0014-4754/96/050487-08$1.50 + 0.20

Spermatozoa: models for studying regulatory aspects of energy metabolism

G. Kamp[a]*, G. Büsselmann[a] and J. Lauterwein[b]

[a]*Institut für Zoophysiologie der Universität Münster, Hindenburgplatz 55, D-48143 Münster (Germany), Fax +49 251 833 876*

[b]*Organisch-Chemisches Institut der Universität Münster, Orléansring 23, D-48149 Münster (Germany)*

Abstract. Spermatozoa are highly specialized cells, and they offer advantages for studying several basic aspects of metabolic control such as the role of adenosine triphosphate-(ATP)-homeostasis for cell function, the mechanisms of fatigue and metabolic depression, the metabolic channelling through the cytoplasm and the organization and regulation of glycolytic enzymes. Spermatozoa of four species with different reproductive modes are introduced and the first results are presented: Spermatozoa of the marine worm *Arenicola marina* are well adapted to external fertilization in sea water with fluctuating oxygen tension: they are motile for several hours in oxygen-free sea water, even when the ATP level is dramatically reduced. Anaerobic ATP production occurs by alanine, acetate and propionate fermentation probably by the same pathways known from somatic cells of this species. Under aerobic conditions the phosphagen system might function like a shuttle for energy-rich phosphate from mitochondria to the dynein-ATPases. Storage of turkey and carp spermatozoa for several hours without exogenous substrates and oxygen results in the degradation of phosphocreatine and ATP to inorganic phosphate and adenosine monophosphate (AMP), respectively. Despite low energy charges, stored spermatozoa of both species are capable of progressive movements. In carp spermatozoa fatigue of motility is not accompanied by the dramatic acidosis one discusses as an important effect in muscle fatigue. Energy metabolism of boar spermatozoa is typically based on glycolysis consuming extracellular carbohydrates and producing lactate and protons. The sperm seem to tolerate low intracellular pH (<6.5). The lack of a phosphagen system (no energy shuttle from mitochondria to the distal dynein-ATPases) is probably compensated by a high glycolytic ATP-production in the mitochondria-free piece of the flagellum.

Key words. ATP-homeostasis; phosphagen function; NMR-spectroscopy; spermatozoa; sperm motility.

Introduction

Lohmann[34] discovered ATP in 1929 but it was in 1941 that Lipman[33] proposed its universal role as an energy 'currency', and in 1962 that Cain and Davies[13] demonstrated the breakdown of ATP during a single muscle contraction. It is now generally accepted that the central role of ATP in all living cells is that of cellular energetic transfer, and ATP-producing pathways and the various ATP-consuming reactions are well known. Interest is now focused on the control mechanisms involved in ATP-production and -consumption (ATP-homeostasis).

Metabolic fluxes are brought about by the catalytic activity of enzymes. Various mechanisms have been demonstrated to modulate enzyme activities in vitro but in many cases their relevance for metabolic control in vivo is still controversial (cf. other reports in this issue). The problems are mainly based on the complexity of metabolic processes in vivo including a network of regulatory interactions and on the difficulty to imitate parts of them in vitro under physiological or at least near-physiological conditions. Comparative studies on cells with highly specialized metabolism, and hence a lower degree of complexity, using non-invasive methods, may be very useful for a better understanding of metabolic regulation.

Spermatozoa provide one example of such an experimental system. These cells offer many advantages for studying basic aspects of metabolic control such as the role of ATP-homeostasis for different cell functions (e.g. biosynthesis, ion transport, movement), the mechanisms of fatigue after motility and of metabolic depression during cell conservation, the metabolic channelling in the cytoplasm and the organization and regulation of glycolytic enzymes:

(1) Spermatozoa pass through distinct metabolic states. During spermiogenesis (cell proliferation and differentiation) they require energy mainly for biosynthetic processes but not for motility. On the other hand, mature spermatozoa have lost their biosynthetic potential, the nucleus is condensed and the Golgi-apparatus has been extruded together with most of the endoplasmic reticulum. Hence, ATP-demand has shifted from biosynthesis to motility. These two periods may be separated by a period of cell conservation which is characterized by a very low metabolic rate. In various species of different phyla (bats, birds, reptiles, insects) it is well known that sperms can also be conserved within the female.

(2) The polarity of spermatozoa, with the mitochondria being located behind the DNA-containing head and in

* Corresponding author.

front of the tail with the contractile apparatus, suggests that metabolic channelling may have developed to improve the intracellular transport.

(3) Spermatozoa can be partially demembranized without loss of motility[22,40] and in some cases without loss of glycolytic enzymes (see below). This enables kinetic studies on enzymes and metabolic pathways to be carried out without destruction of the physiological assembly of enzymes.

(4) Semen usually contains high sperm concentration allowing the continuous observation of metabolic changes by the non-invasive techniques of NMR-spectroscopy. Determination of the $NADH/NAD^+$ ratio by fluorescence analysis and other techniques of cell biology (e.g. fluorescence probes, patch clamp or laser tweezers) are also applicable. Motility can be analyzed by computer-assisted microscopy.

(5) The animal kingdom presents a multitude of reproductive modes, to which the sperms of different species are adapted. This gives us the opportunity to select the appropriate type of sperm cell for investigating a specific question.

We have selected spermatozoa from four species with different reproductive modes in order to analyse how motility is affected by ATP-homeostasis.

Reproductive processes and specialization of spermatozoa

Sexual reproduction is based on fusion of gametes after meiotic division of the genome. Bisexual differentiation of the gametes to spermatozoa and oocytes occurred during the evolution of marine organisms, with a trend from external to internal fertilization[5], the latter being a prerequisite for the evolution of terrestrial life. An interesting evolutionary stage between external and internal fertilization is found in some marine polychaetes[25] or in mouthbreeding fish[41], in which sperms and eggs are released into the water but fertilization takes place inside the female's mouth (or fertilized eggs are taken up into the mouth). Fresh water species show both internal and external fertilization though if the latter takes place, mechanisms are needed to avoid untimely cell lysis due to the entry of water by osmosis.

