THE DISTRIBUTION OF ^{14}C -LABEL IN THE LIPIDS OF RAM SEMEM FOLLOWING THE INTRA-TESTICULAR INJECTION OF $[1-^{14}\text{C}]$ PALMITIC ACID

A. R. NEILL AND C. J. MASTERS

Animal Research Institute, Yeerongpilly, Brisbane, Queensland 4105, and Department of Biochemistry, University of Queensland, St Lucia, Brisbane, Queensland 4067, Australia

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Summary. Two rams were injected intratesticularly with $[1-^{14}\text{C}]$ -palmitic acid, and the amounts and distributions of radiolabel in the sperm and seminal plasma lipids of ejaculates were monitored for 63 days. The residual label remaining in the epididymal spermatozoa and fluid and the testicular tissue of one animal was also examined.

After Day 17, two broad areas of high specific activity were observed in sperm lipids, with maxima occurring on Days 28 and 42. The pattern of labelling of seminal plasma lipids was similar, but the major peaks of activity occurred on Days 31 and 45. By Day 63, both sperm and plasma activities were low.

In spermatozoa, the radioactivity was largely confined to phospholipid fractions, with phosphatidylinositol showing high specific activity relative to other lipids in each ejaculate. The only neutral lipids to have measurable activity were 1,2-diglycerides, but the distribution of label among the various lipid classes altered considerably with time.

A detailed analysis of the distributions of label in ejaculates on Days 28 and 42 revealed that 10 to 12% of the ^{14}C in sperm lipids on each day was present in mono-unsaturated components. The labelling of plasmatogenic aldehydes accounted for 19.3% and 25.8% of the radioactivity in sperm phospholipids on Day 28, but only 9.9% and 9.8% on Day 42 in ejaculates from the two rams.

Testicular tissue differed markedly from epididymal and ejaculated spermatozoa on Day 63, in that triglycerides and 1,3-diglycerides showed high specific activity.

These findings have been discussed in relation to the processes of spermatogenesis in the ram.

INTRODUCTION

Although the incorporation of labelled fatty acid into the spermatozoa of ruminants has been studied previously under conditions *in vivo* (Dawson, 1958a, b), these earlier investigations were aimed principally at evaluating the significance of glycerylphosphorylcholine production in the epididymis, and the rate of synthesis of this component in relation to the appearance of

label in the total sperm lipids. Other workers have used isotopes to elucidate the rates of the various physiological processes occurring within the male reproductive tract, and these studies have yielded useful information on the cycles of germ cell development (Ortavant, 1954, 1956, 1959; Hochereau, Courot & Ortavant, 1964) and the rate of passage of the spermatozoa through the epididymis (Amir & Ortavant, 1968).

It was considered that this knowledge could provide a useful basis for the interpretation of the significance of the metabolism of individual lipids at the various stages of sperm development. It has been shown, for example, that major metabolic and compositional differences exist between the lipids of the whole testis and the testicular spermatozoa in the ram (Scott & Setchell, 1968), the most notable compositional difference being the large increase in the plasmalogen content of testicular spermatozoa. This contrasts with non-ruminant species such as the rat (Scott, Dawson & Rowlands, 1963) in which differences in lipid composition between the spermatozoa and the testis are comparatively small. In the ram, testicular spermatozoa are known to be metabolically more active than ejaculated spermatozoa, and there are marked changes in the phospholipid composition of spermatozoa as they move through the epididymis (Scott, Voglmayr & Setchell, 1967).

In view of these facts, the present study was undertaken. It was anticipated that radioactive label would be incorporated directly into germ cell lipids at the various stages of development occurring at the time of injection. Any delay in the appearance of label in ejaculated spermatozoa and any differences in the distribution of label within spermatozoa could then be interpreted in terms of the stage of spermatogenesis at which label was incorporated, and the initial and subsequent metabolism of sperm lipids before ejaculation.

