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Taurine, Hypotaurine, Epinephrine and Albumin Inhibit Lipid Peroxidation in Rabbit Spermatozoa and Protect Against Loss of Motility

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ABSTRACT

Loss of forward motility of rabbit epididymal spermatozoa in high K^+ phosphate buffer is inhibited by taurine, hypotaurine, epinephrine and bovine serum albumin. Pyruvate and lactate also show this effect. The rate of lipid peroxidation in these spermatozoa, as measured by rate of formation of malonaldialdehyde, is also inhibited by these agents. A close linear correlation between percent inert spermatozoa and malondialdehyde was found, which was independent of the rate of peroxidation. Complete cessation of motility was observed at 0.5 nmol malondialdehyde/10^8 cells in the absence or presence of these agents, which is the same value found in other suspending media in a previous study [Alvarez and Storey (1982) Biol. Reprod. 27:1102–1108]. Albumin was the most effective agent in preventing loss of motility and inhibiting lipid peroxidation. Hypotaurine was the next most effective, followed by taurine, epinephrine, pyruvate and lactate. Hypotaurine reduces the amount of rate of superoxide production, as measured by the rate of reduction of acetylated ferricytochrome *c* by $O_2^{\cdot-}$, from rabbit sperm under these conditions and concomitantly reduces inactivation of the superoxide dismutase in these cells. Since superoxide seems to be the major inducer of lipid peroxidation in rabbit sperm, the protective effect of hypotaurine, which should be readily permeant to the plasma membrane, may be ascribed to scavenging of intracellular superoxide. The mechanism of the protective action of albumin is not known. Rabbit epididymal spermatozoa lose motility over time if Ca^{2+} or Mg^{2+} are omitted from the suspending medium. This loss is not correlated with the rate of lipid peroxidation, which is unaffected by the absence or presence of these ions. The linear correlation between loss of motility and lipid peroxidation no longer holds in the absence of Ca^{2+} and Mg^{2+}, implying motility loss by a different mechanism.

INTRODUCTION

Studies of in vitro fertilization in mammals have been greatly facilitated by refinements in the culture media which maintain gamete viability. This is particularly true of studies with hamster gametes, in which sperm motility can be maintained and fertilization enhanced by addition of bovine serum albumin, catecholamines, and the β-aminoacids, taurine and hypotaurine (Lui and Meizel, 1977; Lui et al., 1977; Cornett and Meizel, 1978; Bavister et al., 1979; Cornett et al., 1979; Mrsny et al., 1979; Bavister, 1979, 1980; Meizel and Working, 1980; Meizel et al., 1980; Meizel, 1981; Leibfried and Bavister, 1981, 1982). Taurine appears to be the sperm motility factor (SMF) active with hamster spermatozoa (Bavister, 1979; Mrsny et al., 1979; Meizel et al., 1980), although hypotaurine seems even more effective (Leibfreid and Bavister, 1981). The mechanism by which these agents maintain motility has apparently not been elucidated (Leibfreid and Bavister, 1982). In our studies of lipid peroxidation in rabbit spermatozoa, we found a close linear correlation between decline of sperm motility and extent of peroxidation, as measured by malondialdehyde (MDA) production (Alvarez and Storey, 1982). This raised the question of whether one mode of action of these agents might be inhibition of lipid peroxidation. The major inducer of lipid peroxidation in rabbit spermatozoa has been shown to be the superoxide species produced by the cells during aerobic incubation (Holland et al., 1982; Alvarez and Storey, 1983), with the perhydroxyl radical $HO_2^{\cdot}$ being about 300-fold more effective than its conjugate base, the

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superoxide anion $O_2^{\cdot-}$ (Alvarez et al., 1983). (In this paper, we use the term "superoxide" to denote both $O_2^{\cdot-}$ and $HO_2^{\cdot}$ in rapid acid-base equilibrium; each species is designated by chemical formula.) Epinephrine reacts with $O_2^{\cdot-}$ to yield adrenochrome (Misra and Fridovich, 1971), so that epinephrine can act as a scavenger for superoxide. In this paper, we report the effects of epinephrine, taurine, hypotaurine and albumin on motility maintenance and superoxide production in rabbit spermatozoa and compare these effects to those obtained with standard scavengers of oxygen species and with effects induced by mono- and diavalent cations.