A general problem of sexual reproduction is that of synchronization, i.e. mature sperms and eggs must be available at the same time. To solve this problem different strategies have been developed, including male and female sexual behaviour patterns, seasonal rhythms, or storage of mature spermatozoa either in the males (e.g. in the epididymidis in mammals) or in females (e.g. in the receptaculum seminis of molluscs, in the reproductive tract of female bats and in the utero-vaginal crypts of birds and reptiles).

Motility of spermatozoa is essential for fertilization. Movement can be achieved by passive transport, however, and aflagellate spermatozoa do exist (e.g. among arthropods[5]). Flagellate spermatozoa can also be transported passively, for example by propulsive contractions of the female reproductive tract (internal fertilization)[42,48] or by water currents (external fertilization). Passive transport reduces the energy needed by flagellate spermatozoa to reach an egg. The composition of the ambient fluid (e.g. the viscosity) and the hydrodynamic structure of the spermatozoa can also influence the energy demand of sperm transport.

In external fertilization spermatozoa are restricted to their own cellular energy fuels. With internal fertilization, however, spermatozoa can utilize fuels supplied by the seminal plasma which is composed of secretions of the testis, epididymis, excurrent ducts and the accessory glands. After insemination the seminal plasma may be complemented by secretions from the utero-vaginal system[37]. Seminal plasma is species-specific and, as shown for mammals, very complex in its chemical composition[28,35]. The physiological function of many compounds contained in seminal plasma is still under discussion, but it appears that only a few of them are used as fuels.

The species selected for our investigation belong to different phyla and show appreciable differences, especially in sperm motility:

(1) The lugworm, *Arenicola marina* (Annelida), is a marine polychaete which lives almost like a sessile animal in the intertidal zone where it has to withstand periods of hypoxia during low tide (facultative anaerobe). Spermatid proliferation and differentiation occur in the large body cavity (coelom) of the worms, from which mature spermatozoa and sperm clusters in different stages of development (fig. 1, a–c) can easily be obtained. Although spawning is synchronized within a population – along the German North Sea coast it takes place in September – spermatozoa still need to survive for several hours in seawater to maximize their chance of meeting an ovum. Duncan[16] found that on a tidal flat epidemic spawning takes place during low tide and concluded that female worms can irrigate their burrows, drawing in a suspension of highly concentrated spermatozoa (passive movement). Accordingly most eggs seem to be fertilized in the female burrows even under hypoxic conditions. The adaptation to fluctuations in oxygen tension to guarantee a continuous development of spermatids in the coelomic cavity during low tide and a prolonged motility of the matured spermatozoa in hypoxic seawater have been investigated.

(2) The carp, *Cyprinus carpio*, provides spermatozoa which are characterized by a separation of the mitochondria from the flagellum (fig. 2, a and b) and the lack of an acrosome[26]. The spermatozoa show no forward motility until diluted with fresh water or hypoosmotic medium[30], when fast movement is immediately activated. Oocytes must be fertilized within a few min-

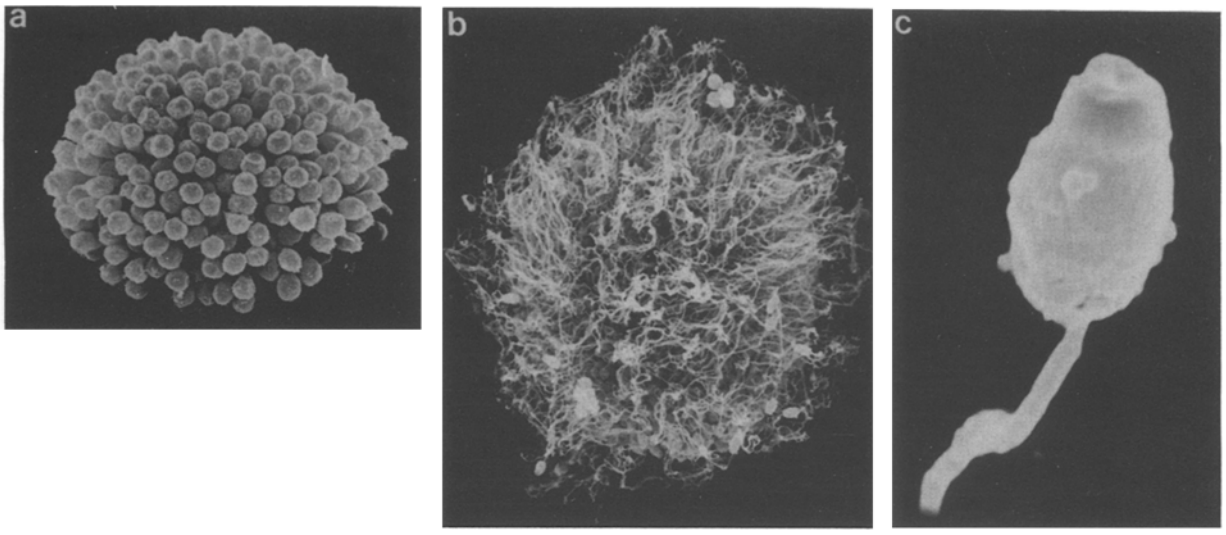

Figure 1. Scanning electron micrographs of the development of lugworm spermatozoa. Spermatogonial clusters (*a* 2000×) proliferate between May and July in the coelomic cavitiy of lugworms. Subsequently flagella develop (*b* 3000×) until mature spermatozoa (*c* 30,000×) are released in September.