MATERIALS AND METHODS

Animals and treatment

Two Merino rams (*Ovis aries*), designated A (54 kg body wt) and B (41 kg body wt), were selected from the flock at the Animal Research Institute, Yeerongpilly. The animals were housed in pens and allowed unrestricted access to a high quality lucerne chaff and water for the duration of the experiment (10 weeks, January to March, 1972). Semen was collected from both rams at 3- and 4-day intervals using the technique of electroejaculation. At the same times, blood was obtained from the jugular veins, coagulation being prevented by the use of heparin. In addition to the above collection periods, ejaculates were obtained 4 hr after the injection of isotope and blood was taken at 2, 6 and 24 hr. The animals were acclimatized to the experimental conditions 1 week before, and the experiment was terminated 63 days after, the administration of radioisotope.

Following the final collections on Day 63, Ram B was castrated. After removing all extraneous tissues and membranes, the epididymides were carefully detached from the testes. The caput and cauda regions were washed with saline and cut with a scalpel blade. The exudates from these areas were collected in small beakers, slight finger pressure being applied around the cut

to assist flow. Tissue contaminants were removed as completely as possible and the exudates diluted with saline.

Spermatozoa and plasma

After subsampling for estimation of sperm count and examination of sperm morphology, ejaculates were separated into plasma and cellular fractions by centrifugation at 450 *g* and 20°C for 20 min. The cells were washed twice with 0.89% (w/v) NaCl and the washings were discarded. The epididymal fluids and cells were separated by the same procedure, whereas blood plasma was obtained after removing the cells by centrifugation at 2070 *g* and 20°C for 10 min.

Lipid was extracted from the plasma samples and the washed spermatozoa by the procedure of Folch, Lees & Sloane Stanley (1957). Solvent was removed from the washed extracts by rotary film evaporation and the residues were dried *in vacuo* over P_2O_5 . Dried samples were stored for up to 7 days in chloroform-methanol (5 : 1, v/v) at -40°C before analysis. No changes occurred during storage.

Lipid fractionation

Lipid extracts, with the exception of those from the blood plasma and the spermatozoa and seminal plasma collected 4 hr after the injection of isotope, were subsampled and fractionated by thin-layer chromatography in order to determine the distributions of radiolabel among the various lipid components.

Neutral lipids were separated on 250- μm thick layers of silica gel G (Merck A.-G., Darmstadt, Germany) that had been activated at 110°C for 30 min. Hexane-diethyl ether-acetic acid (80 : 20 : 1, by vol.) was used as developing solvent. Phospholipids were separated on 500- μm layers of silica gel G that had been activated at 110°C for 1 hr. Plates were developed at 16 cm in chloroform-methanol-water (65 : 25 : 4, by vol.), dried under N_2 , and redeveloped completely (20 cm) in hexane-diethyl ether-acetic acid (80 : 20 : 1, by vol.) in order to remove neutral lipid from the initial solvent front.

Samples of several lipid extracts were methylated using the procedure of Metcalfe, Schmitz & Pelka (1966). The resulting methylated derivatives (methyl esters and dimethyl acetals) were chromatographed on thin-layer plates of silica gel G (250 μm) impregnated with 10% (w/v) AgNO_3 that had been activated at 110°C for 30 min. Benzene-diethyl ether (99 : 1, v/v) was used as developing solvent.

The proportion of label incorporated into the plasmalogenic aldehydes was also determined in several lipid extracts. Lipids were fractionated by two-dimensional thin-layer chromatography on 250- μm thick layers of activated silica gel G. The same solvent (hexane-diethyl ether-acetic acid, 80 : 20 : 1, by vol.) was used in both dimensions. After development in the first dimension, solvent was removed under dry N_2 and the phospholipids remaining at the origin were exposed to HCl fumes to hydrolyse plasmalogens as described by Viswanathan, Phillips & Lundberg (1968). Excess of HCl was removed by exposing the plates to a stream of dry N_2 for 1 hr. The free aldehydes were then separated from the phospholipids by the development of plates in the second dimension.