MATERIALS AND METHODS

Reagents

Malonaldehyde-bis(dimethylacetal) and 2,5-dimethylfuran were from Aldrich Chemical Co. (Milwaukee, WI); prechloracetic acid, mannitol and inorganic salts were from J. T. Baker and of the highest purity available. Cytochrome *c* (horse heart, type VI), xanthine (sodium salt, grade III), xanthine oxidase (grade III), D-glucose, thiobarbituric acid, L-epinephrine, superoxide dismutase (Type I), catalase, 4-5 dihydroxy-1-3-benzene disulfonic acid (Tiron), pyruvate, lactate, bovine serum albumin (Fraction V), taurine, hypotaurine and N-N ethylenediaminotetraacetic acid disodium salt (EDTA) were from Sigma Chemical Co. (St. Louis, MO).

Suspending Media

Three basic media were used for sperm suspension in this study. One was a modification of the high potassium medium of Keyhani and Storey (1973), designated KTP, with the composition: 113 mM KCl, 12.5 mM KH_2PO_4, 2.5 mM K_2HPO_4, 3 mM $MgCl_2$, 20 mM Tris, 1.5 mM D-glucose, 0.4 mM EDTA, 0.6% penicillin/streptomycin, adjusted with HCl to pH 7.4. The high sodium medium, designated NTP, contained 10 mM KCl, 103 mM NaCl, and 15 mM NaH_2PO_4, but was otherwise identical in composition to KTP. The third medium was constituted to simulate the ionic composition of the rabbit oviduct (David et al., 1969) and was designated OSM. Its composition was: 108 mM NaCl, 10 mM KCl, 0.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 40 mM $NaHCO_3$, 0.5 mM $NaHPO_4$, 1.5 mM glucose, 0.4 mM EDTA, and 0.6% penicillin/streptomycin; it was treated with 5% CO_2 in air to bring the pH to the range 7.6–7.8. Variations on the compositions of these media included: omission of Mg^{2+} and/or Ca^{2+} from OSM; omission of Mg^{2+} from KTP and NTP, addition of Ca^{2+} to KTP and NTP, replacement of Na^+ by choline or Li^+ in NTP. In the case of Li^+ substitution, K^+ was omitted from the medium, and concentration sum of Li^+ and Na^+ was 130 mM. All media contained 0.4 mM EDTA in order to chelate heavy metal cations which might act as promoters of lipid peroxidation or accelerate the otherwise negligibly slow Haber-Weiss reaction (Weinstein and Bielski, 1979).

Preparation of Spermatozoa

Spermatozoa were obtained from the caudae of excised epididymides of mature male New Zealand White rabbits by retrograde flushing through the vas deferens with the appropriate medium, followed by washing (Holland and Storey, 1981). The final stock suspensions ranged from $1-5 \times 10^8$ cells/ml. Care was taken to prevent contamination of the sperm sample with hemoglobin from epididymal blood vessels, which interferes with the spectrophotometric procedure used to determine lipid peroxidation.

Lipid Peroxidation

Spontaneous lipid peroxidation was induced by exposure of the spermatozoa to O_2 during aerobic incubation at 37°C of sperm suspensions containing $1-5 \times 10^7$ cells/ml. Production of malondialdehyde (MDA) was determined by a modification of the thiobarbituric acid (TBA) assay (Barber and Bernheim, 1967), in which the TBA concentration was increased to 28 μM and the absorbance of the TBA/MDA chromogen was determined with the DW-2A dual wavelength spectrophotometer using the wavelength pair 534–570 nm. Full details of the procedure have been described by Alvarez and Storey (1982).