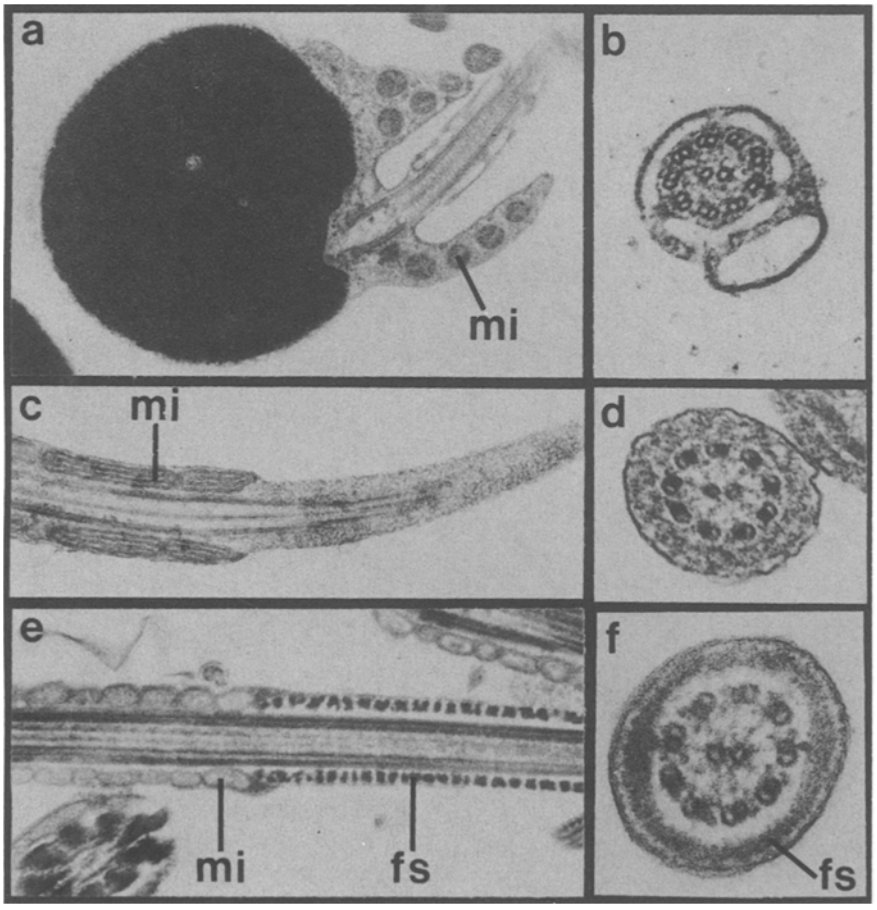

Figure 2. Transmisson electron micrographs of carp (*a* and *b*),turkey (*c* and *d*) and boar (*e* and *f*) spermatozoa. (*c*) and (*e*) show the connection between the sperm midpiece and tail. (*b*), (*d*) and (*f*), transverse sections through the flagellum distal to the midpiece. Mitochondria (mi) of carp spermatozoa (*a*) are separated from the axoneme while midpieces of turkey (*c*) and boar (*e*) spermatozoa show the typical aggregation of mitochondria along the axoneme. A fibrous sheath (fs) is visible in the tail of boar spermatozoa (*e* and *f*). Magnifications: a) 30,600×; b) 91,500×; c) 25,000×; d) 85,500×; e) 30,300×; f) 118,000×.

Table. Enzyme activities in spermatozoa.

		CK	*GAPDH*	*LDH*	*CS*	*HOADH*
Mammals	rat	1.2 ± 1.2 (6)	45 ± 7 (6)	54 ± 11 (5)	6 ± 4 (4)	1 ± 0.4 (3)
	stallion	1.9 ± 1.3 (8)	55 ± 21 (9)	13 ± 3 (8)	1 ± 0.5 (8)	0.5 ± 0.1 (4)
	bull	<0.1 (3)	48 ± 37 (10)	43 ± 17 (9)	4.1 ± 2.4 (9)	0.5 ± 0.1 (9)
	boar	<0.1 (3)	9 ± 8 (16)	4 ± 2 (8)	0.2 ± 0.1 (8)	0.3 ± 0.2 (9)
Bird	turkey	700 ± 143 (3)	16 ± 3 (3)	15 ± 4 (3)	19 ± 4 (3)	6 ± 2 (3)
Fish	carp	40 ± 12 (5)	8 ± 1 (5)	16 ± 4 (5)	3 ± 1 (5)	2 ± 0.4 (5)
Echinoderm	sea urchin	382 ± 123 (3)	1 ± 1 (3)	<0.1 (3)	33 ± 9 (3)	36 ± 16 (3)
Annelid	lugworm	64 ± 9 (3)	28 ± 4 (3)	[a)]8 ± 2 (3)	1.8 ± 0.4 (3)	15 ± 4 (3)

The activities are expressed in μmol min^{-1} g^{-1} cell wet weight (25 °C) and are means ±S.E.M. with the number of independent observations in parentheses.
CK = creatine kinase (EC 2.7.3.2); GAPDH = glyceraldehyde-3-phosphate dehydrogenase (EC1.2.1.12); LDH = lactate dehydrogenase (EC 1.1.1.28); CS = citrate synthase (EC 4.1.3.7); HOADH = hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35); [a)] = alanopine dehydrogenase.

utes because motility of carp spermatozoa is restricted to about three minutes[43]. As shown for trout spermatozoa, cessation of motility is not caused by cell lysis since it also occurs in isoosmotic solutions[6,15]. A study was made to investigate, whether ATP depletion[43], or phenomena similar to muscle fatigue[19,52,56], bring about the rapid loss of motility in carp spermatozoa.

(3) Spermatozoa of the common turkey (*Meleagris gallopavo*, fig. 2, c and d), like sperm from other birds, show prolonged motility (several hours). They are fertile when they leave the efferent ducts of the males, in contrast to mammalian sperms which need capacitation in the oviduct[23,37]. Furthermore, the excurrent ducts of male birds have no accessory glands so that the composition of the seminal fluid is different from that in mammals[1,31]. An interesting feature of turkey spermatozoa is their longevity in the female oviduct. Stored in crypts of the utero-vaginal junction, spermatozoa maintain their fertilization potential for several weeks. The molecular mechanisms bringing about this natural form of conservation of sperm are not known[8]. We will examine the metabolic adaptations required for long term storage of the spermatozoa.

