Br. J. Pharmac. (1981), **73**, 633–638

EFFECTS OF DIVALENT AND LANTHANIDE IONS ON MOTILITY INITIATION IN RAT CAUDAL EPIDIDYMAL SPERMATOZOA

W.M. LEE, A.Y.F. TSANG & P.Y.D. WONG

Department of Physiology, Faculty of Medicine, University of Hong Kong, Hong Kong

1 Sperm motility initiation of rat caudal epididymal spermatozoa *in vitro* has been studied.

2 Spermatozoa flushed out from the cauda epididymis with a sodium-free medium exhibited a transient motility which decreased progessively. At 40 min, the forward motility was completely suppressed. However, if they were resuspended in a sodium containing medium their motility was completely restored to normal within 15 min.

3 This initiation of sperm motility required the presence of extracellular calcium. Maximal stimulation was obtained at a calcium concentration of 10^{-3}M. Above this concentration, further increase in calcium produced a fall in motility and at 10^{-2}M, motility initiation was completely suppressed.

4 The initiation of sperm motility has been shown to depend closely on sodium. Sperm motility initiation alters in a curvilinear fashion with extracellular sodium concentration showing saturation kinetics. High calcium (above 10^{-3}M) was found to depress motility initiation induced by sodium, without affecting the apparent affinity constant for sodium.

5 The effect of Ca^{2+} on sperm motility initiation could be mimicked by Sr^{2+} but not by Mg^{2+}, La^{3+} or Eu^{3+}.

6 In the presence of extracellular calcium (1.27 or 2.54 mM), La^{3+} and Eu^{3+} exerted a dose-dependent inhibition of sperm motility initiation. The IC_{50} values for both ions were about 5×10^{-5}M.

7 The possible mechanism of inhibition by lanthanide ions is discussed.

Introduction

It is now known that spermatozoa once formed in the testis are immature and immotile and it is only during transit through the epididymal duct that they acquire their fertilizing capacity and motility. By the time they reach the cauda epididymidis they have become fully mature. However, they are maintained in the quiescent state during storage in the cauda epididymis. During ejaculation, the epididymal content is mixed with the copious secretion of the accessory glands. This results in the activation of the already mature sperm to full motility, hence facilitating fertilization of the ovum. The mechanism responsible for transforming the spermatozoa from the immotile to the motile state is unknown but inorganic ions may play an important role.

Recently in our laboratory, we have studied the ionic basis of motility initiation in the rat cauda epididymal spermatozoa incubated *in vitro*. We found that during motility initiation, hydrogen ions were released from spermatozoa. There was a strong dependence of acid release and of motility initiation on extracellular sodium (Wong, Lee & Tsang, 1981). In preliminary experiments, it was noted that motility

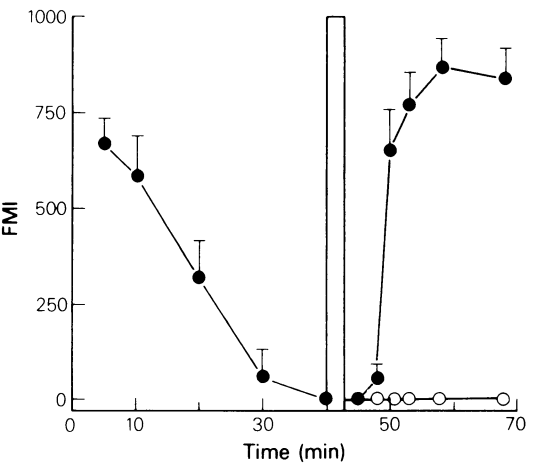

Figure 1 Forward motility (FMI) of spermatozoa after flushing out and incubation in a sodium-free medium. At the column, spermatozoa were resuspended in a sodium-containing medium in the presence (●) or in the absence (○) of Ca^{2+} 1.27 mM. Each point shows the mean of 4 to 7 experiments; vertical lines show s.e. mean.

0007-1188/81/070633-06 $01.00

initiation also required extracellular calcium. In the present study we have therefore investigated in greater detail the role of divalent cations in the initiation of sperm motility.