Spectrophotometric Assays

Formation of adrenochrome during aerobic incubation at 37°C of sperm suspensions in the presence of epinephrine was measured by a composition of the method of Misra and Fridovich (1972). At the designated times of aerobic incubation, samples were placed in crushed ice, chilling the suspension to 0°C. The sperm suspension was centrifuged at $750 \times g$ for 10 min. An aliquot of 1 ml of the supernatant was used for determination of adrenochrome, with the dual wavelength spectrophotometer, using the wavelength pair 480–575 nm and the difference extinction coefficient $\Delta\epsilon = 4.0\ mM^{-1}\ cm^{-1}$ (Misra and Fridovich, 1972).

The rate of $O_2^{\cdot-}$ production was measured by the method of Azzi et al. (1975) using acetylated ferricytochrome *c* at room temperature (24 ± 1°C) with the DW-2A dual wavelength spectrophotometer (American Instrument Co.), using the wavelength pair 550–540 nm and the difference extinction coefficient $\Delta\epsilon$=19 $mM^{-1}\ cm^{-1}$ (Margoliash and Frohwirt, 1959). Full details of the method are given by Holland et al. (1982).

The activity of superoxide dismutase (SOD) in fresh and aerobically incubated spermatozoa was estimated from its ability to inhibit the reaction of acetylated ferricytochrome *c* by $O_2^{\cdot-}$ generated from O_2 oxidation of 200 μM xanthine catalyzed by 10 mU xanthine oxidase (McCord and Fridovich, 1969). Monitoring conditions were as described above for the $O_2^{\cdot-}$ assay. Because $O_2^{\cdot-}$ reacts directly with the sperm cells (Holland et al., 1982), the SOD activity was not expressed in terms of units (McCord and Fridovich, 1969). Instead, the rate of cytochrome reduction in the absence of added sperm, R_0, the rate in presence of added sperm, R_1, and the subsequent rate after addition of 5 mM CN^-, R_2, were recorded. This concentration of CN^- is sufficient to inhibit completely the SOD activity of the cytosolic Cu–Zn enzyme, which in turn makes up 95% of the SOD activity of

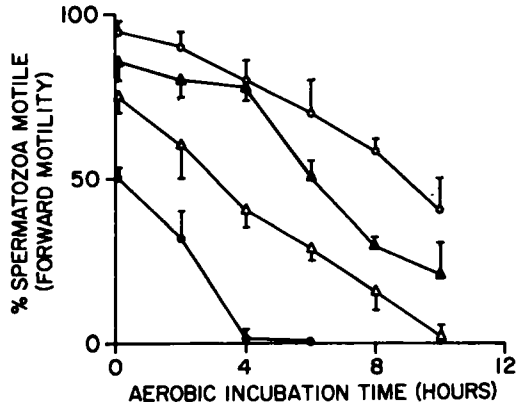

FIG. 1. Effect of added taurine, hypotaurine, and epinephrine on sperm forward motility as a function of aerobic incubation time at 37°C in medium KTP. In this assay, flagellar beating was not counted in the motility score. Sperm suspensions contained 1–5 × 10^7 cells/ml. No additions (control) (●—●); 0.5 mM hypotaurine (○—○); 0.5 mM taurine (▲—▲); 50 μM epinephrine (△—△). Each point is the mean of three experiments; *error bars* are standard deviations.

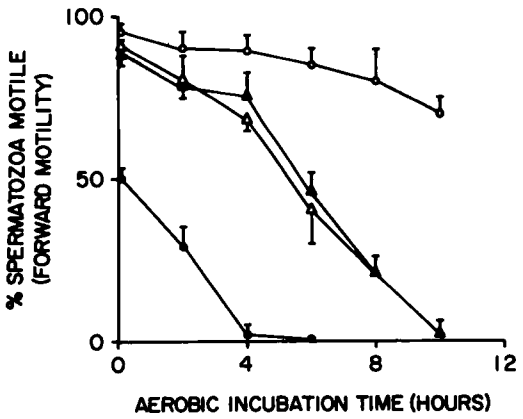

FIG. 2. Effect of added bovine serum albumin, lactate and pyruvate on sperm forward motility as a function of aerobic incubation time at 37°C in medium KTP. Experimental conditions were identical to those for Fig. 1. No additions (control) (●—●); albumin (3 mg/ml) (○—○); 3 mM pyruvate (▲—▲); 3 mM lactate (△—△).

