A carnitine : acetylcarnitine exchange system in spermatozoa

J. Calvin and P. K. Tubbs

*Department of Biochemistry, University of Cambridge, Tennis Court Road,
Cambridge, CB2 1QW, U.K.*

Summary. Hypotonically treated spermatozoa take up L-[^{14}C]carnitine, and release it only by a highly temperature-dependent exchange with external L-carnitine, acetyl-L-carnitine or deoxycarnitine (4-trimethyl-aminobutyrate). The exchange system is probably mitochondrial.

Spermatozoa from several species are known to contain large amounts of carnitine and high levels of carnitine acetyltransferase activity (Marquis & Fritz, 1965; Casillas, 1972). Bovine spermatozoa have been shown to accumulate carnitine during maturation in the epididymis, the concentration increasing more than tenfold during the passage from the testis to the cauda epididymidis (Casillas, 1973). The carnitine concentration in rat epididymal fluid has been reported to be as high as 63 mM (Brooks, Hamilton & Mallek, 1974). As carnitine is present in much higher concentrations in spermatozoa than is Coenzyme A, it has been suggested (Casillas & Erickson, 1975) that the carnitine acetyltransferase system acts as a buffer for the acetyl-CoA:CoA ratio, as in other tissues (Pearson & Tubbs, 1967). The intracellular carnitine is acetylated during incubation with fructose or glucose, and de-acetylated in the absence of substrate (Casillas & Erickson, 1975). Hypotonically treated rabbit spermatozoa rapidly oxidize acetylcarnitine (Keyhani & Storey, 1974), and it is possible that acetyl-carnitine in spermatozoa acts as an important energy store (Harrison, 1976). We have therefore examined whether a carnitine:carnitine (and acetylcarnitine:carnitine) exchange system occurs in spermatozoa, as it does in heart mitochondria (Ramsay & Tubbs, 1975, 1976; Pande, 1975).

Methods

Ram and boar semen was used within 1 hr of ejaculation. Semen from several rams was pooled; only the sperm-rich fraction of boar semen was used. The semen was diluted twofold with 0.25 M-sucrose, containing 10 mM-3-(*N*-morpholino)propanesulphonic acid adjusted to pH 7.2 with KOH, at room temperature. The spermatozoa were washed by centrifugation through a 7.5% (w/v) solution of Ficoll 400 (Pharmacia Fine Chemicals) in the sucrose medium. Hypotonically treated spermatozoa (Keyhani & Storey, 1973) were prepared by resuspending the pellets in ice-cold 10 mM-potassium phosphate, pH 7.4 (four times the original semen volume). The suspension was kept at 0°C for 15 min, and centrifuged at 4°C. The pellet was resuspended in a minimal amount of sucrose medium and incubated with L-[^{14}C]carnitine (250,000 d/min per ml suspension; sp. act. 3.88 $\mu\text{Ci}/\mu\text{mol}$) for 1 hr at 20°C. All subsequent operations were at 0–4°C. The suspension was diluted fourfold with sucrose medium and centrifuged. The spermatozoa were washed twice and resuspended in sucrose medium. Sperm counts were obtained using a haemocytometer.

The radioactivity was displaced from the spermatozoa by exchange with external carnitine or acetylcarnitine as described in the figure legends. The exchange was terminated by addition of 6-trimethylamino-hexanoyl- or 11-trimethylaminoundecanoyl-carnitine (Ramsay & Tubbs, 1976) and the suspension was immediately centrifuged. Samples (200–250 μl) of the supernatant were mixed with 3 ml toluene: Triton X-100 (3:1 v/v) containing 0.5% (w/v) 2,5-diphenyloxazole, and the radioactivity measured in a Nuclear-Chicago Unilux 3B scintillation counter.

L-Carnitine was obtained from Koch-Light Laboratories Ltd, Colnbrook, and DL-[Me- ^{14}C]carnitine (The Radiochemical Centre, Amersham) was resolved to give a solution of the L-isomer (Ramsay & Tubbs, 1975).