(4) Boars, *Sus scrofa*, produce large volumes of ejaculate (0.15–0.5 l) with the relatively low sperm density of 25,000 to 300,000 spermatozoa per μl[36] (for comparison about 7,000,000 per μl in turkey semen, 20,000,000 per μl in carp semen). The seminal plasma of mammals consists of secretions of the epididymidis, the bulbo-urethral gland and the seminal vesicle[37]. For artificial insemination boar semen can be conserved at 16 °C in buffered solutions containing high glucose concentrations without considerable loss of motility up to two days. At 16 °C the cells become immotile which reduces the energy demand. The metabolic changes during cell conservation are investigated.

Boar sperm contain several mitochondria in the middle piece (fig. 2e) but no phosphocreatine[45] (PCr) and no creatine kinase (CK) activity (cf. table). Hence, transport of energy-rich phosphate from the mitochondria to the distal dynein-ATPases by the phosphagen-system, as proposed to be important for spermatozoa of various species (see below), is not necessary for boar sperm motility. In addition, spermatozoa of boars and other mammals show an unusually tight binding of glycolytic enzymes to cell structures[55]. The compartmentation and the assembly of the different glycolytic enzymes as well as the significance of metabolic transport systems have been investigated.

Pathways of energy metabolism in spermatozoa and their compartmentation

Carbohydrates and fat are common fuels for motility in spermatozoa as they are in muscles. Fatty acids can only be utilized aerobically, while carbohydrate can also be used for ATP-production under anaerobic conditions, but has the disadvantage of producing an approximately 18-fold lower yield of ATP per mol glucose. As far as we know, lactate is the main end-product in the spermatozoa of vertebrates and most invertebrates[37] under anaerobic conditions, though lugworm spermatozoa produce alanopine, strombine, succinate, acetate and propionate instead of lactate, in accordance with the metabolic situation in the somatic tissues[17].

ATP can also be regenerated from phosphagens independently of oxygen, but only for a short period of time due to the small amounts present in the intracellular stores and the 1:1 ratio of the phosphagen to ATP stoichiometry. PCr has been demonstrated to be the phosphagen in spermatozoa of various species of vertebrates and invertebrates, but spermatozoa of arthropods and molluscs contain phosphoarginine (PArg)[44,47,49]. In the presence of oxygen the phosphagen system can also function as a shuttle for transporting energy-rich phosphate from mitochondria to the dynein-ATPase[50,51]. Undergoing conformational changes during ATP-hydrolysis, dynein, like myosin in muscle, generates the bending of the flagellum (chemo-mechanical coupling). In this situation, creatine replaces adenosine diphosphate (ADP) as the acceptor of energy-rich phosphate (• ~ P). The cytoplasmic concentration of ADP is extremely low (creatine concentration in muscles is more than 1000 fold higher than that of