intact rabbit sperm (Holland et al., 1982). The difference between R_2 and R_1 therefore gives the activity of the CN^--sensitive SOD. The SOD activity of the aerobically incubated spermatozoa was referred to SOD activity in fresh unincubated sperm, which was taken to be 100% (Alvarez and Storey, 1983).

Motility Assay

Sperm motility was estimated by the modification of the method of Heffner and Storey (1982) as described by Alvarez and Storey (1982), in which duplicate aliquots of the sperm suspension were taken and the average of the percentage motile in both aliquots estimated. Variation between duplicate aliquots was within ±5%. The assay was extended to include a second mode of flagellar motion in rabbit sperm, that of flageller beating. In this mode, there is active flagellar motion and the sperm move, but forward progress is close to nil in this group of sperm.

RESULTS

We have shown previously that lipid peroxidation and loss of motility in rabbit spermatozoa occurs 8-fold more rapidly in the high K^+ medium, KTP, than in the high Na^+ medium, NTP. Protective effects of added agents against these processes are therefore more readily observed in KTP. The effect of added taurine, hypotaurine and epinephrine on sperm forward motility during aerobic incubation in KTP is shown in Fig. 1. At 0.5 mM, hypotaurine and taurine both protect the sperm against loss of motility, with hypotaurine being somewhat more effective. Epinephrine at 50 μM also has a protective effect.

The beneficial effects of bovine serum albumin in maintaining rabbit sperm motility in KTP are shown in Fig. 2. Of all agents tested, this proved the most effective. Both lactate and pyruvate were about equally effective (Fig. 2), with forward motility lost only after 10 h, compared to 4 h for the control suspension.

Evidence that the protective effect of these agents on motility is due to inhibition of lipid peroxidation is peresented in Table 1 and Fig. 3. The rate of MDA production from rabbit sperm suspended in KTP in the absence and

TABLE 1. Lipid peroxidation in epididymal rabbit spermatozoa under aerobic incubation at 37°C in KTP.

Additions	Rate of MDA[a] production (nmol/min−10^8 cells)
None	0.045 ± 0.005
Hypotaurine (0.5 mM)	0.021 ± 0.005
Taurine (0.5 mM)	0.032 ± 0.004
Lactate (3 mM)	0.028 ± 0.006
Pyruvate (3 mM)	0.030 ± 0.002
Albumin (3 mg/ml)	0.0059 ± 0.0001

[a]Malondialdehyde (MDA) determined by the thiobarbituric assay described in *Materials and Methods*. Values are the means ± SD of three determinations.

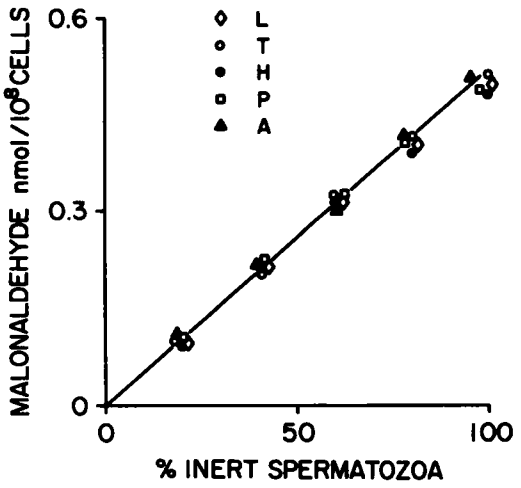

FIG. 3. Linear correlation between malondialdehyde production and percent inert spermatozoa observed during aerobic incubation at 37°C in medium KTP under the experimental conditions containing the added agents described for Figs. 1 and 2. Note that inert spermatozoa means cessation of both forward motility and flagellar beating (Alvarez and Storey, 1982). The *letters next to the symbols* denote the added agents: *L*=3 mM lactate; *T*=0.5 mM taurine; *H*=0.5 mM hypotaurine; *P*=3 mM pyruvate; *A*=albumin 3 mg/ml. The linear regression line calculated through the origin has the form y=0.0050x (*r*=0.996).