Radioactivities

Thin-layer plates were exposed to X-ray film (Kodirex No-screen, Kodak (Aust.) Pty Ltd, Melbourne, Australia). After development, areas of activity were identified and the silica gel containing the labelled lipids was scraped into vials containing 4 ml scintillator fluid (0.4% of 2,5-diphenyloxazole, 0.01% of 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene). Labelled methylated components were recovered from AgNO₃-impregnated silica gel by elution with diethyl ether into the counting vials. Solvent was removed by evaporation before the addition of scintillator fluid. Subsamples of total lipid extracts were also placed in counting vials and the solvent removed by evaporation. Radioactivity was measured by using a Nuclear-Chicago liquid-scintillation counter with absolute activities being determined by the channels ratio technique with the use of an external standard. Between 60 and 70% of plated radioactivity was recovered. Losses appeared to be independent of the lipid class and the results were corrected accordingly.

The identity of the various labelled lipid components was based on the comparative behaviour of standard lipids under the same chromatographic conditions. Specific radioactivity of the total lipid extracts was expressed as d/min/mg of lipid, whereas d/min/ μ mol of glycerol and P was calculated for glycerides and phospholipids respectively. Methods for the determination of these components have been published previously (Neill & Masters, 1972).

Preparation and administration of isotope

Labelled [1-¹⁴C]palmitic acid (1 mCi; 55 mCi/mmol) in benzene was supplied by The Radiochemical Centre, Amersham, Bucks., U.K. The solvent was removed and the acid was treated with 1 ml 0.6% NaOH in ethanol. The solution was heated in a water bath (60 to 70°C) to ensure reaction, after which residual solvent was removed by evaporation, and 5 ml of a salt solution (698.5 mg NaCl and 0.55 ml of 6 M-HCl in 100 ml water) was then added. On the basis of the above reaction being complete, this yielded a 0.89% (w/v) NaCl solution containing the sodium salt of the acid. The solution was emulsified by sonication at 50°C for 2 hr using a Transclean ultrasonic cleaner (Type 66).

The spermatic cords of both rams were each infiltrated with 2 to 3 ml of 2% (w/v) lignocaine hydrochloride (Astra Chemicals Pty Ltd, Chatswood, Aust.), and the radiolabel (total dose/animal of approximately 0.01 mCi/kg body wt) was then injected into the mid-region of each of the four testes (approximately 1.2 ml/testis). Measurement of residual radioactivity indicated that 95% of the total label prepared had been injected.

RESULTS

Less than 1% of the total radioactivity administered was recovered in the total lipid extracts of samples collected from each animal. The radioactivity in blood plasma declined rapidly in the initial stages followed by a slower rate of decline throughout the experiment (Text-fig. 1). The specific activities of blood plasma lipids measured 2 hr and 6 hr after injection were lower than those found at 24 hr, with the specific activity at 6 hr being similar to that at 3 days.

TEXT-FIG. 1. The radiolabelling of ram blood plasma lipids after the intratesticular injection of $[1-^{14}\text{C}]$ palmitic acid. Each ram was injected with approximately 0.01 mCi $[1-^{14}\text{C}]$ palmitic acid (Na^+ salt)/kg body wt. The ^{14}C was recovered in the blood plasma lipids of Ram A (—), 54 kg body wt, and Ram B (---), 41 kg body wt.

TEXT-FIG. 2. The radiolabelling of ejaculated ram sperm lipids after the intratesticular injection of $[1-^{14}\text{C}]$ palmitic acid. Each ram was injected with approximately 0.01 mCi $[1-^{14}\text{C}]$ palmitic acid (Na^+ salt)/kg body wt. The ^{14}C was recovered in the sperm lipids of Ram A (—), 54 kg body wt, and Ram B (---), 41 kg body wt.

