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Role of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa

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Spermatozoa are produced in the testis and undergo post-gonadal modifications in the epididymis to acquire fertilizing ability. In epididymal plasma, high-molecular-weight proteins and such small molecules as free-L carnitine convert the gametes into 'competent' and functional cells. This review summarizes the knowledge pertaining to L-carnitine and the significance of free L-carnitine uptake into the mature spermatozoa of mammals. We provide an overview of the function of free L-carnitine and carnitine esters in the metabolism of eukaryotic cells and review the role of the specific carnitine acyltransferases in mitochondrial transport of fatty acids and in modulating acyl-coenzyme A (CoA) pools in cellular organelles. In mammals, including man, free L-carnitine is taken from blood plasma and concentrated in the epididymal lumen. This epididymal secretion is beneficial for spermatozoa and is not merely an excretory waste. The uptake of free L-carnitine into the spermatozoa and its metabolic outcome are discussed first in in-vivo and then in in-vitro situations. Free L-carnitine goes through the sperm plasma membrane by passive diffusion. Free L-carnitine is acetylated in mature spermatozoa only. The excess acetyl-CoA from the mitochondria is probably stored as acetyl-L-carnitine and modulates the reserves of free CoA essential to the function of the tricarboxylic acid cycle. These properties of L-carnitine of buffering CoA in the mitochondrial matrix are known in somatic cells but are accentuated in this study of the male germinal cells. In the future, a precise measurement of the in-vivo and in-vitro concentrations of free CoA and acetyl-CoA in the cellular compartments of immature and mature spermatozoa might complete these data. The relationship between the endogenous pools of free and acetylated L-carnitine and the percentage of progressive sperm motility indicates a more important metabolic function related to flagellar movement. In conclusion, the potential to initiate sperm motility, which takes place in the epididymis, is probably independent of the carnitine system, while the energy properties of acetyl-L-carnitine can only be relevant in situations of 'energy crisis'. The uptake of 'cytoplasmic' free L-carnitine in mature spermatozoa must be a protective form of mitochondrial metabolism, useful to the survival of this isolated cell.

Key words: acetyl-L-carnitine/epididymal maturation/free CoA/free L-carnitine/mammalian spermatozoa/sperm motility

Introduction

In all mammals, including man, most spermatozoa that leave the testis are infertile. The fertile spermatozoa meet, recognize and fertilize oocytes after several successive biochemical changes during their transit through the male and female genital tracts. This post-gonadal maturation represents the last step of cellular differentiation that changes gonocytes into fertile spermatozoa. In the testis, the genome of the germ

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cell controls the successive cellular transformations and the surrounding somatic cells complete these modifications.

In the male genital tract, the biochemical modifications to spermatozoa occur mainly during epididymal transit. The major changes that have been studied are (i) the relationship between the proteins secreted in the epididymal fluid and the sperm plasma membrane—some mechanisms of transduction have been described; (ii) the increase in nuclear chromatin condensation; (iii) the changes in the periaxonemal and axonemal structures related to the initiation of sperm movement; (iv) the changes of metabolic pathways and enzyme activities of the spermatozoa, which are sometimes stored for several months in the cauda epididymis.

The expression of each modification implicated in the fertilizing ability of spermatozoa results in a heterogeneous cellular population, which progressively becomes more homogeneous. Thereafter, the result of post-gonadal maturation is finally expressed when gamete interaction occurs. The final quality of a single spermatozoon determines whether a fertilized or unfertilized oocyte is the result.

The post-gonadal maturation occurs mainly in the epididymal fluid, where spermatozoa are bathed in plasma containing factors of testicular and epididymal origin. The epithelium of the epididymis takes up material from the blood, removes some testicular factors and produces specific compounds (mainly proteins) which are secreted in the epididymal plasma. Functional modifications of the spermatozoa result from exchanges between the epididymal plasma and spermatozoa, which have been analysed by different experimental approaches as reported in the literature. The study of the mechanism of action of molecules found in the epididymal plasma has intensified in the past decade. Schematically, the main sperm modifications studied by cellular physiologists have included flagellar motility, nuclear chromatin condensation, ability to undergo gamete interaction and effects on embryonic viability. The biochemical studies have mainly concerned the separation and purification of macromolecules (proteins) and the identification of small molecules (ionic or not) involved in the epididymal maturation of spermatozoa (Dacheux *et al.*, 1989, 1990). Free L-carnitine is a highly polar, water-soluble, small quaternary amine (molecular weight = 162) that is classified as a zwitterion. Free L-carnitine is mainly known for its biological importance in mitochondrial β-oxidation of long-chain fatty acids (Fritz, 1963). In mammalian metabolism, all the major roles of free L-carnitine involve conjugation of acyl residues to the β-hydroxyl group on the carnitine molecule, with subsequent translocation from one cellular compartment to another (Marquis and Fritz, 1964). The acetylated form of L-carnitine, acetyl-L-carnitine, is the major acylcarnitine found in animal tissues (Bieber *et al.*, 1982).

In mammalian epididymis, free L-carnitine is taken up from the blood plasma, transported into the epididymal plasma and then into the spermatozoa, where it accumulates as both free and acetylated L-carnitine. The concentrations of free L-carnitine in epididymal plasma and spermatozoa are the highest in the organism (2–100 mmol/l, see Table II). In contrast, for example, the concentrations of free L-carnitine are 50 μmol/l, 1–5 μmol/g and 0.2 mmol/g in rat blood, muscle and testis respectively (Bohmer and Molstad, 1980). Moreover, the initiation of sperm motility occurs in parallel with the increase in concentration of free L-carnitine in the epididymal lumen.

This review summarizes the knowledge concerning (i) the metabolism of free L-carnitine and acylcarnitine esters in eukaryotic cells, epithelial cells from the male genital tract and spermatozoa; (ii) the role of free and acetylated L-carnitine within mammalian spermatozoa during their epididymal maturation; (iii) the relationship between the initiation of sperm motility and the gradual increase in concentration of these molecules outside and inside these cells. Some hypotheses for the mechanism of regulation of this system are proposed.

Metabolism of free L-carnitine and acylcarnitine esters in eukaryotic and germinal cells

Eukaryotic cells

Free L-carnitine (β-hydroxy-γ-*N*-trimethylaminobutyric acid) was first isolated from beef muscle in 1905 and its structure was firmly established in 1927. It has been shown to be an essential nutrient (vitamin) for the mealworm *Tenebrio molitor (*Fraenkel, 1948) and a growth factor for a mutant yeast strain, *Candida pintolopesii* ATCC 26014 (Travassos *et al.*, 1961; Travassos and Sales, 1974; Green and Lewin, 1993). In fact, these properties have a common element because free L-carnitine is known to control transport of acetyl and acyl groups across the mitochondrial inner membrane. Free L-carnitine may be obtained from food or produced from lysine and methionine by liver, kidney and brain. Its concentration in blood is regulated and stable, ~10–50 μmol/l in rats (Hinton and Setchell, 1980; Marciani *et al.*, 1991) and humans (Li *et al.*, 1992).