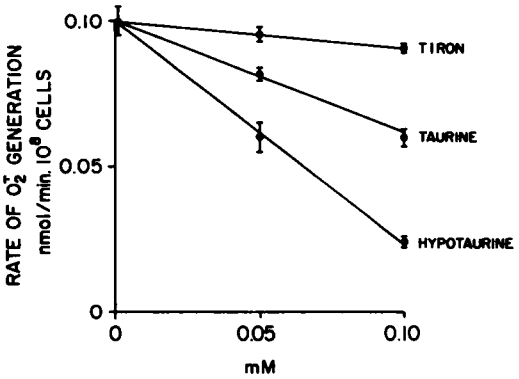

FIG. 4. Effects of Tiron, taurine and hypotaurine concentration on the rate of superoxide generation by rabbit spermatozoa, as measured by the rate of reduction of acetylated ferricytochrome *c* by O_2^-. The sperm were suspended at 5 × 10^7 cells/ml in medium NTP containing 90 μM acetylated ferricytochrome *c*. Each point is the mean of three experiments; *error bars* are standard deviations.

presence of the various agents is shown in Table 1. All the agents reduced the rate of production of this by-product of lipid peroxidation. Among the small molecules, hypotaurine had the greatest inhibitory effect, but albumin reduced lipid peroxidation by 8-fold to the rate observed in NTP (Alvarez and Storey, 1982). The loss of both forward motility and flagellar beating, designated as increasing percent inert spermatozoa, gave a linear correlation with the amount of MDA produced, independent of the rate of MDA production, with all agents tested (Fig. 3). This correlation is identical to the one originally obtained with KTP and NTP alone, and yields the same value of 0.5 nmol MDA/10^8 cells as the index of lipid peroxidation at which the whole sperm population becomes inert (Alvarez and Storey, 1982).

Since superoxide produced by rabbit sperm appears to be the chief inducer of spontaneous lipid peroxidation in these cells, the protective agents should exert their effect by reacting with superoxide. The ability of taurine and hypotaurine to reduce the amount of superoxide produced by the cell, as measured by reaction of O_2^- with acetylated ferricytochrome *c*, is shown in Fig. 4. Hypotaurine was the most effective, reducing the amount of superoxide by 75% at 0.1 mM (above this concentration of hypotaurine, the rate of cytochrome reduction becomes too slow to measure accurately). Hypotaurine also protects the sperm SOD from inactivation by superoxide (Alvarez and Storey, 1983; Table 2). Also shown is the effect of 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron) which has been shown to be a scavenger of O_2^- (Miller, 1970; Miller and Rapp, 1975). Over the concentration range used for Fig. 4, Tiron has little effect on superoxide production, and at 0.5 mM does not protect the sperm against motility

TABLE 2. Cyanide-sensitive SOD activity as a function of aerobic incubation time in medium KTP in the absence and presence of hypotaurine.[a]

Incubation (h)	Hypotaurine	
	0 mM	0.5 mM
4	61	71
6	48	64
8	24	50

[a]The SOD activity is expressed as the percent CN^--sensitive activity, compared to that obtained with fresh spermatozoa prior to incubation taken as 100%. Values are the means of two determinations. Details of the assay are given in *Materials and Methods*. Sperm concentration was 1.0 × 10^8 cells/ml.