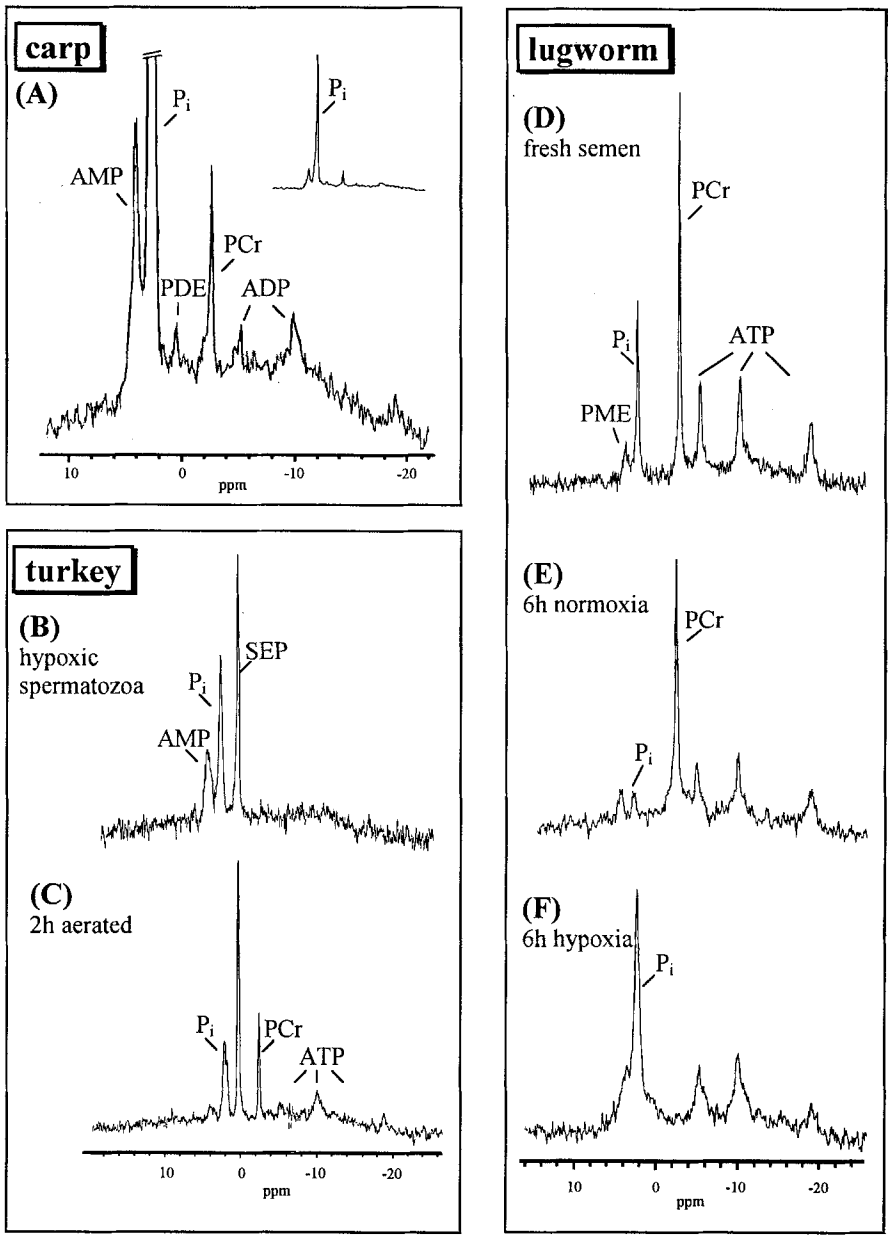

Figure 3. ^{31}P-NMR spectra of spermatozoa from carp, turkey and lugworm. (*A*) Immotile carp spermatozoa of undiluted semen after 3 h incubation at 0 °C. The inset shows the whole signal for inorganic phosphate in relation to other peaks. (*B*) Turkey spermatozoa washed with extender (30 mM HEPES, 20 mM KCl, 5 mM $MgCl_2$, 125 mM NaCl, pH 7.4) and monitored without air supply (hypoxic conditions). (*C*) Turkey spermatozoa from the same sample as (*B*), but intermittently aerated and containing 20% Fluosol (Alpha Therapeutic) as oxygen carrier. The spectrum is based on acquisition cycles each consisting of: 2 min aeration −0.5 min pause −5 min recording. (*D*) Freshly prepared lugworm semen obtained in September when most of the spermatozoa are mature. (*E*) Lugworm semen stored for 6 hours at 12 °C with air supply and (F) without aeration. Spectra were recorded as described by Kamp and Juretschke[27] using a Bruker 360 MHz machine with a maximum recording time of about 30 min. P_i = inorganic phosphate; PCr = phosphocreatine; PDE = phosphodiester; PME = phosphomonoester; SEP = serine ethanolamine phosphodiester; external-calibration with 85% H_3PO_4 = 0 ppm.

ADP[21]), and could therefore be a limiting factor in the transport of •~P over the long distance from the mitochondria to the distal part of the flagellum. Flagellum lengths of about 22 µm in porcupine sperms, or 2270 µm in the amphibian Discoglossus[32,18], give rise to much longer distances than those between mitochondria and myofibrils in muscles.

Evidence for a phosphagen shuttle to overcome diffusion-limitation exists for spermatozoa of humans and chickens[53,54], sea urchins[50], horseshoe crabs[47] and lugworms[29]. In contrast, spermatozoa of bulls and boars do not contain phosphagen systems[9,45]. Sperms of stallions and rats display only very low CK activities, whereas non-mammalian spermatozoa do show the high CK activity (table) that is typical for cells with a phosphagen system.

As an alternative to a phosphagen shuttle, a shuttle in which AMP replaces ADP has been proposed[21]. For

most cells and tissues this shuttle is certainly of low significance because the cytoplasmic AMP concentration is rather lower than the ADP concentration. In spermatozoa, however, the cytoplasmic AMP level exceeds the level of ADP under hypoxic conditions. This was clearly demonstrated by ^{31}P-NMR spectroscopy for intact turkey and carp spermatozoa (fig. 3, A, B and see below). Nevertheless it remains doubtful whether the elevated AMP concentration is sufficient to replace creatine in spermatozoa.

Another alternative to a phosphagen shuttle derives from the observation that spermatozoa without or with only low CK activities have relatively high activities of glycolytic enzymes, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH), but low activities of enzymes representing aerobic pathways such as hydroxyacyl-CoA dehydrogenase (HOADH) and citrate synthase (CS). On the other hand, CK-rich spermatozoa show considerable capacities for oxidation (turkey, carp and lugworm spermatozoa). Sea urchin spermatozoa appear to be completely dependent on mitochondrial ATP production from fat (low GAPDH and LDH activities, high HOADH and CS activities[38,39]) and show high CK activities. An adaptation in carbohydrate metabolism possibly substitutes for a shuttle in some spermatozoa. If ATP in the distal part of the flagellum were preferentially produced glycolytically, an energy shuttle from the mitochondria would not be necessary. This organization would, however, require the regeneration of glycolytic NADH in the distal part of the flagellum because, owing to the low concentration of NADH, the transport of NADH to the mitochondria would be subject to the same problem as were described for ADP. In addition to the typical shuttle systems for the reoxidation of cytosolic NADH (glycerolphosphate shuttle, malate/aspartate shuttle), mammalian spermatozoa seem to contain another system. In several mammalian spermatozoa, a mitochondrial-specific LDH-C_4 (in previous reports called LDH X) has been found[4,20]. It appears that lactate produced in the distal part of the flagellum is transferred to the mitochondria where pyruvate plus NADH are formed which can be oxidized in the mitochondria[20]. Due to the lower ATP yield of glucose fermentation (2Mol ATP/Mol glucose) compared to the complete oxidation of glucose (38 Mol ATP/glucose), aerobic lactate accumulation is to be expected if glycolytic ATP-production accounts for more than 5% of total. Indeed, several vertebrate spermatozoa reveal a high rate of aerobic lactate production (aerobic glycolysis)[36].

In this context it may also be of interest that glycolytic enzymes of boar and other mammalian spermatozoa show unusual binding to cell structures while spermatozoa of non-mammalian species do not[55]. For example, 90% of the GAPDH activity was found in the sediment after centrifugation of disintegrated boar spermatozoa. Neither changes of buffer ion concentration or composition nor addition of various detergents were successful in solubilizating the enzyme. A brief incubation with trypsin, however, detached large amounts of the enzyme from cell fragments and correspondingly the total GAPDH activity increased severalfold (unpubl. results). It is likely that glycolytic enzymes are associated with a special structure of mammalian spermatozoa, the fibrous sheath, that runs along the distal part of the flagellum close to the dynein ATPases (fig. 2e, f). A localization of glycolysis at this special cell structure could explain how sufficient ATP can be supplied in the distal part of the flagellum. Immunocytochemical studies are in progress to verify this suggestion.