Methods

Male Sprague-Dawley rats (weighing 300 – 350 g) of proven fertility were prepared for microperfusion of the cauda epididymis as described previously (Wong & Yeung, 1978; Wong, Au & Ngai, 1979; Au & Wong, 1980). After cannulation of the duct the caudal epididymal content was flushed out with a sodium-free medium (SFM) which contained (mM): choline Cl 148, $CaCl_2$ 1.27 and Tris (pH7.02) 5. The solution had a calculated osmolarity of 295 mosmol/l. After flushing out, spermatozoa were sedimented by centrifugation at 700 *g* for 2 min and incubated in the same solutions containing bovine serum albumin (2 mg/ml) and glucose (2 mg/ml) at 35°C. Each incubation medium had a sperm concentration of 10^7/ml. During incubation, the forward motility of the spermatozoa was measured at timed intervals as described below.

The motility of spermatozoa was completely suppressed at the end of 40 min (see Results) and these are referred to as 'choline arrested' spermatozoa. They were then sedimented by centrifugation at 700 *g* and resuspended in a sodium containing medium (SCM) which had the same ionic content as the SFM except that choline chloride (148 mM) was replaced by NaCl. In some experiments, $CaCl_2$ was omitted to produce a Ca^{2+}-free SCM. Since the 'choline arrested' spermatozoa regained their motility upon resuspension in SCM, this period of resuspension in SCM is referred to as the 'initiation' period.

In some experiments, the 'choline arrested' spermatozoa were resuspended in the SCM containing various concentrations of Ca^{2+}. When Mg^{2+}, Sr^{2+}, La^{3+} or Eu^{3+} was used to replace Ca^{2+}, the $CaCl_2$ was omitted from the SCM and different concentrations of these ions were included. On other experiments, motility initiation of the rat caudal spermatozoa was studied in SCM containing different concentrations of Mg^{2+}, Sr^{2+}, La^{3+} or Eu^{3+} but in the presence of Ca^{2+} (1.27 or 2.54 mM).

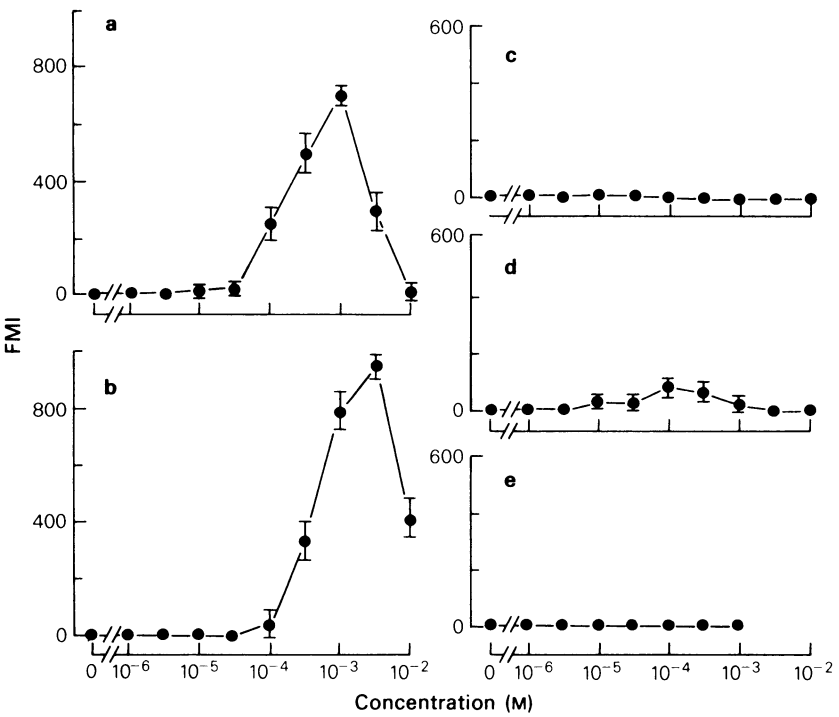

Figure 2 Effects of (a) Ca^{2+}, (b) Sr^{2+}, (c) Mg^{2+}, (d) La^{3+} and (e) Eu^{2+} on the initiation of sperm motility. Spermatozoa were first incubated in sodium-free medium to suppress motility and then subsequently washed and resuspended in a Ca^{2+}-free sodium-containing medium with different concentrations of these ions. The FMI was measured after 15 min resuspension in various solutions. Each point shows the mean of 3–16 experiments; vertical lines show s.e. mean.

different concentrations of Mg^{2+}, Sr^{2+}, La^{3+} or Eu^{3+} but in the presence of Ca^{2+} (1.27 or 2.54 mM).