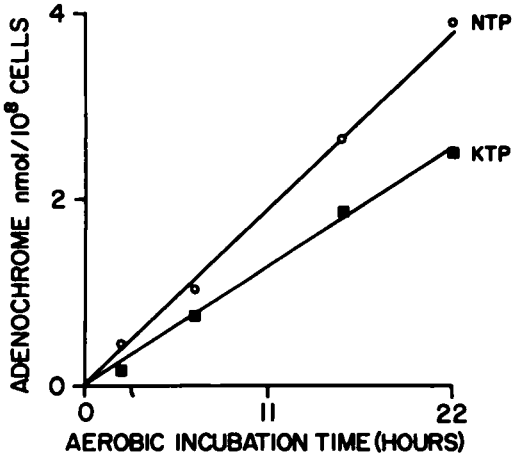

FIG. 5. Formation of adrenochrome from epinephrine during aerobic incubation of rabbit spermatozoa at 37°C in media KTP and NTP. The sperm were suspended at 0.8–1.0 × 10^8 cells/ml; initial concentrations of epinephrine were 50 μM. Each point is the mean of two experiments.

loss. The assays shown in Fig. 4 were done in NTP to improve the accuracy of the measurements. The export rate of superoxide is greater in NTP for a given concentration of sperm cells than it is in KTP, and so is more readily measured. This difference is due to reaction of superoxide with the cells, which is greater in KTP than in NTP (Alvarez and Storey, 1982). Adrenochrome formation from epinephrine was measured as a function of time over long incubation times in both NTP and KTP (Fig. 5). More chromagen was produced in NTP, as expected. The formation of adrenochrome indicates that this agent also acts by reacting with $O_2^{\cdot}$ formed by the spermatozoa. The assay with acetylated ferricytochrome *c* fails in the presence of epinephrine due to interference from the chromogen formed. Albumin interferes with both the acetylated ferricytochrome *c* and the adrenochrome assays for $O_2^{\cdot}$, so that the effect of this agent on its production rate could not be assessed.

While the experimental results obtained during our studies of lipid peroxidation in rabbit sperm strongly support superoxide as the principal inducer of this process (Alvarez and Storey, 1982, 1983; Alvarez et al., 1983), two other oxygen species should be considered as candidates for inducer: singlet oxygen $^1O_2$ and hydroxyl radical $HO^{\cdot}$. Mannitol is considered to be an effective and specific scavenger for $HO^{\cdot}$, while 2,5-dimethylfuran is considered to be a scavenger for $^1O_2$ (Kellogg and Fridovich, 1975). Neither mannitol at 50 mM nor 2,5-dimethylfuran at 1 mM had a protective effect on sperm motility under our experimental conditions.

A close correlation between sperm motility and lipid peroxidation as determined by MDA production was shown in Fig. 3 for medium KTP, without and with the additions inhibiting the rate of peroxidation listed in Table 1. The same close correlation had previously been established for the media NTP and OSM and for hybrids of NTP and KTP in which the relative proportions of Na^+ and K^+ were varied (Alvarez and Storey, 1982). These media all contain the