ATP-homeostasis during motility

In mammalian spermatozoa sugars seem to be used for ATP production but not glycogen stores[2], and consequently motility depends to some extent on exogenous fuels[36]. In the presence of glucose and oxygen, motility and ATP levels of boar spermatozoa decrease only marginally during 5 hours' incubation at 34 °C (unpubl. results). Nevertheless ^{31}P-NMR spectra of intact boar spermatozoa show no signals for ATP, although ATP can easily be detected in spectra of perchloric acid extracts[45]. Similar phenomena were also found for spermatozoa of other mammalian species (ram, goat and bull)[45]. The authors conclude that the binding of ATP to cell structures might result in a broadening of NMR-signals, rendering ATP 'invisible'. NMR also failed to detect PCr[45], and the absence of the phosphagen has been confirmed using biochemical methods (Brooks[9] and unpubl. results).

Exposure of boar spermatozoa to hypoxia at 34 °C in the presence of glucose resulted in a decrease in motility and a concomitant drop in the content of ATP to below 30% of control values within 5.5 h as well as in an intracellular acidosis ($pH_i < 6.5$), as was detected using in vivo ^{31}P-NMR spectroscopy (unpubl. results). After reoxygenation for 30 min following hypoxia, motility and the ATP level were only slightly improved, and the total concentration of all adenylate nucleotides was reduced by up to 50% of that in normoxic boar spermatozoa. Probably, AMP is preferentially degraded to urea during reoxygenation if oxygen availability enables the reaction catalysed by xanthine oxidase to occur. It is likely that posthypoxic AMP degradation contributes to the intolerance of boar spermatozoa to hypoxia. The acidosis during hypoxia seems to be less harmful, because boar spermatozoa which were kept immotile in a commonly used diluent for two days at 16 °C could be reactivated by warming up without significant loss of motility though they also showed a marked acidosis (unpubl. results).

Carp spermatozoa showed a 50 to 80% drop in the ATP level[7,43] after progressive movements, and thereafter the spermatozoa appeared to be fatigued. Fatigue is accompanied by a dramatic increase of inorganic phosphate (P_i) and a corresponding decrease of PCr, but no decrease of pH_i was observed by ^{31}P-NMR (unpubl. results). Trout spermatozoa show similar changes in phosphorus metabolites, but the pH_i does decrease[46]. These results suggest that motility depends on consumption of PCr and ATP. On the other hand, motility could also be initiated in carp spermatozoa after storage of undiluted semen on ice for four hours, although these cells showed low intracellular concentrations of PCr and ATP compared with the high P_i level(fig. 3A). A phosphomonoester (PME) identified as AMP is also visible in NMR-spectra of sperms that are able to swim. The fact that initiation of motility is still possible at relatively low ATP and high P_i levels suggests that dynein ATPases function over a wide range of the free energy change of ATP hydrolysis.

Turkey and lugworm spermatozoa remain motile for several hours even if exogenous substrates and oxygen are wanting. Motile lugworm spermatozoa incubated without exogenous substrates but with a continuous air supply are able to keep their ATP level approximately constant for several hours. Hypoxia, however, results in a loss of PCr and in a marked drop in the ATP level, as measured by biochemical analysis (not shown) and in the case of PCr by in vivo-^{31}P-NMR-spectroscopy (fig. 3, D–F). In hypoxic turkey spermatozoa (fig. 3B) AMP was identified by NMR as the main product of ATP breakdown[12]. Re-aeration induced the appearance of the PCr signal and, simultaneously with an increase in ADP and ATP resonances, the AMP resonance disappeared (fig. 3, B and C).

Spermatozoa from horseshoe crab[44] (*Limulus polyphemus*), sea urchin[14] (*Strongylocentrotus purpuratus*) and trout (*Salmo gairdneri*)[46] also show the typical high levels of phosphagen in fresh, quiescent spermatozoa but lack PCr and ATP resonances after motility or hypoxia. In ^{31}P-NMR spectra of rooster[11] and human semen[3], no phosphagen was detected. The authors assumed that these spermatozoa lack a phosphagen shuttle[45] but this was disproved by Wallimann et al.[53,54]. Possibly the NMR experiments were performed under hypoxic conditions, which would explain the discrepancy.

The motility of hypoxic turkey and lugworm spermatozoa seems to be unaffected by a considerable decrease in the ATP level and a concomitant increase in the P_i level. This suggests that the free energy change of ATP hydrolysis remains sufficient for sperm movement even when the ATP level is low and the P_i level is high. These results agree with Brown's[10] hypothesis that ion transport and muscle contraction or cell motility are less sensitive to the ATP/ADP ratio than 'less immediately essential processes' such as protein synthesis and glyconeogenesis (processes not found in mature spermatozoa).

Outlook

Our first comparative investigations on spermatozoa of species from different phyla suggest general and species-specific features in sperm energy metabolism. One general feature might be the ability of spermatozoa to perform flagellar beats with unusually low ATP levels and high AMP, ADP and in some cases high P_i concentrations. This raises questions about the significance of ATP-homeostasis for cell functions (contractility, ion and metabolite transport or biosynthesis) and for ATP-production (e.g. control of hexokinase or 6-phosphofructokinase activities). Biochemical, fluorometric and NMR-spectroscopic techniques have been used for determinations of the cytoplasmic concentrations of important regulatory metabolites and ions, so that possible effects on the kinetics of the enzymes involved can be examined. The kinetic parameters of structure bound glycolytic enzymes in boar spermatozoa will be compared with those of the purified enzymes, to make a contribution to the general question of enzymologists: how far does enzyme assembly in intact cells affect kinetic properties?