Measurement of sperm motility

The progressive forward motility of the spermatozoa was measured by the photographic tracking method (Hoskins, Johnson, Brandt & Acott, 1979; Wong, Tsang, Lee & Li, 1980). The sperm suspension (5 μl) was placed on a prewarmed (35°C) haemocytometer and a photograph taken with Kodak 135 Tri-X film at exposure time of 1 s under a 10 × 10 dark field illumination (Olympus BHA). This procedure was repeated for three different fields and the whole procedure took less than 20 s. The developed negatives were projected onto a screen with a final magnification of 720 times. Sperm tracks were measured directly from the screen and converted into micrometres. The forward motility index (FMI) was calculated from the equation:

$$FMI = n/N\,(\bar{v}) \times 100$$

where n is the number of sperm tracks, N is the total number of sperm and $\bar{v}$ is the mean velocity in micrometres per s calculated from the three fields. In all experiments, during the course of motility initiation (except when the spermatozoa were immotile), the fraction of motile sperm, n/N was quite constant and had values from 0.5 – 0.7. $\bar{v}$ ranged from 0 to 30 μm/s.

Results

Effect of calcium on sperm motility initiation

When spermatozoa were flushed out from the cauda epididymis with the sodium-free medium (SFM), they exhibited a transient motility which then decreased progressively. At the end of 40 min the motility was reduced to zero. However, when the 'choline-arrested' spermatozoa were resuspended in a sodium containing medium (SCM) which contained calcium (1.27 mM), the forward motility was restored within 15 min. If resuspended in a sodium containing medium without extracellular calcium, their motility was not restored (Figure 1).

The curve relating calcium concentration and motility initiation is shown in Figure 2. The ability to initiate sperm motility rose with increasing calcium concentration and the peak value was obtained at 10^{-3}M. Further increase in calcium concentration produced a fall in motility and at 10^{-2}M, initiation was completely suppressed.

Effects of other cations in motility initiation

It is of interest to see whether other divalent and lanthanide ions can replace calcium in motility initiation. The effects of Mg^{2+}, Sr^{2+}, La^{3+} and Eu^{3+} were studied. In these experiments the $CaCl_2$ was omitted and various concentrations of these ions were added. It was found that among the ions studied, only Sr^{2+} could effectively replace calcium in the sperm motility initiation (Figure 2). The concentration-response relationship for Sr^{2+} was similar to that for Ca^{2+}.

Interaction between calcium and sodium

The motility initiation of the rat cauda epididymal spermatozoa has been shown to be dependent on the extracellular sodium concentration (Wong *et al.*, 1981). The motility attained during initiation increased in a curvilinear fashion with sodium concentration, with maximal motility reached at a sodium concentration of 60 mM. The concentration of sodium giving rise to 50% motility was approx. 25 mM. Increasing extracellular calcium concentration (above 1 mM) was found to depress motility activation induced by all sodium concentrations (Figure 3).

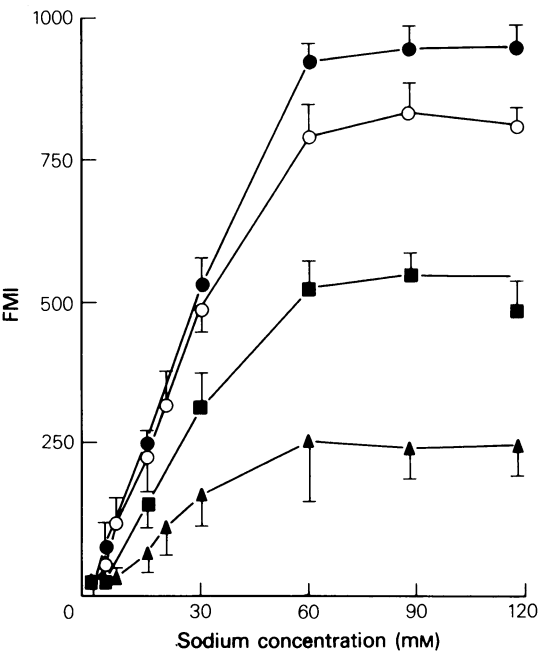

Figure 3 Effect of different calcium concentrations on the dependence of sperm motility initiation on extracellular sodium ions. Spermatozoa were first incubated in sodium-free medium to suppress motility and then subsequently resuspended in solutions containing different sodium concentrations (choline chloride was used to substitute for sodium) and in the presence of 1.27 (●); 2.54 (○); 3.76 (■) or 5.08 (▲) mM Ca^{2+}. The FMI was measured after 15 min resuspension in various solutions. Each point shows the mean of 3–7 experiments; vertical lines show s.e. mean.