Only the L isomer of carnitine is biologically active. In mammals, the concentration of this component and its derivatives within an organ or a cell is called the ‘total carnitine concentration’ or ‘total carnitine pool’. This comprises both free L-carnitine and acylcarnitine esters resulting from reaction with different fatty acids catalysed by carnitine acyltransferases. Table I summarizes different types of acyltransferases identified in cellular compartments and in different cells. The acyltransferases are specific for the length of the fatty acid chains. The reaction of acylation is as follows:

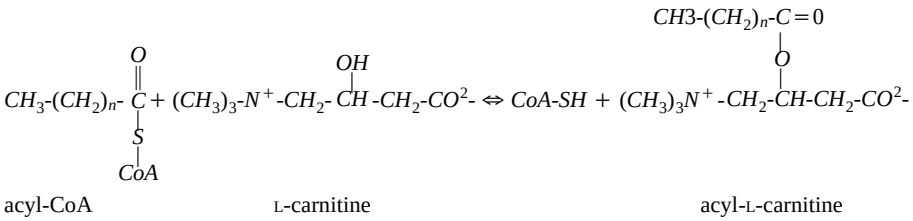

Table I. The carnitine acyltransferases. From Ramsay and Arduini (1993)

Acronym	Name	Location	Acyl chain specificity	Malonyl-CoA sensitivity
CAT	carnitine acetyltransferase	mitochondria, peroxisomes endoplasmic reticulum soluble or membrane bound	C_2–C_6	no
CPT-1	outer carnitine palmitoyltranferase	mitochondria, facing the cytosol (probably the outer membrane)	C_6–C_{18}	yes
CPT-2	inner carnitine palmitoyltransferase	mitochondrial inner membrane	C_8–C_{18}	no
COT	peroxisomal carnitine octanoyltransferase	peroxisomes (membrane-bound?)	C_6–C_{22}	yes
CPT (ER)	microsomal carnitine acyltransferase	endoplasmic reticulum	C_8–C_{22}	yes
CPT (RBC)		plasma membrane	C_6–C_{22}	yes
CPT(SR)		sarcoplasmic reticulum	C_6–C_{22}	yes
	carnitine/acylcarnitine translocase	mitochondrial inner membrane	C_2–C_{22}	no

ER = endoplasmic reticulum; RBC = red blood cells; SR = sarcoplasmic reticulum.

The inner mitochondrial membrane is not permeable to long-chain fatty acids or acyl-coenzyme A (CoA) derivatives. Acylcarnitine esters transport these fatty acyl groups across the inner mitochondrial membrane and thus play a crucial role in regulating β-oxidation. Figure 1 summarizes the exchanges of these components between the extracellular compartment and plasma membrane, cytoplasm, outer and inner mitochondrial membranes and matrix of eukaryotic cells.

The transport of free L-carnitine through the plasma membrane

Physiological concentration of free L-carnitine (μmol/l) is achieved by uptake from the blood using a specific and active transport mechanism through the plasma membrane of numerous tissues. Most of these tissues use free fatty acids as a major energy source (cardiac and skeletal muscles, for example). The kinetic characteristics of free L-carnitine accumulation differ according to the type of tissue. Active transport of free L-carnitine has an ionic regulation which is very often sodium dependent (Borum, 1983; Brass, 1992). In contrast, both enteral carnitine administration in humans and in-vitro studies of free L-carnitine transport in human muscle cell culture showed that pharmacological concentrations (mmol/l) accumulated slowly by passive diffusion in organs and cells (Li *et al.*, 1990, 1992; Tein *et al.*, 1990; Marciani *et al.*, 1991; Martinuzzi *et al.*, 1991). Free L-carnitine thus enters cells via either passive or active transport, depending on whether physiological or pharmacological concentrations of the molecule are present (Figure 1).

The carnitine acyltransferases and their role in modulating acyl-CoA pools

The total pool of L-carnitine accumulated by eukaryotic cells is distributed among different intracellular compartments (Figure 1). The ratios of acylcarnitine esters and free CoA are variable within the cytoplasm and mitochondrion. The concept of modulating the mitochondrial ratio of acyl-CoA:free CoA by free L-carnitine is now well established in eukaryotic cells (Brass and Hoppel, 1980; Lysiak *et al.*, 1988). The role of free L-carnitine in the regulation of CoA concentration within the mitochondrial matrix is summarized in Figure 1.

Each carnitine acyltransferase catalyses the acyl transfer between CoA and the carnitine pools in a distinct cellular compartment, because each is specific for an individual organelle and membrane and responds to local energy needs. Briefly, carnitine acetyltransferase activity (CAT) regulates the transport of short-chain fatty acids (C_2–C_6)

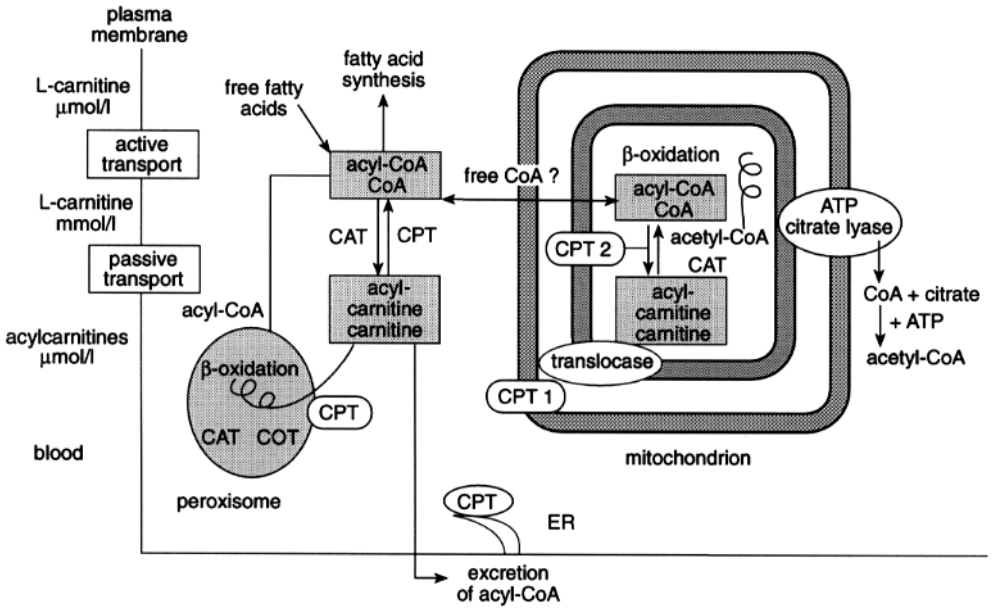

Figure 1. Metabolism of free L-carnitine, free coenzyme A (CoA) and the location of the carnitine acyltransferases in the eukaryotic cell. The abbreviations for the acyltransferases are given in Table I. Other abbreviations: ER, endoplasmic reticulum, ATP, adenosine triphosphate.