Boar spermatozoa provide an interesting model for studying other aspects of the compartmentation and control of glycolysis. Immunocytochemical investigations are in progress to examine whether a 'job sharing' in ATP supply exists, between glycolysis in the distal part of the flagellum and mitochondria in the middle piece. This might also be of general interest because 'job sharing' in ATP supply is one possible explanation of the phenomenon of aerobic glycolysis[24].

Acknowledgments. We thank D. Westhoff for providing electron micrographs and Dr. A. G. Meyer for critically reading the manuscript. The supply of boar semen by the local breeders association GFS (Ascheberg), of carp semen by K. Liller and A. Sander (RWE-Limnotherm, Bergheim) and of turkey semen by B. Inhestern (Reken) are acknowledged with appreciation. This work is supported by the Deutsche Forschungsgemeinschaft (Ka 583/4-1) and the NRW government.

1 Ahluwalia, B. C., and Graham, E. F., Free amino acids in the semen of the fowl and turkey. J. Reprod. Fert. *12* (1966) 365–368.
2 Anderson, W. A., and Personne, P., The localization of glycogen in the spermatozoa of various invertebrate and vertebrate species. J. Cell Biol. *44* (1970) 29–51.
3 Arrata, W. S. M., Burt, T., and Corder, S., The role of phosphate esters in male fertility. Fert. Steril. *30* (1978) 329–333.
4 Baccetti, B., Pallini, V., and Burrini, A. G., Localization and catalytic properties of lactate dehydrogenase in different sperm models. Expl Cell Res. *90* (1975) 183–190.
5 Baccetti, B., Evolution of the sperm cell, in: Biology of Fertilization, vol. 2, pp. 3–58. Eds. C. B. Metz and A. Monroy. Academic Press, London 1985.
6 Billard, R., and Cosson, M.-P., The energetics of fish sperm motility. in: Controls of Sperm Motility: Biological and Clinical Aspects, pp. 153–173. Ed. C. Gagnon. CRC Press, Boca Raton (USA) 1990.
7 Billard, R., Cosson, J., Perchec, G., and Linhart, O., Biology of sperm and artificial reproduction in carp. Aquaculture *129* (1995) 95–112.

8 Brillard, J. P., Sperm storage and transport following natural mating and artificial insemination. Poult. Sci. *72* (1993) 923–928.

9 Brooks, D. E., Examination of bull semen and of the bull and rabbit testis for the presence of creatine phosphate and arginine phosphate. J. Reprod. Fert. *26* (1971) 275–278.

10 Brown, G. C., Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem. J. *284* (1992) 1–13.

11 Burt, C. T., and Chalovich, J. M., Serine ethanolamine phosphodiester: a major component in chicken semen. Biochim. biophys. Acta *529* (1978) 186–188.

12 Büsselmann, G., Kamp, G., and Lauterwein, J., ^{31}P-NMR studies on turkey semen. Abstract XVIth ICMRBS Intern. Conference of Magnetic Resonance in Biological Systems, p. 63. Veldhoven (1994).

13 Cain, D. F., and Davies, R. E., Breakdown of adenosine triphosphate during a single contraction of working muscle. Biochem. biophys. Res. Comm. *8* (1962) 361–366.

14 Christen, R., Schackmann, R. W., Dahlquist, F. W., and Shapiro, B. M., ^{31}P-NMR analysis of sea urchin sperm activation. Expl Cell Res. *149* (1983) 289–294.

15 Christen, R., Gatti, J.-L., and Billard, R., Trout sperm motility. Eur. J. Biochem. *166* (1987) 667–671.

16 Duncan, A., The spawning of *Arenicola marina* (L.) in the British Isles. Proc. zool. Soc. Lond. *134* (1960) 37–156.

17 Elsing, A., Untersuchungen zum Energiestoffwechsel von Spermazellen des marinen Anneliden *Arenicola marina*. Diploma thesis, University of Münster 1994.

18 Fioroni, P., Allgemeine und vergleichende Embryologie der Tiere. Springer, Berlin 1987.

19 Fitts, R. H., Cellular mechanisms of muscle fatigue. Physiol. Rev. *74* (1994) 49–94.

20 Gallina, F. G., Gerez de Burgos, N. M., Burgos, C., Coronel, C. E., and Blanco, A., The lactate/pyruvate shuttle in Spermatozoa: Operation in vitro. Archs Biochem. Biophys. *308* (1994) 515–519.

21 Gellerich, F. N., Kapischke, M., Kunz, W., Neumann, W., Kuznetsov, A., Brdiczka, D., and Nicolay, K., The influence of the cytosolic oncotic pressure on the permeability of the mitochondrial outer membrane for ADP: implications for the kinetic properties of mitochondrial creatine kinase and for ADP channelling into the intermembrane space. Molec. cell. Biochem. *133/134* (1994) 85–104.

22 Gibbons, B. H., and Gibbons, I. R., Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with triton X-100. J. Cell Biol. *54* (1972) 75–97.

23 Howarth, B., and Palmer, M. B., An examination of the need for sperm capacitation in the turkey. J. Reprod. Fertil. *28* (1972) 443–445.

24 Ishida, Y., Riesinger, I., Wallimann, T., and Paul, R. J., Compartmentation of ATP synthesis and utilization in smooth muscle: role of aerobic glycolysis and creatine kinase. Molec. cell. Biochem. *133/134* (1994) 39–50.

25 Jamieson, B. G. M., and Rouse, G. W., The spermatozoa of the Polychaeta (Annelida): an ultrastructural review. Biol. Rev. *64* (1989) 93–157.

26 Jamieson, B. G. M., Fish Evolution and Systematics: Evidence from Spermatozoa, p. 141. Cambridge University Press. Cambridge 1991.

27 Kamp, G., and Juretschke, H. P., Hypercapnic und hypocapnic hypoxia in the lugworm *Arenicola marina*: a ^{31}P-NMR study. J. expl Zool. *252* (1989) 219–227.