Results and Discussion

Preliminary experiments showed that ram spermatozoa which had been rapidly cooled ('cold-shocked') retained radioactivity after incubation with L-[14 C]carnitine despite extensive washing; however, if unlabelled L-carnitine was present during the incubation the L-[14 C]carnitine uptake was much reduced. For example, in one experiment cells which were resuspended after washing to the original semen volume contained 172 and 10,800 d/min per ml after incubation in the presence or absence, respectively, of 20 mM-unlabelled L-carnitine. This competition suggested the existence of a carnitine uptake system in spermatozoa which could be saturated.

Treatment of rabbit epididymal spermatozoa with cold 10 mM-phosphate disrupts the plasma membrane, but apparently leaves the mitochondria morphologically and metabolically undamaged (Keyhani & Storey, 1973). Boar spermatozoa treated in this way were compared with untreated cells. After incubation with L-[14 C]carnitine for 1 hr at 20°C, both suspensions were cooled to 4°C over a period of 4 hr, washed in the usual way and resuspended to give approximately 2.6×10^9 cells/ml. The hypotonically treated spermatozoa contained 2167 d/min/ 10^9 cells and the untreated preparation 367 d/min/ 10^9 cells. The carnitine exchange system is therefore probably mitochondrial.

In all further experiments the spermatozoa were hypotonically treated as described above.

As shown in Text-fig. 1, the exchange of internal and external carnitine is highly temperature-dependent, and in the absence of external carnitine leakage of radioactivity is negligible at the lower temperatures. In another experiment the exchange given by 8.2 mM-L-carnitine at 10°C was completely inhibited by 5 mM-11-trimethylaminoundecanoyl-L-carnitine. Variation of the external carnitine concentration affected the rate of efflux of L-[14 C]carnitine from ram spermatozoa at 6°C (Text-fig. 2), and the K_m was about 12 mM.

Text-fig. 1. The temperature-dependence of the carnitine exchange system in spermatozoa. Hypotonically treated ram spermatozoa (1.3×10^9 cells/ml) containing L-[14 C]carnitine (9545 dpm/ml) were incubated at various temperatures with 4.1 mM-L-carnitine. Control samples were incubated without carnitine. The exchange reaction was terminated as described in the text, and the [14 C]carnitine content of the supernatants determined. In the case of the 0°C and 10°C experiments points for times longer than 6 min are not shown for reasons of scale. Exchange with 4.1 mM-L-carnitine is shown at 20°C (○), 10°C (●) and 0°C (△); control experiments (leakage) were at 20°C (□); 10°C (▲) and 0°C (■).

As shown in Text-fig. 3(a) acetyl-L-carnitine also exchanges very rapidly with internal L-[14 C]-carnitine, and other experiments showed that the K_m was similar to that for L-carnitine itself. D-Carnitine was not a substrate for the exchange, but deoxycarnitine (4-trimethylaminobutyrate) effectively displaced internal L-[14 C]carnitine (Text-fig. 3b). This shows that acetylation of carnitine is unnecessary for the transport process, which could, however, enable the mitochondria to import (and presumably export) acetyl groups (Text-fig. 3a).

Text-fig. 2. Double reciprocal plot showing the effect of external carnitine concentration on the efflux of L-[¹⁴C]carnitine from spermatozoa. Hypotonically treated ram sperm suspensions (9.1×10^8 cells/ml) containing 7761 d/min per ml were incubated with various concentrations of external L-carnitine at 6°C and initial rates of [¹⁴C]carnitine efflux were measured.