Table 1. The distribution of ^{14}C in the lipids of ejaculated spermatozoa of Rams A and B after intratesticular injection of $[1-^{14}\text{C}]$ -palmitic acid

Days after injection	Specific activity (d/min/ μmol)											
	1,2-Diglycerides		Total phospholipid		Cardiolipin, phosphatidic acid and cerebroside		Ethanolamine phosphoglyceride		Choline phosphoglyceride		Sphingomyelin	
	A	B	A	B	A	B	A	B	A	B	A	B
3	—*	62100	—	300	300	—	100	—	100	—	4100	—
7	8500	500	—	—	—	—	—	—	—	—	2700	1300
10	2600	1000	—	—	—	—	—	—	—	—	—	700
14	600	13800	—	—	—	—	1400	—	—	—	4200	4400
17	600	3300	100	600	—	—	200	1900	100	1400	5200	10900
21	27100	36700	3200	6200	—	—	2600	7100	3800	1000	900	22400
24	14300	22500	3100	5500	4300	—	2100	3600	900	3500	1000	176100
28	33900	28900	7400	9000	—	—	8200	8500	6600	7700	3000	43900
31	13900	25200	4900	7200	—	—	—	2700	800	—	4500	74900
35	9500	23900	4400	8500	17500	—	6300	3700	3000	11800	—	241000
38	25800	25200	6800	3900	—	—	3800	7300	3000	10400	3000	61500
42	32700	65700	8600	11600	17800	—	1700	1100	6300	5800	5800	155200
45	11400	8800	4900	4700	—	—	5700	11600	6800	10000	5600	44100
49	10200	8400	3800	4700	2400	—	2200	4200	3000	7300	74900	40800
52	9300	8000	2100	2100	5400	—	2800	3500	3600	4800	3800	9500
56	8700	3900	1400	1200	—	—	1300	1600	1600	4100	4000	7500
59	4300	4300	1300	1400	2100	—	1300	900	1100	2100	1700	5100
63	6600	7000	1000	1100	3500	—	900	700	700	3500	3100	8500
							500	600	600	2700	3400	5300

The rams (A, 54 kg and B, 41 kg body wt) were injected with approximately 0.01 mCi $[1-^{14}\text{C}]$ palmitic acid (Na^+ salt)/kg body wt. Results are expressed as d/min/ μmol of glycerol and P for diglycerides and phospholipids respectively. Concentrations of lipids were based on previous estimates (Neill & Masters, 1973). Radioactivity was observed in:

lysophosphatidylcholine on Days 24, 38 and 42 in Ram A and Days 7, 17, 31, 38 and 42 in Ram B;
triglycerides on Days 38, 52 and 56 in Ram A and Days 17, 31, 35, 38, 52 and 56 in Ram B;
free fatty acids on Days 3, 7, 10 and 38 in Ram A and Days 3, 7, 10, 17 and 38 in Ram B.

* Radioactivity was observed but not measured.

The distribution of ^{14}C in ejaculated sperm lipids

Little radioactivity was present in ejaculates collected to Day 17 after the injection of label (Text-fig. 2). Thereafter, two broad peaks of activity were evident for the serially collected samples of spermatozoa to Day 63, the first reaching a maximum on Day 28 and the second having a higher maximum on Day 42 in each animal. After Day 49, activity fell rapidly to levels observed initially. Throughout the experiment, spermatozoa were motile and no morphological abnormalities were apparent.

Table 2. Distribution of ^{14}C within the combined methyl esters and dimethyl acetals of ejaculated ram sperm lipids after intratesticular injection of $[1-^{14}\text{C}]$ palmitic acid

Methylated component	Radioactivity (%)			
	Spermatozoa from Ram A		Spermatozoa from Ram B	
	28 days*	42 days	28 days	42 days
Saturated	82.0	84.8	75.9	81.7
Mono-unsaturated	9.6	10.1	11.9	11.6
Origin	8.4	5.1	12.2	6.7

Labelled lipid extracts of washed spermatozoa were methylated and the methylated derivatives were separated by argentation thin-layer chromatography. The results are expressed as a percentage of the total radioactivity recovered.

* Days after injection.

Table 3. The radiolabelling of the plasmalogenic aldehydes of ejaculated ram sperm phospholipids after intratesticular injection of $[1-^{14}\text{C}]$ palmitic acid

Days after injection	Radioactivity in aldehydes (%)	
	Spermatozoa from Ram A	Spermatozoa from Ram B
28	19.3	25.8
42	9.9	9.8

Neutral lipids were separated from phospholipids by thin-layer chromatography, plasmalogens were hydrolysed by HCl fumes and free aldehydes were separated from phospholipids by development of plates in the second dimension. The results are shown as the radioactivity in aldehydes expressed as a percentage of the radioactivity in the unhydrolysed phospholipid.