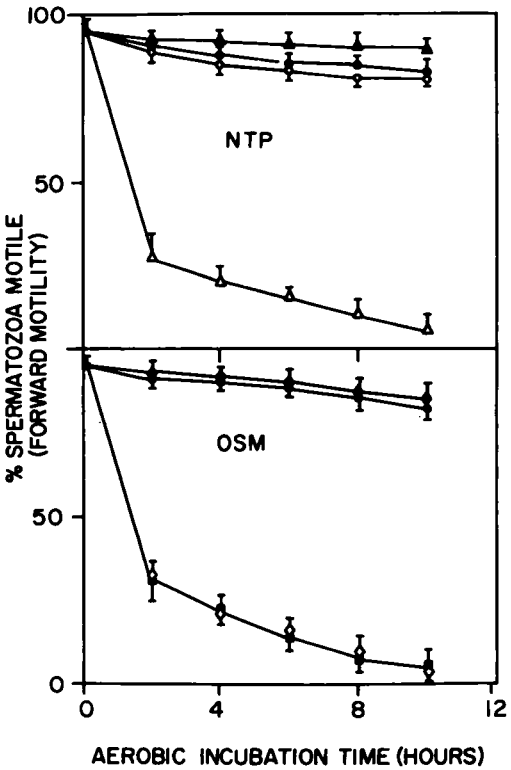

FIG. 6. Effect of varying Mg^{2+} and Ca^{2+} content of media NTP and OSM on sperm motility during aerobic incubation at 37°C. The experimental conditions were those described for Fig. 1. For medium NTP: no change (●—●); plus 1 mM Ca^{2+} (○—○); plus 2 mM Ca^{2+} (▲—▲); 0 mM Mg^{2+} (△—△). For medium OSM: no change (●—●); increase Mg^{2+} from 0.5 to 3.0 mM (◆—◆); 0 mM Mg^{2+} and Ca^{2+} (■—■); 0 mM Ca^{2+} (◇—◇). Each point is the mean of three experiments; *error bars* are standard deviations.

divalent ion, Mg^{2+}. In addition, medium OSM also contains Ca^{2+}. In view of the observation that Ca^{2+} and/or Mg^{2+} are necessary to maintain motility in the spermatozoa from a number of mammalian species (Morton et al., 1978; Heffner and Storey, 1981), the question of whether the divalent ion effect on motility was related to or distinct from lipid peroxidation was addressed. Omission of Mg^{2+} from NTP had a deleterious effect on rabbit sperm motility, as did omission of Ca^{2+} alone and Ca^{2+} plus Mg^{2+} from OSM (Fig. 6). Addition of Ca^{2+} to NTP showed an apparent slight improvement in motility, which was not statistically significant (Fig. 6). Increasing the concentration of Mg^{2+} from 0.5 mM to 3 mM in OSM had no effect (Fig. 6). There was however, no change in the rate of lipid peroxidation rate upon omission of the divalent cations or upon increasing their concentration in the different media. The effect of changing the species of monovalent cation on loss of motility and lipid peroxidation was also examined. Choline could be substituted completely for Na^+ in NTP with no perturbation of the correlation between lipid peroxidation and loss of motility. The substitution of Li^+ for Na^+ in NTP had a profoundly deleterious effect on motility without affecting the rate of lipid peroxidation (Fig. 7). At 10 mM Li^+, motility caused by the second hour of aerobic incubation, yet the rate of lipid peroxidation at this and lower concentrations of Li^+ was indistinguishable from that in NTP alone.

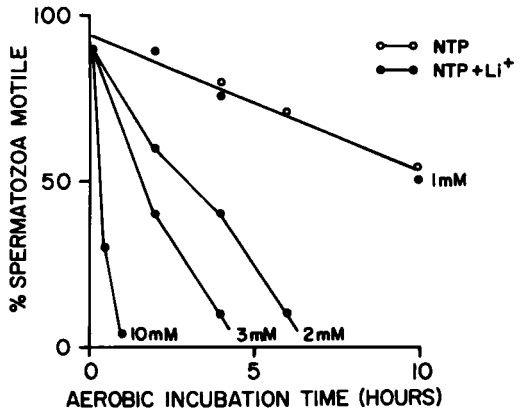

FIG. 7. Effect of Li^+ on the motility of rabbit sperm during aerobic incubation at 37°C in medium NTP. Experimental conditions were those described for Fig. 1. Concentrations of Li^+ are as indicated on each curve.

