconversions of phosphatidyl inositol and phosphatidic acid involved in the response to acetylcholine in the salt gland*. In: *Metabolism and Physiological Significance of Lipids*, p. 423. Eds. R. M. C. Dawson and D. M. Rhodes. John Wiley, London.
- KARNOVSKY, M. L. & WALLACH, D. F. H. (1961) The metabolic basis of phagocytosis. III. Incorporation of inorganic phosphate into various classes of phosphatides during phagocytosis. *J. biol. Chem.* **236**, 1895.
- MILLS, S. C. & SCOTT, T. W. (1969) Metabolism of fatty acids by testicular and ejaculated ram spermatozoa. *J. Reprod. Fert.* **18**, 367.
- MORRIS, L. J. (1964) Specific separations by chromatography on impregnated thin-layers. *Lab. Pract.* **13**, 284.
- OWENS, K. (1966) A two dimensional thin-layer chromatographic procedure for the estimation of plasmalogens. *Biochem. J.* **100**, 354.
- PAYNE, E. & MASTERS, C. J. (1968) Incorporation of ^{14}C -labelled fatty acids into the lipids of bovine spermatozoa. *Life Sciences*, **7**, 935.
- PURSEL, V. G. & GRAHAM, E. F. (1967) Phospholipids of bovine spermatozoa and seminal plasma. *J. Reprod. Fert.* **14**, 203.
- SCOTT, J. W., VOGLMAYR, J. K. & SETCHELL, B. P. (1967) Lipid composition and metabolism in testicular and ejaculated ram spermatozoa. *Biochem. J.* **102**, 456.