and is located on the inner face of the mitochondrial matrix (Table I and Figure 1). This activity is also linked to endoplasmic reticulum and peroxisomes of eukaryotic cells. Carnitine octanoyltransferase activity (COT) is found only in peroxisomes. In mitochondria, COT activity is associated with mitochondrial CAT and CPT and the existence of a separate enzyme has not been demonstrated (Clark and Bieber, 1981). COT has a broad substrate specificity (C_6–C_{22}), with the highest activity towards hexanoyl-CoA, but is also active against the longer chain acyl derivatives. Carnitine palmitoyltransferase activity (CPT), which facilitates fatty acid transport (C_6–C_{18}), is represented by CPT-1 and CPT-2, which are located on the outer and inner mitochondrial membranes respectively. Other CPT activities have been located in endoplasmic and sarcoplasmic reticulum and, more recently, in the plasma membrane of red blood cells (RBC). CPT_{RBC} is implicated in fatty acid turnover of membrane phospholipids of red blood cells (see the review by Ramsay and Arduini, 1993). A precursor for fatty acid synthesis, malonyl-CoA, inhibits all of these enzymes except CAT, CPT-2 and translocase (Table I). The molecular genetics of the components of the carnitine system are now known. A cDNA encoding for the CAT of the yeast *Saccharomyces cerevisiae* has been isolated by screening a yeast cDNA λgt 11 library with antibody. The coding sequence consists of 670 residues, which amount to a molecular mass of 77 300 kDa. A search in the protein database revealed that, besides the known carnitine acyltransferases, choline acyltransferases are highly homologous to yeast CAT (Kispal *et al.*, 1993). The ethanol-inducible *YAT1* gene from *S. cerevisiae* encodes a presumptive mitochondrial outer membrane CAT which is a protein of 688 amino acids displaying significant sequence similarity to vertebrate L-carnitine acyltransferases and yeast inner mitochondrial membrane L-CAT (Schmalix and Bandlow, 1993).

The intracellular regulation of carnitine transport by mitochondrial translocase

β-oxidation of fatty acids is accomplished within the mitochondrial matrix (Figure 1). Only the long-chain acylcarnitines generated outside the inner mitochondrial membrane are able to carry long-chain fatty acyl groups into the matrix, where exchange of acyl group between acylcarnitine and acyl-CoA produces free L-carnitine. This free L-carnitine is exported out of the mitochondrion to the cytoplasm by translocase activity, which exchanges carnitine for acylcarnitine (see the review by Ramsay and Arduini, 1993).

Relationship between CAT activity and mitochondrial free CoA concentration

In general, lack of CoA can limit the mitochondrial capacity for energy production. The CoA concentration regulates numerous enzyme activities implicated in metabolic pathways (tricarboxylic acid cycle, β-oxidation, detoxification of organic acids, oxidative degradation of amino acids) and is a substrate for pyruvate dehydrogenase. In particular, pyruvate dehydrogenase is inhibited by a high

acyl-CoA:CoA ratio (Bremer, 1969). Free L-carnitine has been shown to relieve this inhibition concomitant with the decrease in the acyl-CoA:CoA ratio (Brass, 1992). Two examples illustrate this system of regulation: (i) the importance of free L-carnitine and CAT *in vivo* is apparent from the parallel accumulation of acetyl-CoA and acetyl-L-carnitine during muscular exercise (Constantin-Teodosiu *et al.*, 1991); (ii) it has been demonstrated that carnitine administration results in the activation of pyruvate dehydrogenase and hence improved glucose disposal in diabetics (Calpaldo *et al.*, 1991). In cardiac cells, acetyl-L-carnitine in the mitochondrial matrix is exported (via translocase) to the cytoplasm with other acylcarnitines. The CAT activity located in endoplasmic reticulum and/or peroxisomes changes cytoplasmic acetyl-L-carnitine to free L-carnitine and acetyl-CoA, which is used for synthesis of malonyl-CoA, a precursor for fatty acid synthesis. The cytoplasmic accumulation of acetyl-CoA from the mitochondria thus causes a decrease in the amount of CoA available to activate free fatty acid.

Table II. Concentrations of free L-carnitine (mmol/l) measured in the rete testis and epididymal plasma of different species

Species	Rete testis	Epididymis										Ref.
		Initial	Caput			Corpus			Cauda			
		segment	Proximal	Median	Distal	Proximal	Median	Distal	Proximal	Median	Distal	
Guinea pig	–	–	–	–	–	–	–	–	–	–	67	1
Hamster	–	–	–	–	–	–	–	–	–	–	30	1
	<1	–	–	–	–	–	–	2.4	8.7	–	10.1	2
	–	–	–	0.019	–	–	0.015	–	–	0.36	–	6
Rat	–	–	–	–	–	–	–	–	–	–	54	1
	<1	–	<1.3	–	19.1	–	22.2	30.8	51.7	–	53.7	2
	–	–	–	–	–	–	–	–	–	–	63	9
Rabbit	–	–	–	–	–	–	–	–	14.5	–	19.0	2
	–	–	–	–	–	–	–	–	–	–	43	1
Ram	–	–	–	–	–	–	–	–	–	–	17	1
	0.03	–	–	–	–	–	–	11.2	10.6	–	14.5	2
	–	–	–	–	–	–	–	–	–	–	12.6	4
	0.1	0.1	0.1	0.2	0.8	1.0	1.5	3.5	4.5	–	8.5	3
Boar	0.01	–	–	–	5.8	–	4.1	15.6	17.9	–	16.1	2
	–	–	–	–	5.8	–	9.8	–	17.0	–	–	5
	–	–	–	–	–	–	–	–	–	–	11	1
	–	–	–	–	–	–	–	–	–	–	14.5	8
	13.5[a]	–	9.8[a]	16.3[a]	38.8[a]	106.5[a]	185.1[a]	228.2[a]	–	696.2[a]	–	8
Dog	–	–	–	–		–	–	–	–	–	20	1
Bull	–	–	–	–		–	–	–	–	–	2.0	6
	–	–	–	–		–	–	–	–	–	2.0	1
Horse	–	–	–	–		–	–	–	–	–	11.0	1
Monkey	–	–	–	–		–	–	–	–	–	80.0	1
Man	–	–	–	–		–	–	–	–	–	6	7 and 1
	–	–	–	–		–	–	–	–	–	5	10[b]

–, no attempt was made to measure free L-carnitine concentrations.
[a]nmol/mg proteins in diluted fluid by Krebs Ringer Bicarbonate (KRB) medium.
[b]Measurement in ductus deferens.

References: (1) Setchell and Brooks (1988), (2) Hinton *et al.* (1979a), (3) Besançon *et al.* (1985), (4) Vogmayr *et al.* (1977), (5) Mann (1959), (6) Casillas (1973), (7) Turner (1979), (8) Jeulin *et al.* (1987), (9) Brooks *et al.* (1974), (10) Hinton and Setchell (1981).

The acetyl-L-carnitine molecule is widely distributed. In the mutant yeast strain *Candida pintolopesii*, mitochondrial acetyl-L-carnitine transports acetyl groups which are used in respiration or for biosynthesis (Green and Lewin, 1993). In mammals, the transport of acetyl-CoA from the mitochondrion to the cytoplasm is performed by two pathways: the first is dependent on free L-carnitine, carnitine translocase and cytoplasmic CAT; the second is energy consuming and uses citrate as a carrier and is catalysed by ATP citrate lyase (Figure 1).

$$\text{acetyl-CoA} + \text{ADP} + \text{Pi} + \text{oxaloacetate} \xrightarrow[Mg^{2+}]{\text{citrate lyase}} \text{citrate} + \text{ATP} + \text{CoA}$$

Citrate is carried into the cytoplasm, where it is a precursor of acetyl-CoA and oxaloacetate in a reaction which requires CoA and ATP. The free L-carnitine pathway is able to carry acetyl-CoA out of the mitochondrion without ATP. This property of free L-carnitine is called 'the ability for buffering or trapping excessive production of acetyl-CoA'.