Effects of other ions in the presence of calcium

It has been shown that the other divalent and lanthanide ions can interact with calcium in many physiological processes. Experiments were performed in which the effects of Mg^{2+}, Sr^{2+}, La^{3+} and Eu^{3+} were studied in the presence of calcium. It was found that Mg^{2+} and Sr^{2+} had no effect on the calcium-dependent motility initiation, whereas La^{3+} and Eu^{3+} produced a concentration-dependent inhibition on the motility initiation. The IC_{50} values for La^{3+} and Eu^{3+} were approx. 5×10^{-5}M. The inhibition curves for both ions were similar whether the calcium concentration was 1.27 or 2.54 mM (Figure 4).

Discussion

There is compelling evidence to show that calcium ions are involved in many physiological processes. This is particularly true of excitation-contraction coupling (Frank, 1962; Ebashi, 1974), excitation-secretion coupling (Douglas & Poisner, 1964; Matthews, 1970) and cell division (Duffus & Patterson, 1974). Elegant studies with ciliated protozoa have also established a role of calcium in determining the orientation and frequency of the ciliary beat (Naitoh & Kaneko, 1972). Recently, we have been interested in the ionic basis of initiation of sperm motility. We found that motility activation of the rat caudal epididymal spermatozoa requries the presence of extracellular sodium. In the present study, a role for calcium in the motility initiation has also been established.

In the absence of extracellular Ca^{2+}, the initiation of sperm motility was abolished. The requirement for calcium may be attributed to an influx of calcium ions essential for activation of flagellar movement in the rat caudal spermatozoa. This phenomenon is analogous to the influx of calcium into smooth muscle and cardiac muscle cells prior to muscle contraction. Storey (1975) and Babcock, First & Lardy (1976)

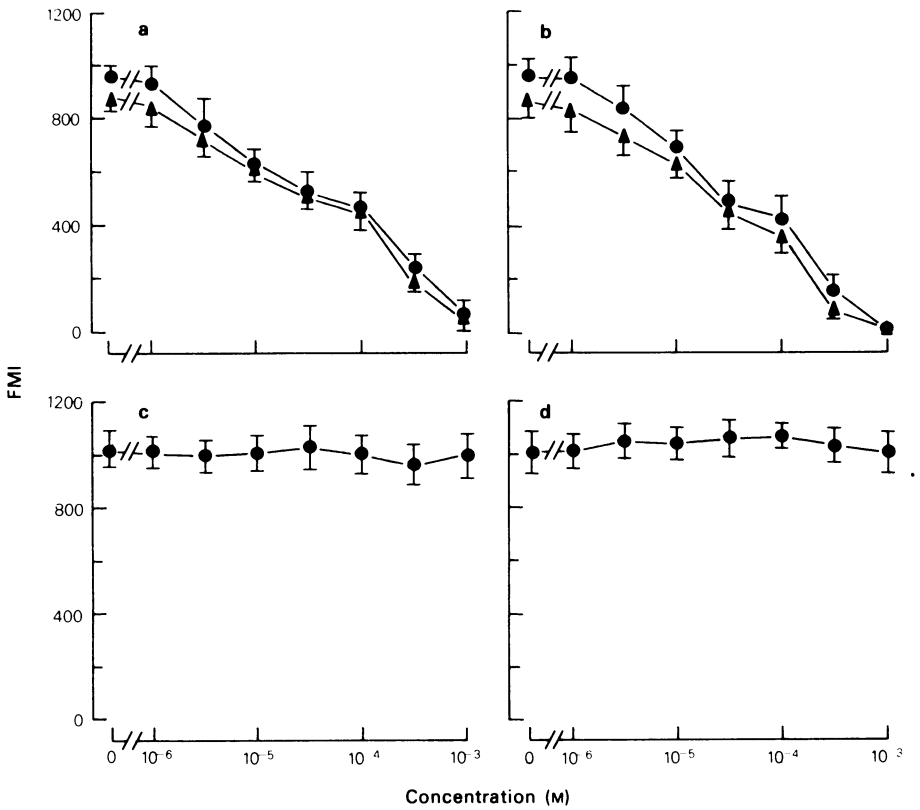

Figure 4 Effects of (a) La^{3+}, (b) Eu^{3+}, (c) Mg^{2+} and (d) Sr^{2+} on the motility initiation of the rat caudal epididymal sperm in the presence of 1.27 (●) or 2.54 (▲) mM Ca^{2+}. Spermatozoa were first incubated in sodium-free medium to suppress motility and then subsequently resuspended in various solutions. The FMI was measured after 15 min of resuspension. Each point shows the mean of 3–5 experiments; vertical lines indicate s.e. mean.