From the results presented in Table 1, it is evident that the specific activities of sperm phospholipids in general reflected the specific activities of the total sperm lipids (Text-fig. 2). However, the relative significance of labelled lipid fractions within each subsequent ejaculate differed markedly. The activity in sperm lipids to Day 10 was confined mainly to free fatty acids, diglycerides

and phosphatidylinositol. Ethanolamine phosphoglycerides from the spermatozoa of each ram exhibited highest specific activity on Day 28, and choline phosphoglycerides on Day 42, these maxima corresponding to the peaks of activity in the total sperm lipids (Text-fig. 2). The maximum activity in phosphatidylinositol, however, was evident on Day 31. This particular phospholipid had high specific activity throughout the whole experiment. Except on Days 7 and 21 in spermatozoa from Rams A and B respectively, the fraction containing cardiolipin, phosphatidic acid and cerebrosides was labelled in ejaculates collected from Day 35 to Day 63 only. The specific activity of sphingomyelin was variable but, in general, it increased relative to that of other lipid fractions,

TEXT-FIG. 3. The radiolabelling of ram seminal plasma lipids after the intratesticular injection of $[1-^{14}\text{C}]$ palmitic acid. Each ram was injected with approximately 0.01 mCi $[1-^{14}\text{C}]$ palmitic acid (Na^+ salt)/kg body wt. The ^{14}C was recovered in the seminal plasma lipids of Ram A (—), 54 kg body wt, and Ram B (---), 41 kg body wt.

particularly in the later stages of the experiment. The lipoprotein fractions also contained low activity. Phosphatidylserine, however, could not be resolved from the major phospholipid classes under the conditions of analysis. The specific activity of sperm 1,2-diglycerides was highest on Days 28 and 42 for Rams A and B respectively. No other sperm lipids contained radioactivity except as indicated in Table 1.

The extent of metabolism of labelled palmitic acid in the sperm lipids collected on Day 28 and Day 42 was also studied. Methylated derivatives (esters and acetals) were formed and analysed by argentation thin-layer chromatography (Table 2). Saturated components predominated, there being slightly increased proportions of these in the spermatozoa ejaculated on Day 42. Significant and similar amounts of label were recovered in monosaturated components on each day. The origin of chromatograms also contained activity, which decreased in significance on Day 42. The component containing this

activity at the origin was not identified, but was probably autoxidized fatty acid. A high proportion of the label was recovered in plasmalogenic aldehydes (Table 3), this having greater significance in ejaculates collected on Day 28 compared with Day 42.

The distribution of ^{14}C in seminal plasma lipids

The specific activity of seminal plasma lipids increased markedly after Day 17 (Text-fig. 3), with some variability evident in the fluids collected from each ram; however, corresponding peaks of activity were evident in ejaculates from each animal on Days 31 and 45. Analysis of the seminal plasma lipids revealed that, after Day 17, radioactivity was confined to choline and ethanolamine phosphoglycerides and sphingomyelin.

The distribution of ^{14}C in testicular and epididymal sperm lipids

After the final collections on Day 63, Ram B was castrated, and the testicular and epididymal sperm lipids were recovered in order to determine the distribution of residual ^{14}C -label. The lipids of the epididymal fluids expressed from the head and tail of the epididymis were also analysed. The specific activities of the lipid extracts were: testis, 300 d/min/mg of lipid; spermatozoa and fluid from the head of the epididymis, 500 and 600 d/min/mg of lipid; and spermatozoa and fluid from the tail of the epididymis, 900 and 700 d/min/mg of lipid, respectively.

As with the seminal plasma lipids, label in the epididymal fluids was confined to the ethanolamine and choline phosphoglycerides and sphingomyelin. The percentage distributions of radioactivity in the lipids of these fluids were: ethanolamine phosphoglycerides, 10.6 and 19.6; choline phosphoglycerides, 33.3 and 25.0 and sphingomyelin, 56.1 and 55.4, for the head and tail of the epididymis respectively.