In conclusion, the cellular concentrations and compartmentalization of free CoA, acetyl-CoA, free L-carnitine, acetyl-L-carnitine and acylcarnitine esters in eukaryotic cells are important variables in regulating metabolic pathways.

The male genital tract

The male genital tract contains several compartments which maintain the highest free L-carnitine concentrations in the body [1–80 mmol/l in epididymal tissue (Casillas, 1972), seminal plasma (Frenkel *et al.*, 1974; Bohmer and Johansen, 1978; Wetterauer and Heite, 1978; Soufir *et al.*, 1981, 1984, 1988; Tomamichel and Bandhauer, 1986; Szerman-Joly *et al.*, 1987) and spermatozoa (Brooks, 1980)]. In mammals, the origin of free L-carnitine in the seminal plasma and spermatozoa is mainly epididymal.

Free L-carnitine and acylcarnitines in epididymal plasma

Free L-carnitine is taken up from blood plasma into the epididymal lumen. The epithelial cells secrete L-carnitine into the lumen of the tubule by active transport using a specific carrier which in rats (Hinton and Setchell, 1980; Yeung *et al.*, 1980; Cooper *et al.*, 1986a) and monkeys (Cooper *et al.*, 1986b) is regulated by androgens. The free L-carnitine concentration in the lumen of the cauda epididymis of rat and boar (Brooks, 1979a,b; Hinton *et al.*, 1979a) is 2000-fold greater than the blood plasma concentration. The gradual increase in free L-carnitine concentrations along the length of the epididymis is variable with the species (Table II) and is discussed below.

Homeostasis of free L-carnitine and acylcarnitine concentrations occurs in blood plasma. This equilibrium results from exchanges between free L-carnitine and acylcarnitine esters and a process of reabsorption by kidney tubules, with specific thresholds of concentration for each component (Marzo *et al.*, 1991; Li *et al.*, 1992). Data concerning the acylcarnitine concentrations in epididymal plasma indicated that long-chain acylcarnitine esters were not detectable either in human seminal plasma (Fraenkel and Lewin, 1979) or in epididymal plasma from bulls (Casillas, 1973; Van Dop *et al.*, 1977), rats (Brooks *et al.*, 1974) and rams (Golan *et al.*, 1982), whereas low concentrations of short-chain acylcarnitine esters were found in seminal plasma and fluid from deferent duct of rams and humans (Lewin and Bieber, 1979; Lewin *et al.*, 1979; Brooks, 1979a,b, 1987; Golan *et al.*, 1982). Acetyl-L-carnitine is the major acylcarnitine ester found in the epididymis (Table III).

Free L-carnitine and acylcarnitines within the epididymal spermatozoa

Spermatozoa collected from the first region of the epididymal lumen are immotile *in vivo* or *in vitro* after dilution in a saline medium and have an undetectable or a very low free L-carnitine content (Tables IV and V). During transit from the caput to the cauda region of the epididymis, the free L-carnitine concentration of epididymal plasma increases and spermatozoa accumulate free L-carnitine, which is immediately acetylated (Figure 2 and Tables VI and VII). This process is observed in most species. The free L-carnitine and acetyl-L-carnitine concentrations within cauda epididymal spermatozoa have been estimated to be 20–100 mmol/l (Huston *et al.*, 1977b; Brooks, 1979b). Recently, Jeulin *et al.* (1994) and Jeulin (1994) have demonstrated in boar epididymal spermatozoa that physiological concentrations of free L-carnitine and acetyl-L-carnitine (mmol/l), which are commonly found in the epididymal lumen, accumulated *in vitro* within spermatozoa by passive diffusion. The caput spermatozoa were unable to acetylate free L-carnitine, but the boar cauda epididymal spermatozoa produced an excess of acetyl-CoA that acetylated free L-carnitine. The reaction was catalysed by a sperm CAT that was found in spermatogenic cells from primary spermatocytes (Vernon *et al.*, 1971). The high concentrations of acetyl-L-carnitine measured within cauda epididymal spermatozoa of the boar and ejaculated human spermatozoa reflect an elevated production of acetyl-CoA. This excess of acetyl-CoA production can result from an increase in either pyruvate dehydrogenase activity or β-oxidation, together with limited entry of acetyl-CoA into the tricarboxylic acid cycle.

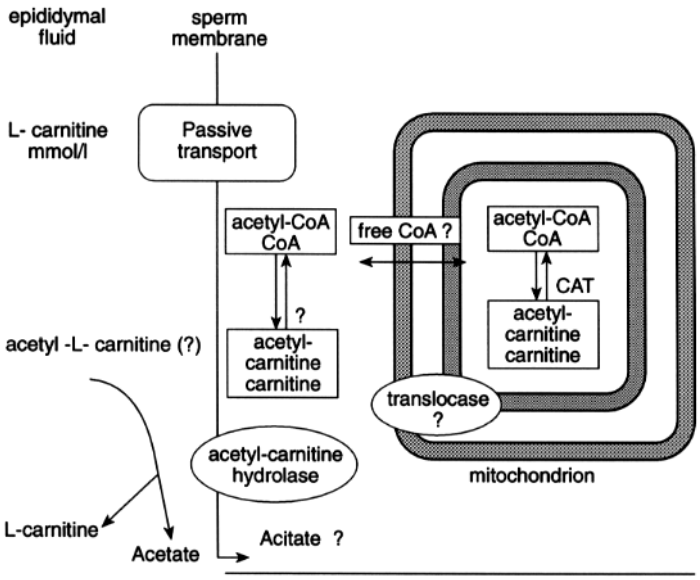

Figure 2. Metabolism of free L-carnitine, free coenzyme A (CoA), acetyl-CoA and the location of carnitine acyltransferases in the mammalian spermatozoa.

Table III. Concentrations of acetyl-L-carnitine (mmol/l) measured in epididymal and deferential duct plasma of different species

Species	Rete testis	Epididymis											Ref.
		Initial	Caput			Corpus			Cauda			Deferent	
		segment	Proximal	Median	Distal	Proximal	Median	Distal	Proximal	Median	Distal	duct	
Rat	–	–	–	–	–	–	–	–	–	–	3–6	–	1
Hamster	–	–	–	4.2[a]	–	–	0.5[a]	–	–	156[a]	–	–	2
Rabbit	–	–	16[b]	–	36[b]	15[b]	–	17[b]	180[b]	–	155[b]	–	3
Ram	–	–	–	0	–	–	+	–	–	++	–	+++	4
Boar	5.0[c]	–	6.7[c]	15.2[c]	7.6[c]	7.2[c]	8.8[c]	–	13.8[c]	–	7.8[c]	–	6
	–	–	–	–	–	–	–	–	–	–	0.2	–	7
Man	–	–	–	0	–	–	0	–	–	+	–	+++	4 and 5
												+	8

[a]nmol/epididymis, short-chain acylcarnitine, epididymal fluid.
[b]nmol/epididymis, acetyl-L-carnitine, epididymal tissue.
[c]nmol/mg proteins.
+ = acetyl-L-carnitine was detected using bioautography (Golan *et al.*, 1982, 1983).
– = no attempt was made to measure acetyl-L-carnitine concentrations.
References: (1) Brooks *et al.* (1974), (2) Casillas *et al.* (1984), (3) Casillas and Chaipayungpan (1979), (4) Golan *et al.* (1982), (5) Golan *et al.* (1983), (6) Jeulin *et al.* (1987), (7) Jeulin *et al.* (1994), (8) Soufir and Jeulin (1985).