DISCUSSION

The effect of hypotaurine in reducing superoxide production by rabbit sperm and in protecting the sperm SOD from inactivation provides further support for our hypothesis that SOD is the primary enzymic defense against spontaneous lipid peroxidation in these cells (Alvarez and Storey, 1983). The observation that hypotaurine is effective in this respect, while Tiron is not, is consistent with the concept that superoxide produced intracellularly is the primary inducer of peroxidation (Holland et al., 1982; Alvarez and Storey, 1982, 1983). Hypotaurine exists in the zwitterionic form at pH 7.4, and so has no net change. It should be readily permeant to the sperm plasma membrane while Tiron, with two negative charges, should be impermeant. Hypotaurine could act as an intracellular superoxide scavenger which would not only inhibit lipid peroxidation but also inactivation of SOD by both superoxide and H_2O_2 (Sinet and Garber, 1981). We have reported that SOD inactivation correlates closely with motility loss and lipid peroxidation in these cells, and that exogenous SOD and catalase, alone or in combination, do not protect the sperm against the deleterious effects (Alvarez and Storey, 1983). Since neither of these proteins can traverse the sperm plasma membrane, this result is also consistent with the hypothesis that intracellular superoxide is the principal inducer of peroxidation. The finding inconsistent with the hypothesis is the remarkable protective effect of albumin (Fig. 2; Table 1). This protein is too large to traverse the membrane. It may adsorb to the plasma membrane and inhibit, by a mechanism yet unknown, the peroxidation of membrane lipids. Blank et al. (1976) have provided evidence for albumin binding to rabbit spermatozoa and a similar binding of albumin seems to occur with mouse spermatozoa (Florman and Storey, 1981). The interactions between sperm and untreated, reprecipated, or delipidated albumin are sufficiently complex (Lui and Meizel, 1977; Lui et al., 1977; Go and Wolf, 1981; Quinn et al., 1982) that elucidation of this mechanism will require extensive investigation.

The effect of omitting Ca^{2+} and Mg^{2+} and of adding Li^+ on sperm motility provide the exceptions to the linear correlation between lipid peroxidation and motility loss (Fig. 3; Alvarez and Storey, 1982, 1983). The role of Ca^{2+} in maintaining motility in spermatozoa of

other mammalian species is well documented (Morton et al., 1978; Heffner et al., 1980), and Mg^{2+} can substitute for Ca^{2+} in this regard. Rabbit sperm are usually classed among those sperm not requiring exogenous Ca^{2+} for motility maintenance (Storey, 1975; Morton et al., 1978). Under our experimental conditions of aerobic incubation, the sperm are at a low cell concentration, which may promote loss of Ca^{2+} or Mg^{2+} bound to plasma membrane sites (Heffner and Storey, 1981). The effects of Li^+ on rabbit sperm motility may also be explicable in terms of loss of Ca^{2+} or Mg^{2+} from sperm plasma membrane sites: Wallace and Scarpa (1983) have found with isolated bovine parathyroid cells that Li^+ competes for Ca^{2+} binding sites on the plasma membrane of these cells. This effect of Li^+ on rabbit sperm motility may prove useful in immobilizing sperm in certain experimental situations (Saling, 1982).

The effects of epinephrine, taurine and hypotaurine on protecting rabbit sperm from loss of motility due to spontaneous lipid peroxidation suggest that this activity may play a role in their efficacy in promoting motility in hamster sperm. This antiperoxidation activity can only be a small part of their activity in that system, however, since these compounds are effective with hamster sperm in the micromolar range (Liebfreid and Bavister, 1982). With 20 μM taurine, Mrsny et al. (1979) reported maintenance of motility of hamster sperm at the 70–90% level and activation of the sperm to their characteristic whiplash flagellar movement at the same level. From Fig. 4 it is evident that this concentration of taurine has little inhibitory effect on $O_2^{\cdot-}$ production and hence little protective effect against peroxidation. The experiments of Mrsny et al. (1979) were carried out with albumin at 12 mg/ml, a concentration which in our system would offer ample protection against peroxidation (Table 1). The low concentrations of taurine are apparently working through specific sites on the hamster sperm. The effect of adrenergic antagonists also suggests that epinephrine may act on these sperm through specific sites (Meizel, 1981). At higher concentrations, taurine, epinephrine and hypotaurine may provide an antiperoxidative effect, which is not mutually exclusive to site-specific activity. Our results imply that hypotaurine should be about twice as effective as taurine, if the antiperoxidation effect were of importance in hamster sperm.

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