Within the testis, the specific activity of the 1,3-diglycerides was 67-, 4- and 2-times that of the total phospholipids, 1,2-diglycerides and triglycerides respectively. This contrasted with the labelling of spermatozoa from both sites within the epididymis where neither 1,3-diglycerides nor triglycerides contained any detectable radioactivity.

DISCUSSION

Previous workers have established that radiolabelled fatty acids are rapidly incorporated into the pools of testicular lipid metabolism after being injected directly into the testis: much of the fatty acid is then actively catabolized and/or transported out of the testis within a comparatively short time, but significant radioactivity is still present in the testis after several weeks (Nakamura & Privett, 1969; Bridges & Coniglio, 1970; Coniglio, Bridges, Aguilar & Zseltvay, 1972). In the present work, some of the [$1\text{-}^{14}\text{C}$]palmitic acid which was injected into the ram testis would have been incorporated into the metabolism involving the synthesis of lipids in the developing germ cells and accessory gland secretions. Soon after injection, labelled lipids would appear in these cells and secretions in the reproductive tract and the time taken for the recovery of radio-

activity in ejaculated semen may be correlated with the physiological processes affecting spermatozoa and plasma within the reproductive tract.

In this connection, it may be noted that spermatogenesis in the ram takes about 49 days and can be divided into cycles of 10.4 days (see Roosen-Runge, 1962). The spermatozoa are shed into the lumen of the seminiferous tubules and transported to the efferent ducts and thence to the epididymis. Epididymal passage takes from 11 to 13 days with only slight variation according to frequency of ejaculation (Amir & Ortavant, 1968).

In the present work, the radioactivity of accessory gland secretions could be expected to parallel blood plasma activity. The analysis of ejaculates to Day 17 indicates that the incorporation of labelled fatty acid into spermatozoa during epididymal passage or as the result of the metabolism of seminal plasma

Table 4. The contributions of individual phospholipid classes to palmitoyl and stearoyl phospholipid species of ram testis and spermatozoa

Phospholipid	Palmitoyl species in:		Stearoyl species in:	
	Testis	Spermatozoa	Testis	Spermatozoa
Phosphatidylcholine	60	76	47	12
Phosphatidylethanolamine	9	6	20	39
Phosphatidylinositol	6	3	16	4
Sphingomyelin	3	21	7	37

The contribution of each phospholipid class to the fatty acid ester and amide content of the total phospholipid in both testis and ejaculated spermatozoa was calculated from published data (Scott & Setchell, 1968; Scott *et al.*, 1967). These findings, together with information on the percentage composition of fatty acids in the phosphoglycerides and sphingomyelin of testis and spermatozoa (Neill, 1972; Neill & Masters, 1973), were used to estimate the distributions of palmitic and stearic acids among phospholipid classes. The fatty acid ester contributions of alkyl ether lipid to particular phosphoglycerides were apportioned on the basis of plasmalogen distributions.

The results are expressed as the percentage contribution of palmitic acid or stearic acid in individual phospholipid classes to the total pools of each acid in testis and sperm phospholipid.

lipids after ejaculation was minimal; consequently the marked increase in the radioactivity of semen after Day 17 would seem to have originated as a result of the metabolism associated with spermatogenesis.

From these data, it is possible to calculate that spermatozoa with a high specific activity on Day 28 would have been undergoing the initial stages of spermiogenesis soon after the injection, and spermatozoa ejaculated on Day 42 would have been entering the pachytene state of meiosis at about the same time.