The long-chain acylcarnitine esters are undetectable in human spermatozoa (Golan *et al.*, 1983, 1984). This suggests either a very low production of these components or a very rapid turnover (see Figure 2).

The carnitine acyltransferases within the epididymal spermatozoa

The activity of ATP citrate lyase, which transports acetyl-CoA from the mitochondrion to the cytoplasm, is not detectable in rat cauda epididymal spermatozoa (Brooks, 1978). This suggests a possible role for free L-carnitine/acetyl-L-carnitine in transporting acetyl groups out of mitochondria. Two acyltransferases, CAT and CPT, have been measured in cauda epididymal spermatozoa from rats, rams and humans (Brooks, 1979a). The localization of CPT within spermatozoa has not been determined, while a mitochondrial CAT activity has been found in epididymal spermatozoa of rats (Marquis and Fritz, 1965; Brooks,

1978), rams and bulls (Day-Francesconi and Casillas, 1982). Whether this enzyme (CAT) is bound to the inner or outer mitochondrial membrane has not yet been determined. Vernon *et al.* (1971) have detected activity of this enzyme in diplotene primary spermatocytes, and a mitochondria-bound activity of CAT has been suggested. The role of CPT activity in fatty acid metabolism in spermatozoa is not well understood. Information concerning the effects of free L-carnitine on oxidation of long-chain fatty acids in spermatozoa is conflicting. For example, the rate of oxidation of palmitate decreased upon addition of free L-carnitine to incubated bovine ejaculated spermatozoa (Hamilton and Olson, 1976), but increased in the presence of bovine epididymal spermatozoa (Casillas, 1972). Palmitate entered the intact spermatozoa from bovine epididymis with difficulty, but was oxidized after sonication or permeabilization of the plasma membrane with Filipin, which allowed direct access to the mitochondrion (Casillas, 1972; Huston *et al.*, 1977a,b). In another report, palmitate was oxidized by rat epididymal spermatozoa without exogenous carnitine (Geer *et al.*, 1975). [U-^{14}C]Palmitate is rapidly incorporated into the phospholipid membranes of bovine spermatozoa (Neill and Masters, 1971) and then into diglycerides following a longer incubation time (Neill and Masters, 1972). Moreover, it has been suggested that the mitochondria of rabbit epididymal spermatozoa might lack outer membrane CPT because they are able to oxidize palmitoylcarnitine but not palmitoyl-CoA in the presence of free L-carnitine (Storey and Keyhani, 1974). A carnitine translocase-like activity has been described by Calvin and Tubbs (1976), who demonstrated exchanges between mitochondrial acetyl-L-carnitine or free L-carnitine and cytoplasmic free L-carnitine. This exchange system was temperature dependent and probably mitochondrial, and was found in ejaculated boar and ram spermatozoa which were subjected to hypo-osmotic treatment.

Acetate, acetyl-CoA and free CoA within the epididymal spermatozoa

Boar ejaculated spermatozoa which had been washed and incubated with pyruvate produced acetate (Brooks and Mann, 1973). This surprising production of acetate increased with epididymal maturation (Inskeep and Hammerstedt, 1982). Cauda spermatozoa from the boar, incubated in the presence of glucose, produced lactate, CO_2 and more acetate (+25%) than did spermatozoa from corpus or caput. The incubation of cauda spermatozoa with free L-carnitine (mmol/l) increased acetate production in the medium (Inskeep and Hammerstedt, 1982).

The mitochondria of eukaryotic cells produce acetyl-CoA from pyruvate (when carbohydrates are used for energy) or from free fatty acids (β-oxidation). All the enzymes involved in these metabolic pathways have been identified in rat epididymal spermatozoa (Geer *et al.*, 1975; Brooks, 1978) and are functional. CAT activity is not limiting in spermatozoa (Brooks, 1978), and the rate of acetylation of free L-carnitine depends on the patterns of substrates used by spermatozoa (Casillas and Erickson, 1975; Milkowski *et al.*, 1976; Van Dop *et al.*, 1977, 1978; Carter *et al.*, 1980; Shalev *et al.*, 1986). Acetyl-CoA production might therefore be expected to vary very rapidly in relation to the different environments experienced by the male gametes as they are transported along the male and female genital tracts.

Table IV. Objective measurements of percentage of motile spermatozoa recovered[a] from different epididymal regions

Species	Epididymis											Ref.
	Initial	Caput			Corpus			Cauda			Deferent	
	segment	Proximal	Median	Distal	Proximal	Median	Distal	Proximal	Median	Distal	duct	
Rat	–	18	–	53	–	45	52	52	–	63	54	1
	–	9	–	9	–	12	–	–	18	–	–	2
Ram	2	13	–	46	54	63	72	–	77	84	92	3
	–	–	38	42	45	80	85	–	80	85	–	4
Boar	3	10	–	33	60	74	75	–	81	82	77	3
Bull	5	19	–	23	36	56	47	–	61	84	83	3
Goat	6	22	–	48	40	54	73	–	77	83	83	3
Horse	–	17	–	27	55	68	77	–	84	83	90	3

[a]Without distinction between patterns of motility, i.e. vibrating, circular or straight trajectories.
– = no attempt was made to measure percentage of motile spermatozoa.
References: (1) area change frequency, Hinton *et al.* (1979b), (2) capillary migration assay, Turner and Giles (1981), (3) laser light scattering method, Paquignon *et al.* (1983), (4) multiple moving exposure microphotography, Chevrier and Dacheux (1992).

Table V. Percentages of progressively motile spermatozoa (circular or straight trajectories) measured (by objective method) in different epididymal regions

Species	Epididymis										Ref.
	Initial	Caput			Corpus			Cauda			
	segment	Proximal	Median	Distal	Proximal	Median	Distal	Proximal	Median	Distal	
Rat	–	–	50	–	55	–	55	75	–	55	1
Ram	–	–	–	15	15	50	65	–	78	75	2
Boar	0	0	4	5	7	8	32	–	60	55	3 – 6
Man	–	–	–	0	–	21	–	–	22	–	4
	–	0	–	8	–	45	–	–	50	55	5

– = no attempt was made to measure percentage of motile spermatozoa.
References: (1) manual counting of videotape images (50 images/s), Yeung *et al.* (1992), (2) multiple moving exposure microphotography, Chevrier and Dacheux (1992), (3) phase-contrast microscopy, Jeulin *et al.* (1987), (4) CellSoft, Mathieu *et al.* (1992), (5) video recordings, Dacheux *et al.* (1987), (6) microphotography, Bork *et al.* (1988).