have shown that the calcium ionophore A23187 can increase sperm metabolism and motility by inducing calcium influx into spermatozoa. We found that the effect of calcium in the motility activation could be replaced by strontium but not by magnesium and the lanthanide ions. This observation has parallels in the physiology of muscle contraction in that strontium can effectively replace calcium in excitation-contraction coupling by binding at the calcium sites within the muscle cells (Edwards, Lorković & Weber, 1966).

The action of calcium on motility initiation was concentration-dependent, stimulating at low concentrations but inhibiting it at high concentrations (above 1 mM, Figure 2). The latter effect probably reflects an action on the membrane. There is evidence that high calcium decreases the permeability of nerve membrane (Frankenhaeuser & Hodgkin, 1957), muscle membrane (Lüttgau & Niedergerke, 1958), red cell membrane (Bolingbroke & Maizels, 1959) and transporting epithelia (Cuthbert & Wong, 1971) to sodium and it is possible that a similar mechanism may be operative in our system. There is evidence that motility initiation requires an influx of sodium ions into sperm. First, sperm motility initiation is closely dependent on extracellular sodium concentration. Secondly, amiloride, a drug which specifically blocks sodium influx into many cells exerts a dose-dependent inhibition on sperm motility initiation (Wong *et al.*, 1981). Our present results show that increasing extracellular calcium concentration depressed the sodium-induced motility activation (Figure 3). The sodium concentration giving rise to 50% motility was not affected by high calcium but the maximal motility produced by sodium was reduced. It is possible that calcium may interact with sodium sites in the membrane leading to a decrease in motility activation.

This study also demonstrates that the lanthanides, La^{3+} and Eu^{3+} inhibited motility stimulation in the presence of calcium (Figure 4). Lanthanum has been found to inhibit the action of calcium in many other tissues. Examples include blockade of transmitter release from motor nerve terminals (Miledi, 1971), inhibition of histamine release from mast cells (Foreman & Mongar, 1972) and the contractility of cardiac muscle (Wong, Hwang & Yeung, 1976). These effects are probably mediated by lanthanum blocking the sites for calcium entry in the cell membrane. Another possibility is that these lanthanide ions entered the spermatozoa and exerted an inhibitory effect on the metabolic pathway which is essential for motility activation. Alternatively, these ions might also inhibit the generation of cyclic adenosine 3′,5′-monophosphate which has been proposed as the intracellular mediator of motility initiation in mammalian sperm (Hoskins & Casillas, 1975; Cascieri, Amann & Hammerstedt, 1976). In this context, the inhibition of adenyl cyclase activity by lanthanum has been demonstrated in other tissues (Wong, Bedwani & Cuthbert, 1972).

We are grateful to Professor A.W. Cuthbert for his useful comments on the manuscript. This work was supported by the Committee of the Medical Faculty Research Grant Fund, University of Hong Kong. The skilful technical assistance of Mr C.M. Li is gratefully acknowledged.

References

AU, C.L. & WONG, P.Y.D. (1980). Luminal acidification by the perfused rat cauda epididymidis. *J. Physiol.*, **309,** 419–427.

BABCOCK, D.F., FIRST, N.L. & LARDY, H.A. (1976). Action of ionophore A23187 at the cellular level. Separation of effect at the plasma and mitochondrial membranes. *J. biol. Chem.*, **251,** 3881–3886.

BOLINGBROKE, V. & MAIZELS, M. (1959). Calcium ions and the permeability of human erythrocytes. *J. Physiol.*, **149,** 563–585.

CASCIERI, M., AMANN, R.P. & HAMMERSTEDT, R.H. (1976). Adenine nucleotide changes at initiation of bull sperm motility. *J. biol. Chem.*, **10,** 787–793.

CUTHBERT, A.W. & WONG P.Y.D. (1971). The effect of metal ions and antidiurectic hormone on oxygen consumption in toad bladder. *J. Physiol.*, **219,** 39–56.