In a previous study of the incorporation of fatty acids into spermatozoa, Dawson (1958a) found that a single peak of activity for both labels occurred in sperm lipids 21 days after the simultaneous intravenous injection of [^{32}P]P_i and [1- ^{14}C]stearate in a ram. The peak position varied from 21 to 26 days in four rams injected with [^{32}P]P_i alone; the dual label experiment was discontinued apparently after 35 days when the ^{14}C activity in the spermatozoa was rising again. The timing of these maxima differ somewhat from those obtained for palmitate in the present experiments (28 and 42 days), but several observations derived from the available literature on lipid compositions in ram spermatozoa and testis (Scott *et al.*, 1967; Scott & Setchell, 1968; Neill, 1972; Neill

& Masters, 1973), suggest that palmitate and stearate would be metabolized differently within the reproductive tract of the ram. Phosphoglycerides, for example, are the major esterified lipids of both spermatozoa and testis, but the data in Table 4 indicate that a greater difference occurs in stearate species between testis and spermatozoa than in the case of palmitate species. With sphingomyelin species, which form the other major phospholipid component of the spermatozoa, the marked relative increase in sperm content is similar for each of these fatty acid constituents. Assuming that the cellular lipid within the seminiferous tubules contributes significantly to the total testis lipid, then these results indicate that the aim of metabolism during the later stages of sperm development within the reproductive tract would be to increase the levels of stearylphosphatidylethanolamine, and to a lesser extent palmitoylphosphatidylcholine, in ejaculated spermatozoa. This could occur by one or more of the following processes; increased synthesis of these species during spermiogenesis, their selective retention by the spermatids in association with the lipid cycle of the Sertoli cells, and their selective retention by spermatozoa during maturation within the epididymis.

Additionally, after the injection of labelled palmitate, proportionally greater activity was recovered in the ethanolamine phosphoglycerides of spermatozoa undergoing spermiogenesis soon after injection, than in spermatozoa at an earlier stage of development. The reverse relationship was found with choline phosphoglycerides. Although different fatty acid species of the same phospholipid may have different turnover rates (Collins, 1963), these present observations, when taken in conjunction with the above information, are again consistent with the apparent disparity between stearate incorporation (Dawson, 1958a) and palmitate incorporation in ram spermatozoa *in vivo*.

Although the differences in the activities of lipid species in spermatozoa from various ejaculates appear to indicate differences in lipid metabolism at the various stages of spermatogenesis, it is difficult to ascertain the contribution of the relative turnover of these individual species in the present context. Obviously, the more stable lipids may appear increasingly significant in later ejaculates, and such behaviour could explain the consistently higher proportions of label in the sphingomyelin fraction of sperm lipids collected after Day 42. Conversely, those lipids which had a very rapid turnover would be expected to contain little or no activity in the ejaculated spermatozoa.

The activity of 1,2-diglycerides and phosphatidylinositol in all ejaculates was, however, contrary to this latter proposal. Phosphatidylinositol is known to exhibit marked metabolism in processes associated with the movement and activity of cell membranes (Dawson, 1966), and contains very high specific activity in ram testis after the intra-arterial injection of $[^{32}\text{P}]\text{P}_i$ (Scott & Setchell, 1968). The rapid incorporation of fatty acids into this phospholipid and the 1,2-diglycerides of spermatozoa *in vitro* (Neill & Masters, 1972, 1973) was suggested as being associated with rapid cytostructural rearrangements within the cell membrane. From present observations, the significance of the metabolism of these lipids appears to be established during spermatogenesis also, and is consistent with the complex morphological changes during sperm development.

The dynamic turnover of plasmalogens is regarded as slow compared with most lipids. The higher proportion of ^{14}C in sperm vinyl ethers on Day 28 than on Day 42 suggests, therefore, an active synthesis of plasmalogens towards the final stages of sperm development within the reproductive tract. Since the plasmalogen content is much higher in testicular spermatozoa than in testicular tissue (Scott & Setchell, 1968), plasmalogen synthesis would be most active during spermiogenesis.

Within ejaculated semen, the peaks of plasma activity closely paralleled the major peaks of sperm activity. These phenomena appear to be interrelated; otherwise, a single peak of activity corresponding to the metabolism in secretory processes at the time of injection would have been expected. Analyses within the epididymis point to the epididymal fluid as the major source of seminal plasma activity. The possible absorption or dissolution of spermatozoa within the epididymis (Amann & Almquist, 1962) may offer, in part, an explanation for these findings; however, reasons for the delay of one collection period between corresponding activities in the spermatozoa and plasma remain obscure.

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