Table VI. Concentrations of free L-carnitine (nmol/10^8 spermatozoa) measured in sperm extracts from different epididymal regions

Species	Epididymis									Ref.
	Caput			Corpus			Cauda			
	Proximal	Median	Distal	Proximal	Median	Distal	Proximal	Median	Distal	
Rat	–	–	–	–	–	–	–	–	60	1
	–	–	–	–	–	–	–	–	62	2
Hamster	–	–	–	–	–	41.6	91.6	172	169	3
	–	40.9	–	–	61.5	–	–	135	–	3
Rabbit	5.2	–	6.5	4.9	–	5.4	12.4	–	13.1	4
Ram	–	10	19	38	–	30	43	–	60[a]	5
Bull	10	–	7	–	28	–	49	–	100	6
	–	–	–	–	–	–	–	–	38	7
	–	–	–	–	–	–	–	–	90	8
Boar	5	4	10	11	10	18	–	40	51[a]	9

[a]If we assume that the water space of ram spermatozoa is 2.5 µl/10^8 spermatozoa (Inskeep and Hammerstedt, 1982), the concentration of free L-carnitine within spermatozoa is estimated to be 24 mmol/l, and this concentration is equilibrated outside and inside (epididymal plasma 17 mmol/l). The same observations were made for boar spermatozoa (see Table II, refs 1, 2 and 8).
– = no attempt was made to measure free L–carnitine concentrations.
References: (1) Marquis and Fritz (1965), (2) Brooks *et al.* (1974), (3) Casillas *et al.* (1984), (4) Casillas and Chaipayungpan (1979), (5) Inskeep and Hammerstedt (1982), (6) Casillas (1973), (7) Milkowski *et al.* (1976), (8) free L-carnitine + acetyl-L-carnitine (mmol/l), Huston *et al.* (1977b), (9) Jeulin *et al.* (1987).

The concentrations of acetyl-CoA and free CoA are low in spermatozoa. Acetyl-CoA was not detected in washed epididymal spermatozoa of rat (Brooks *et al.*, 1974). However, Casillas and Erickson (1975) reported a high concentration of free L-carnitine in bull and monkey spermatozoa that was 2000-fold greater than that of free CoA. These authors concluded that the CAT system was able to buffer the acetyl-CoA produced. Lower ratios of acetyl-L-carnitine:acetyl-CoA have been found in other tissues, 20:1 in rat heart and diaphragm, 5–10:1 in kidney and 2:1 in liver (Pearson and Tubbs, 1967). The intracellular concentrations of free L-carnitine and free-CoA might be good indicators of the buffering role of free L-carnitine. The concentrations of both of these components in each cellular compartment (mitochondrion and cytoplasm) might modulate this property.

The ratio of acetyl-L-carnitine:free L-carnitine concentration within the spermatozoa may provide a measure

of the buffering capacity of carnitine. At equilibrium, the value of the equilibrium constant (*K*) in the reaction catalysed by CAT determines the ratios of acetyl-L-carnitine:free L-carnitine and acetyl-CoA:free CoA found in a particular cellular compartment.

$$\frac{\text{(acetyl-CoA)}}{\text{(CoA)}} = K\frac{\text{acetyl-L-carnitine)}}{\text{(L-carnitine)}}$$

Fritz *et al.* (1963) showed in an in-vitro system at equilibrium that the constant *K* was equal to 0.6 at 35°C. The difference in free energy between acetyl-L-carnitine and acetyl-CoA is low. Green and Lewin (1993) clarified the understanding of the regulation of this ratio of acetyl-CoA:CoA using a simplified model in yeast. Briefly, the wild-type strain *Candida pintolopesii* ATCC 22987 did not need free L-carnitine to grow because the intracellular ratio acetyl-CoA:CoA seemed to be regulated by a mechanism which differed from the system L-carnitine:CAT. The apparent equilibrium constant (K_{app}), calculated from the measured ratios of acetyl-CoA:CoA and acetyl-L-carnitine:free L-carnitine was equal to 0.77, not very different from the value determined by Fritz *et al.* (1963). On the other hand, the mutant yeast ATCC 26014, whose growth rate in culture was stimulated by free L-carnitine, showed a different value of K_{app} (7.7). If we calculate K_{app} from the acetyl-CoA, free CoA, free L-carnitine and acetyl-L-carnitine measured by Casillas and Erickson (1975) in bulls and monkeys, we observe high variations related to the presence of substrates in the medium (0.18–64). These results, which require confirmation, suggest that the CAT system might not have been in equilibrium in these conditions.

These data show that intracellular free L-carnitine accumulated by spermatozoa might participate in a buffering role, trapping excess mitochondrial acetyl-CoA as acetyl-L-carnitine. This system would protect the activity of pyruvate dehydrogenase, a key enzyme for mitochondrial respiration, which is inhibited by excess acetyl-CoA. Exchanges of free L-carnitine and acetyl-L-carnitine between the mitochondrion and the cytoplasm (via a translocase-like enzyme) can be considered as a carrier system that allows cytoplasmic storage of acetyl groups (Figure 2). The mode of transport of acetate, short-chain fatty acid and acyl-CoA derivatives from the cytoplasm to the mitochondrial matrix of spermatozoa is not known. Acetate and short-chain fatty acids in the cytoplasm of eukaryotic cells are transported across the inner mitochondrial membrane without the help of free L-carnitine. All these data suggest that, in a germinal cell, the cytoplasmic and mitochondrial pools of acetyl-L-carnitine might play an important role in sperm metabolism and require further investigations.

Table VII. Concentrations of acetyl–L–carnitine (nmol/10^8 spermatozoa) measured in sperm extracts from different epididymal regions and ejaculates

Species	Epididymis									Ejaculates	Ref.
	Caput			Corpus			Cauda				
	Proximal	Median	Distal	Proximal	Median	Distal	Proximal	Median	Distal		
Rat	–	–	–	–	–	–	–	–	40	–	1
	–	–	–	–	–	–	–	–	35	–	2
Hamster	–	70.7	–	–	32.2	–		49.9	–	–	3
Bull	–	–	–	–	–	–	–	–	22	–	4
	–	0	–	–	–	–	–	–	20	–	5
	–	–	–	–	–	–	–	–	25	–	6
Ram	–	13	12	45	–	66	220	–	320	–	7
	–	–	–	–	–	–	–	–	–	109	9
Boar	9	5	10	8	5	11	–	35	57	–	8
	–	–	–	–	–	–	–	–	–	123	9
Man	–	–	–	–	–	–	–	–	–	93–130	10
	–	–	–	–	–	–	–	–	–	70	11

– = no attempt was made to measure acetyl-L-carnitine concentrations.
References: (1) Marquis and Fritz (1965), (2) Brooks *et al.* (1974), (3) Casillas *et al.* (1984), (4) Casillas and Erickson (1975), (5) Casillas (1973), (6) Milkowski *et al.* (1976), (7) results expressed as μmol/10^8 spermatozoa, Inskeep and Hammerstedt (1982), (8) Jeulin *et al.* (1987), (9) Brooks (1979a), (10) Jeulin *et al.* (1988), (11) Golan *et al.* (1986).