DOUGLAS, W.W. & POISNER, A.M. (1964). Calcium movement in the neurohypophysis of the rat and its relation to the release of vasopressin. *J. Physiol.*, **172,** 19–30.

DUFFUS, J.H. & PATTERSON, L.J. (1974). Control of cell division in yeast using the ionophore, A23187 with calcium and magnesium. *Nature, Lond.*, **251,** 626–627.

EBASHI, S. (1974). Regulatory mechanism of muscle contraction with special reference to the Ca-troponin-tropomyosin system. In *Essays in Biochemistry*, ed. Campbell, P.N. & Dickins, F. pp. 1–36. New York: Academic Press.

EDWARDS, C., LORKOVIĆ, H. & WEBER A. (1966). The effect of the replacement of calcium by strontium on excitation-contraction coupling in frog skeletal muscle. *J. Physio.*, **186,** 295–306.

FOREMAN, J.C. & MONGAR, J.L. (1972). Dual effect of lanthanum on histamine release from mast cells. *Nature, New Biol.*, **240,** 255–256.

FRANK, G.B. (1962). Utilization of bound calcium in the action of caffeine and certain multivalent cations on frog's skeletal muscle. *J. Physiol.*, **163,** 254–268.

FRANKENHAEUSER, B. & HODGKIN, A.L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.*, **137,** 218–244.

HOSKINS, D.D. & CASILLAS, E.R. (1975). Function of cyclic nucleotides in mammalian spermatozoa. In *Handbook of Physiology; Endocrinology*, Vol. V: Male Reproductive System, ed. Hamilton, D.W. & Greep, R.O.

pp. 000–000. Washington, D.C.: American Physiological Society.

HOSKINS, D.D., JOHNSON, D., BRANDT, H. & ACOTT, T.S. (1979). Evidence for a role for a forward motility protein in the epididymal development of sperm motility. In *The Spermatozoon*, ed. Fawcett, D. & Bedford, M.J. pp. 43–53. Baltimore-Munich: Urban & Schwarzenberg.

LÜTTGAU, H.C. & NIEDERGERKE, R. (1958). The antagonism between Ca and Na ions on the frog's heart. *J. Physiol.*, **317,** 84–92.

MATTHEWS, E.K. (1970). Calcium and hormone release. In *Calcium and Cellular Functions*, ed. Cuthbert, A.W. pp. 163–182. London: Macmillan.

MILEDI, R. (1971). Lanthanum ions abolish the 'calcium response' of nerve terminals. *Nature, Lond.*, **229,** 410–411.

NAITOH, Y. & KANEKO, H. (1972). Reactivated triton-extracted models of paramecium: modification of ciliary movement by calcium ions. *Science*, **176,** 523–524.

STOREY, B.T. (1975). Energy metabolism of spermatozoa. IV. Effect of calcium on respiration of mature epididymal sperm of the rabbit. *Biol. Reprod.*, **13,** 1–9.

WONG, P.Y.D., AU, C.L. & NGAI, H.K. (1979). Some characteristics of salt and water transport in the rat epididymis. In *The Spermatozoon*, ed. Fawcett, D. & Bedford, M.J. pp. 57–63. Baltimore-Munich: Urban & Schwarzenberg.

WONG, P.Y.D., BEDWANI, J.R. & CUTHBERT, A.W. (1972). Hormone action and the levels of cyclic AMP and prostaglandins in the toad bladder. *Nature, New Biol.*, **238,** 27–31.

WONG, P.Y.D., HWANG, J.C. & YEUNG, C.H. (1976). Interaction between lanthanum and calcium in isolated guinea-pig heart. *Eur. J. Pharmac.*, **36,** 253–256.

WONG, P.Y.D., LEE, W.M. & TSANG, A.Y.F. (1981). The effects of extracellular sodium on acid release and motility initiation in rat caudal epididymal spermatozoa *in vitro*. *Expl. Cell Res.*, **131,** 97–104.

WONG, P.Y.D., TSANG, A.Y.F., LEE, W.M. & LI, C.M. (1980). secretion of the rat cauda epididymidis. *Arch. Ardro.*, **5,** 327–336.

WONG, P.Y.D. & YEUNG, C.H. (1978). Absorptive and secretory functions of the perfused rat cauda epididymidis. *J. Physiol.*, **275,** 13–26.

(Received October 23, 1980.
Revised December 15, 1980.)