28 Kamp, G., and Lauterwein, J., Multinuclear magnetic resonance studies of boar seminal plasma. Biochim. biophys. Acta *1243* (1995) 101–109.

29 Kamp, G., Englisch, H., Müller, R., Westhoff, D., and Elsing, A., Comparison of two different phosphagen systems in the lugworm *Arenicola marina*. J. comp. Physiol. *165* (1995) 496–505.

30 Krasznai, Z., Marian, T., Balkay, L., Gaspar, R., and Tron, L., Potassium channels regulate hypo-osmotic shock-induced motility of common carp (Cyprinus carpio) sperm. Aquaculture *129* (1995) 123–128.

31 Lake, P. E., Male genital organs. in: Form and Function in Birds, Vol. 2. pp. 1–61. Eds. A. S. King and J. McLelland. Academic Press, London 1981.

32 Lamming, G. E. (Ed.), Marshall's Physiology of Reproduction, 4th edition, vol. 2. Churchill Livingstone, Edinburgh 1990.

33 Lipmann, F., Metabolic generation and utilization of phosphate bond energy. Adv. Enzymol. *1* (1941) 99–162.

34 Lohmann, K., Über die Pyrophosphatfraktion im Muskel. Naturwiss. *31* (1929) 624–625.

35 Lynch, M. J., Masters, J., Pryor, J. P., Lindon, J. C., Spraul, M., Foxall, P. J. D., and Nicholson, J. K., Ultra high field NMR spectroscopic studies on human seminal fluid, seminal vesicle and prostatic secretions. J. Pharm. Biomed. Anal. *12* (1994) 5–19.

36 Mann, Th., Metabolism of semen. Adv. Enzymol. *9* (1949) 329–390.

37 Mann, Th., and Lutwak-Mann, C. (Eds.), Male Reproductive Function and Semen. Springer, Berlin 1981.

38 Mita, M., and Yasumasu, I., Metabolism of lipid and carbohydrate in sea urchin spermatozoa. Gamete Res. *7* (1983) 133–144.

39 Mita, M., Diacyl choline phosphoglyceride – the endogenous substrate for energy metabolism in Sea Urchin spermatozoa. Zool. Sci. *9* (1992) 563–568.

40 Okuno, M., and Brokaw, C. J., Inhibition of movement of triton-demembranated sea-urchin sperm flagella by Mg^{2+}, ATP^{4-}, ADP and P_i. J. Cell Sci. *38* (1979) 105–123.

41 Oppenheimer, J. R., Mouthbreeding fishes. Anim. Behav. *18* (1970) 493–503.

42 Overstreet, J. W., and Katz, D. F., Interaction between the female reproductive tract and spermatozoa, in: Controls of Sperm Motility: Biological and Clinical Aspects, pp. 63–75. Ed. C. Gagnon. CRC Press, Boca Raton (USA) 1990.

43 Perchec, G., Jeulin, C., Cosson, J., Andre, F., and Billard, R., Relationship between sperm ATP content and motility of carp spermatozoa. J. Cell Sci. *108* (1995) 747–753.

44 Robitaille, P. A., Robitaille, P.-M. L., and Brown, G. G., ^{31}P-NMR studies of *Limulus polyphemus*: spermatozoa at rest and after motility. J. expl Zool. *238* (1986) 89–98.

45 Robitaille, P.-M. L., Robitaille, P. A., Martin, P. A., and Brown, G. G., Phosphorus-31 nuclear magnetic resonance studies of spermatozoa from the boar, ram, goat and bull. Comp. Biochem. Physiol. *87B* (1987) 285–296.

46 Robitaille, P.-M. L., Mumford, K. G., and Brown, G. G., ^{31}P-NMR nuclear magnetic resonance study of trout spermatozoa at rest, after motility, and during short-term storage. Biochem. Cell Biol. *65* (1987) 474–485.

47 Strong, S. J., and Ellington, W. R., Horseshoe crab sperm contain a unique isoform of arginine kinase that is present in midpiece and flagellum. J. expl Zool. *267* (1993) 563–571.

48 Taggart, D. A., A comparison of sperm and embryo transport in the female reproductive tract of marsupial and eutherian mammals. Reprod. Fert. Dev. *6* (1994) 451–472.

49 Thoai, R. M., and Robin, Y., Guanidine compounds and phosphagens. in: Chemical Zoology, pp. 163–203. Eds. M. Florkin and B. T. Scheer. Academic Press, New York 1979.

50 Tombes, R. M., and Shapiro, B. M., Metabolite channeling: a phosphorylcreatine shuttle to mediate high energy phosphate transport between sperm mitochondrion and tail. Cell *41* (1985) 325–334.

51 Tombes, R. M., and Shapiro, B. M., Energy transport and cell polarity: relationship of phosphagen kinase activity to sperm function. J. expl Zool. *251* (1989) 82–90.

52 Thompson, L. V., and Fitts, R. H., Muscle fatigue in the frog semitendinosus: role of high-energy phosphates and P_i. Am. J. Physiol. *263* (1992) C803–C809.

53 Wallimann, Th., Moser, H., Zurbriggen, B., Wegmann, G., and Eppenberger, H. M., Creatine kinase isoenzymes in spermatozoa. J. Muscle Res. Cell Motil. *7* (1986) 25–34.

54 Wallimann, Th., and Hemmer, W., Creatine kinase in non-muscle tissues and cells. Molec. Cell Biochem. *133/134* (1994) 193–220.

55 Westhoff, D., Bohne, W., and Kamp, G., Unusual binding of a glycolytic enzyme to structures of mammalian spermatozoa. in: Proceedings of the German Zoological Society 87th Meeting in Jena. Fischer, Stuttgart 1994.

56 Wilkie, D. R., Muscular fatigue: effects of hydrogen ions and inorganic phosphate. Federation Proc. *45* (1986) 2921–2923.