Acetate, acetyl-L-carnitine, acetylcarnitine hydrolase and the sperm membrane

Bruns and Casillas (1989, 1990) have proposed a physiological function to explain the high concentration of acetyl-L-carnitine measured in cauda epididymal plasma of rabbit and hamster. Their model is supported by the fact that bovine spermatozoa are not permeable to free L-carnitine in cauda epididymis (Casillas, 1973). Acetyl-L-carnitine hydrolase activity located on the outer face of sperm membrane hydrolyses acetyl-L-carnitine to an acetate moiety which enters the sperm cell and free L-carnitine, which accumulates in the fluid (Figure 2). Purified vesicles of plasma membrane and purified extract from bovine epididymal spermatozoa have been shown to contain the acetyl-L-carnitine hydrolase activity (Bruns and Casillas, 1989, 1990). Evidence concerning the physiological role of this enzyme activity is conflicting. In an in-vitro study on boar and rat spermatozoa, Jeulin *et al.* (1994) and Jeulin (1994) observed that exogenous acetyl-L-carnitine entered boar caput spermatozoa by passive diffusion without a high rate of hydrolysis of the compound. Approximately 3% of the acetyl-L-carnitine added to the medium was hydrolysed to free L-carnitine in 20 min by boar caput and cauda spermatozoa, whereas an acetyl-L-carnitine hydrolase activity on the outer face of the plasma membrane of rat spermatozoa hydrolysed 15% of acetyl-L-carnitine to carnitine during a 30 min incubation (Jeulin, 1994). Enzymatic activity, if present, is therefore higher in rat than in boar spermatozoa.

Distribution of free L-carnitine and acetyl-L-carnitine in epididymal plasma and spermatozoa

Concentrations of free L-carnitine and acetyl-L-carnitine in epididymal plasma and spermatozoa

Tables II and III summarize the data concerning the distribution of free L-carnitine and acetyl-L-carnitine in the rete testis and epididymal plasma found in different species. A large variation in the site on the epididymal duct, where the free L-carnitine concentration begins to increase, is observed (frequently, it is located between the proximal and the distal parts of the caput epididymis). The concentration of free L-carnitine increases gradually and the difference between minimum and maximum concentrations differs with the species. Concentrations of acetyl-L-carnitine increased in epididymal plasma from the cauda region (Casillas and Chaipayungpan, 1979; Casillas *et al.*, 1984) of rabbit and hamster, whereas no increase in the concentrations of acetyl-L-carnitine was measured in the epididymal plasma of the boar (Jeulin *et al.*, 1987). Only traces of acetyl-L-carnitine were found in cauda epididymal plasma of man using bioautography, which is not a quantitative method (Golan *et al.*, 1982, 1983). In humans, the origin of seminal acetyl-L-carnitine is probably ampullar and/or deferential (Soufir and Jeulin, 1985) and in the ram, it is partially from the distal epididymis but mainly of vesiculo-deferential origin (Golan *et al.*, 1982, 1983). Substantial differences found between species and by different authors in the values for comparable epididymal regions are mainly related to the very small volumes (0.1–1.0 µl) of the contents collected from each lumen. This has made it difficult to microanalyse free L-carnitine and acylcarnitine concentrations. However, in all species, the free L-carnitine concentrations in cauda fluid are 2000-fold greater than the blood plasma concentration.

Potential for initiation of sperm motility and concentrations of free L-carnitine in the epididymal plasma

Spermatozoa which enter the epididymis are immotile and their free L-carnitine content is very low or undetectable. During their transit through the epididymis, they become capable of initiating flagellar motion and accumulate a very high concentration (mmol/l) of free L-carnitine from the luminal fluid. *In vivo*, spermatozoa first express motility in the ejaculate, while *in vitro*, the initiation of sperm motility can be observed directly in the epididymal fluid (Armstrong *et al.*, 1994) or after dilution in a saline medium (Dacheux and Paquignon, 1980a,b; Cooper, 1986). Several patterns of sperm motility can be seen: non-progressive and progressive, with circular or straight trajectories, during a time period of one or several seconds. Since the 1980s, many groups have tried to explain the relationship between the accumulation of free L-carnitine in spermatozoa and their ability to move (Tanphaichitr, 1977; Hinton *et al.*, 1981; Turner and Giles, 1981; Golan *et al.*, 1984; Casillas and Chaipayungpan, 1979, 1982; Johansen and Bohmer, 1979; Jeulin *et al.*, 1981, 1987, 1994; Jeulin, 1994). Two major approaches have been used: (i) the percentage of spermatozoa that are motile and their patterns of flagellar movement have been studied in epididymal and ejaculated spermatozoa in the presence of millimolar concentrations of exogenous free L-carnitine and acetyl-L-carnitine and (ii) the ability of spermatozoa from different regions of the epididymal duct to initiate motility *in vitro* in saline medium has been correlated with their cellular content of free L-carnitine and acetyl-L-carnitine. The data on sperm motility characteristics were often dissociated from the biochemical data. Tanphaichitr (1977) and Jeulin *et al.* (1981), using subjective and objective measurement methods respectively, observed a significant increase in the

percentage of human spermatozoa that were motile after adding acetyl-L-carnitine. Jeulin *et al.* (1987) reported parallel increases in the percentage of progressive sperm motility and free L-carnitine and acetyl-L-carnitine contents of distal corpus spermatozoa of the boar. A time-lag between the increase of external and internal free L-carnitine concentrations of boar epididymal spermatozoa is explained by the release of free L-carnitine during washing procedures used to separate the spermatozoa (Jeulin *et al.*, 1994). Table IV summarizes data on percentages of motile spermatozoa measured objectively during epididymal transit by different methods. The first three methods (area change frequency, capillary migration and laser light scattering) did not allow study of the different patterns of sperm movement. These methods showed only that a low percentage of motile cells appeared in the proximal or mid caput, depending on the species (Table IV). These spermatozoa were described as non-progressive with a strongly curved midpiece (Chevrier and Dacheux, 1992) after analysis of microphotographs obtained by the Multiple Moving exposure method. The micropuncture of each epididymal region provided sperm samples which were analysed after dilution in a saline medium. Table V shows a large increase in the percentage of straightline progressive trajectories observed at the level of mid caput in rats and mid corpus in rams, boars and humans. If we compare these observations with the accumulation of free L-carnitine in the epididymal fluid (Table II), we find a relationship between the two phenomena in rats but not in boars or rams. Armstrong *et al.* (1994) demonstrated definitively that the intracellular signal transduction mechanism of initiation of motility of rat spermatozoa is regulated by Ca^{2+} and guanylate and adenylate cyclases and requires the cAMP-dependent phosphorylation of a protein (Tash *et al.*, 1984). The carnitine system is not related to the initiation of sperm motility. Bruns and Casillas (1989, 1990) proposed that the high concentrations of acetyl-L-carnitine measured in rabbit and hamster epididymal plasma (Table III) functioned to stimulate sperm motility. Acetyl-L-carnitine might produce (by hydrolysis) free L-carnitine in the fluid as well as acetate which could be used by spermatozoa for energy and motility. The following findings argue against this hypothesis. The percentage of motile human ejaculated spermatozoa was increased when acetyl-L-carnitine was added *in vitro* to the semen (Tanphaichitr, 1977; Jeulin *et al.*, 1981). This result has been emphasized recently because the flagellar movement of ATP-depleted rat spermatozoa was stimulated *in vitro* by addition of acetate or acetyl-L-carnitine to the medium (Jeulin, 1994). Free L-carnitine alone had no effect on flagellar movement of these ATP-depleted rat spermatozoa (Jeulin and Schoevaert, 1992; Jeulin and Soufir, 1992; Jeulin, 1994; Jeulin *et al.*, 1994). Apparently, acetyl-L-carnitine stimulates the motility of previously active spermatozoa that have become energy depleted, but there is no evidence for a role in the initiation of movement.