Text-fig. 3. Displacement of internal L-[¹⁴C]carnitine by acetyl-L-carnitine (a) and the effect of external D-carnitine and deoxycarnitine on the efflux of L-[¹⁴C]carnitine from spermatozoa (b). (a) Hypotonically treated ram spermatozoa (1.2×10^9 cells/ml) containing 11,520 d/min per ml were incubated with external acetyl-L-carnitine at 5.5°C. The exchange was terminated as described in the text, and the [¹⁴C]carnitine content of the supernatants determined. Exchange is shown with 6.4 mM acetyl-L-carnitine (○) and 1 mM acetyl-L-carnitine (□); a control sample was incubated without acetyl-L-carnitine (△). (b) Hypotonically treated boar spermatozoa (1.7×10^9 cells/ml) containing 8950 d/min per ml were incubated at 0°C with 2 mM-D-carnitine (▲) or 2 mM-deoxycarnitine (●).

In its sensitivity to temperature, inhibition by 11-trimethylaminoundecanoyl-carnitine and ability to use deoxycarnitine the exchange system described in this paper (and also observed in bull and rat spermatozoa) resembles that found in heart mitochondria (Ramsay & Tubbs, 1975, 1976).

We thank Miss Rona R. Ramsay for her help, Dr H. M. Dott, Dr E. J. C. Polge, Mr G. Foster and Mr. K. Elsome, of the A.R.C. Animal Research Station, Cambridge, for providing us with bull, boar and ram semen, and the M.R.C. for a Research Studentship to J.C.

References

- BROOKS, D.E., HAMILTON, D.W. & MALLEK, A.H. (1974) Carnitine and glycerylphosphorylcholine in the reproductive tract of the male rat. *J. Reprod. Fert.* **36**, 141–160.
- CASILLAS, E.R. (1972) The distribution of carnitine in male reproductive tissues and its effect on palmitate oxidation by spermatozoal particles. *Biochim. biophys. Acta* **280**, 545–551.
- CASILLAS, E.R. (1973) Accumulation of carnitine by bovine spermatozoa during maturation in the epididymis. *J. biol. Chem.* **248**, 8227–8232.
- CASILLAS, E.R. & ERICKSON, B.J. (1975) The role of carnitine in spermatozoan metabolism: substrate-induced elevations in the acetylation state of carnitine and coenzyme A in bovine and monkey spermatozoa. *Biol. Reprod.* **12**, 275–283.
- HARRISON, R.A.P. (1976) The metabolism of mammalian spermatozoa. In *Frontiers in Reproduction and Fertility Control*. MIT Press, Cambridge, Mass. (in press).
- KEYHANI, E. & STOREY, B.T. (1973) Energy conservation capacity and morphological integrity of mitochondria in hypotonically treated rabbit epididymal spermatozoa. *Biochim. biophys. Acta* **305**, 557–569.
- KEYHANI, E. & STOREY, B.T. (1974) Energy metabolism of spermatozoa. II. Comparison of pyruvate and fatty acid oxidation by mitochondria of rabbit epididymal spermatozoa. *Fert. Steril.* **25**, 857–864.
- MARQUIS, N.R. & FRITZ, I.B. (1965) The distribution of carnitine, acetylcarnitine and carnitine acetyltransferase in rat tissues. *J. biol. Chem.* **240**, 2197–2200.
- PANDE, S.A. (1975) A mitochondrial carnitine acyl-carnitine translocase system. *Proc. natn. Acad. Sci. U.S.A.* **72**, 883–887.
- PEARSON, D.J. & TUBBS, P.K. (1967) Carnitine and derivatives in rat tissues. *Biochem. J.* **105**, 953–963.
- RAMSAY, R.R. & TUBBS, P.K. (1975) The mechanism of fatty acid uptake by heart mitochondria: an acyl-carnitine-carnitine exchange. *FEBS Letters*, **54**, 21–25.
- RAMSAY, R.R. & TUBBS, P.K. (1976) Effects of temperature and some inhibitors on the carnitine exchange system of heart mitochondria. *Eur. J. Biochem.* (in press).

Received 19 May 1976