Potential for initiation of sperm motility and concentrations of free and acetylated L-carnitine in the epididymal spermatozoa

All authors agree that spermatozoa accumulate free L-carnitine from the epididymal plasma during their transit (Table VI). Jeulin *et al.* (1994) demonstrated that, *in vitro*, free L-carnitine enters via passive diffusion through the plasma membranes of immature and mature spermatozoa of the boar, whereas Casillas (1973) showed that, *in vitro*, bovine cauda spermatozoa, unlike caput epididymal spermatozoa, were not permeable to free L-carnitine. In rams and boars, a high acetyl-L-carnitine content is found only in mature epididymal spermatozoa (Table VII; Inskeep and Hammerstedt, 1982; Jeulin *et al.*, 1994) while, in hamster spermatozoa, the acetyl-L-carnitine concentration is high, but constant, in all areas of the epididymis (Casillas *et al.*, 1984). A relationship has been observed between the potential initiation of progressive sperm motility and a large increase in concentration of both free L-carnitine and acetyl-L-carnitine within boar spermatozoa (Jeulin *et al.*, 1987). These data suggest that mature and viable spermatozoa which have accumulated free L-carnitine from epididymal fluid are also capable of acetylating it. We believe that the potential to initiate sperm motility is independent of and not related to the carnitine system. The substantial differences between species in the concentrations of free and acetylated L-carnitine might be explained by the various methods used to isolate spermatozoa from epididymal plasma. Passive diffusion of free L-carnitine through the plasma membrane of epididymal spermatozoa (Jeulin *et al.*, 1994) changes its concentration within spermatozoa in relation to the length of time of incubation in a saline medium lacking free L-carnitine.

Role of free L-carnitine accumulation in epididymal and ejaculated spermatozoa

A buffer system which controls free CoA concentrations and protects cellular functions

The free L-carnitine accumulation by epididymal spermatozoa confers new physiological functions upon the gametes. The plasma membrane of epididymal spermatozoa allows passive diffusion of free L-carnitine from the fluid, during the transit time of 1–10 days. This mechanism of membrane transport is identical to those of somatic cells when the free L-carnitine concentration is millimolar. In

rabbit spermatozoa only was free L-carnitine accumulation facilitated by androgens such as dihydrotestosterone (Casillas and Chaipayungpan, 1982). The free L-carnitine accumulated by epididymal spermatozoa is rapidly acetylated only in cauda epididymis. These findings might be related to the change in sperm metabolism which occurs during transit (decrease of lipid synthesis and increase of oxidative phosphorylation; Brooks, 1979b; Dacheux *et al.*, 1979; Dacheux and Paquignon, 1980a,b; Cooper, 1986). Increase of acetyl-CoA production, activation of pyruvate dehydrogenase or degradation of fatty acids might explain this excess of acetyl-CoA production buffered as acetyl-L-carnitine. An excess of acetyl-CoA production might inhibit several key enzymes, particularly pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Thus, the carboxylic acid cycle enzymes might be protected by the acetyl-L-carnitine/free L-carnitine system, while free CoA is restored and energy stored as acetyl groups of acetyl-L-carnitine. In the future, a precise measurement of the in-vivo and in-vitro concentration of free CoA in caput and cauda spermatozoa, incubated with and without free L-carnitine, might allow this hypothesis to be tested.

After ejaculation, the rate of diffusion of free L-carnitine through the sperm membrane seems to be changed. Carter *et al.* (1988) have found a human seminal protein which inhibits accumulation of free L-carnitine in bovine caput spermatozoa. Moreover, Bohmer and Johansen (1978) have shown that ejaculated human spermatozoa are not permeable to free L-carnitine, and several washings and incubation during sperm swim-down migration did not change the free L-carnitine and acetyl-L-carnitine concentrations of ejaculated human spermatozoa (Jeulin *et al.*, 1988). This newly acquired property of the sperm membrane might protect the gametes during transport in the female tract. In chiroptera, Krutzch *et al.* (1984) measured free L-carnitine concentrations in epididymal and uterine tissues. They observed large variations related to the seasonal period. A high epididymal concentration of free L-carnitine during hibernation (beginning in November) may have a role in extended sperm survival and a high concentration was also found in uterine tissue during the ovulation time (April) of female bats.

A buffer system implicated in the storage of energy as acetyl-L-carnitine, a donor molecule of acetyl groups

The endogenous storage of free L-carnitine and particularly acetyl-L-carnitine inside mature and ejaculated spermatozoa seems to be a guarantee of gamete viability with fully functional metabolic pathways. The acetyl-L-carnitine concentration of ejaculated human spermatozoa was correlated with their motility (Johansen and Bohmer, 1979). The ratio acetyl-L-carnitine:free L-carnitine was higher in progressively motile human spermatozoa than in living but immotile cells (Golan *et al.*, 1984). Motile spermatozoa selected by migration through layer of bovine swim-down migration screen albumin had a higher content of acetyl-L-carnitine and free L-carnitine than the residual sperm population which did not migrate (Jeulin *et al.*, 1988). The same ratio of acetyl-L-carnitine:free L-carnitine was measured in both of those sperm populations, which differed only in the total concentration of free and acetylated L-carnitine. Moreover, Milkowski *et al.* (1976) showed that bovine epididymal spermatozoa incubated in the absence of substrates and stimulated by caffeine used endogenous acetyl-L-carnitine stores to produce a burst of energy for motility. A brief increase of O_2 consumption was observed in parallel with a decrease of acetyl-L-carnitine and increase of free L-carnitine within the spermatozoa. The same observation has been made for ejaculated human spermatozoa selected by swim-up migration and incubated for 2 h in the absence of exogenous substrates (Jeulin, 1994). Smith *et al.* (1985) suggested that acetyl-L-carnitine may be used to replace the energy storage function of high-energy phosphate compounds in mammalian spermatozoa. In boar, ram, goat and bull spermatozoa, there are no high-energy molecules such as phosphoarginine or phosphocreatine which could act as energy transfer substances (Robitaille *et al.*, 1987). A similar mechanism of action of acetyl-L-carnitine for the protection of neuronal mitochondria has been proposed. Administration of acetyl-L-carnitine to patients with Alzheimer's disease resulted in detectable improvement of memory (Carta *et al.*, 1993).

Are the epididymal secretions always useful?

Some molecules are found in very high concentrations in epididymal fluid**.** The concentrations of free L-carnitine and endothelin-1 are 100- to 2000-fold higher in epididymal and seminal plasma than in blood. These 'pharmacological' concentrations in the epididymal fluid might also reflect the embryological origin of the epididymis, which is derived from the primitive mesonephros and may therefore be indicative of excretory waste related to kidney function. Although endothelin-1 does not act on spermatozoa (Kamada, 1994), in contrast, we conclude that epididymal secretion of free L-carnitine is useful for spermatozoa. We have presented solid arguments that support the hypothesis that free L-carnitine acts on the tricarboxylic acid cycle by buffering CoA concentrations in the mitochondrial matrix and that, in the form of acetyl-L-carnitine, it also serves as a store of readily available acetyl groups. These properties of L-carnitine are

known in somatic cells but are emphasized in this study of the male germinal cells that bear the gene inheritance.

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