# Induction of Motility in Immature Bovine Spermatozoa by Cyclic AMP Phosphodiesterase Inhibitors and Seminal Plasma

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## ABSTRACT

Motility can be induced in immature spermatozoa from the bovine caput epididymidis by cyclic 3',5'-adenosine monophosphate (cAMP) phosphodiesterase inhibitors (PDIs) and bovine seminal plasma. PDIs alone indicate flagellar activity without forward progression. Initiation of maximal movement without forward progression requires the presence of high levels of PDIs and is characterized by a distinct lag phase, the duration of which depends on the nature and concentration of the inhibitor. With theophylline (10 mM), for example, the cells reach maximal activity after 12—13 min. That theophylline owes its efficacy to an inhibition of phosphodiesterase is indicated by the fact that cyclic AMP levels increase 3- to 4-fold after the addition of theophylline and reach a maximal level (700 pmoles/$10^9$ sperm) several minutes before peak flagellar activity.

The same time lag occurs with the PDIs papaverine (100 $\mu$M), SQ80002 (50 $\mu$M), and SQ20006 (10 mM) but with the cyclic AMP derivative, dibutyryl cyclic AMP (5 mM), the lag phase is considerably longer (40 min) and the peak stimulation (5 percent) is smaller than with PDIs. Adding 10 mM glucose shortens the lag phase and increases the maximal response to Bt$_2$cAMP (5 mM), SQ80002 (25 mM), and theophylline (25 mM).

Seminal plasma alone has no effect on either motility or intracellular cAMP levels but when 8 percent seminal plasma (optimum level) is added to 25 mM theophylline, 50 percent of the caput sperm show flagellar activity, and 80 percent of these move forward in a rotating fashion at about 58 $\mu$m/sec. When 4 percent bovine serum albumin (BSA) is added to 25 mM theophylline 40 percent of the caput sperm become motile, but less than one-half of these cells (20 percent of total) move forward in a circular kind of motion which diminishes after 10 min and is lost after 35 min.

These data support the view (Hoskins et al., 1974) that an increase in the intrasperm level of cAMP is an important, but not the sole, factor in the normal development of sperm motility in the bovine epididymis.

## INTRODUCTION

Cyclic 3',5'-adenosine monophosphate (cAMP) phosphodiesterase inhibitors have been shown to increase the motility of washed spermatozoa from the bovine cauda epididymidis (Garbers et al., 1971) by raising intracellular cAMP levels (Garbers et al., 1973). These observations led us to suggest (Hoskins et al., 1974) that the development of motility in the bovine epididymis is also related to an increase in the level of cellular cAMP since immotile sperm from the caput portion of the bovine epididymis contain only one-half as much of this nucleotide as do motile sperm from the cauda (Hoskins et al., 1974). In the study reported here, we attempted to induce motility in caput sperm by raising the intracellular level of cAMP with several phosphodiesterase inhibitors.

## MATERIALS AND METHODS

Bovine testicles were obtained from a local slaughterhouse and delivered to the laboratory within a few hours of slaughter. Spermatozoa were recovered (Hoskins et al., 1975) from the distal caput areas of the attached epididymides which correspond to region 2 in the schema drawn by Casillas (1973). Longitudinal incisions, approximately 1/8 inch apart, were made in 2 or 3 excised sections and the tissues incubated for 10 min in 15 ml of incubation medium at room temperature. The incubation medium consisted of 42 mM KCl, 103 mM NaCl, 5 mM MgSO$_4$, 10 mM KH$_2$PO$_4$, and 10 mM Tris-HCl, pH 7.2 and was used to wash and suspend, as well as recover, the spermatozoa. Sperm were washed 3 times at room temperature by suspension in 10 ml incubation media and centrifugation in 15 ml conical centrifuge tubes at 800 X g for 5 min. After each sedimentation, the washing media was removed, approximately 5 ml of additional medium added, and the uppermost layer of caput sperm separated from contaminating erythrocytes and

tissue debris by gentle liquid pressure agitation with a Pasteur pipette. The sperm-containing solution was then transferred to a second centrifuge tube. After the final wash the sperm were resuspended and the suspension was distributed into a number of tubes so that on final centrifugation each tube contained either approximately $10^7$ pelleted spermatozoa for motility studies or $10^9$ cells for the determination of cAMP content. After removal of the washing media by aspiration the tubes were transferred to a $37°$ water bath.

Flagellar activity was initiated by suspension of the cells in 5 ml of incubation medium at $37°$ containing the concentration of phosphodiesterase inhibitor or dibutyryl cAMP indicated in the figures and 10 mM glucose.

To stimulate forward progression 0.45 ml of seminal plasma was added to the 5 ml of activation medium. Spermatozoa were separated from whole semen, generously supplied by All-West Breeders, Inc., Burlington, Washington, by centrifugation at 800 × g for 5 min. Seminal plasma was obtained by centrifuging the resultant supernatant at 1500 × g for 30 min at $4°$. The cell-free plasma was stored in 5 ml aliquots in liquid nitrogen and thawed samples were not refrozen. Samples were removed at the times indicated on the figures and motility was evaluated microscopically using dark field optics. The percentage of sperm, in a field of at least 200, showing flagellar motion with and without forward progression was determined.

Concentrations of theophylline as high as 50 mM were obtained by heating the compound in incubation medium with stirring at $50°$ for 15 min before cooling to $37°$. Papaverine was dissolved in dimethyl sulfoxide (DMSO) at a level of 100 mM so that the addition of 5 $\mu$l of solution gave a final inhibitor concentration of 100 $\mu$M and a DMSO concentration of 0.1 percent. This concentration of DMSO has no effect on sperm motility. Solutions of the inhibitor SQ20006 (1-ethyl-4-hydrazino-1 $H$-pyrazolo [3,4-b] pyridine-5-carboxylic acid, ethyl ester, hydrochloride) in the incubation medium were neutralized to pH 7.2 and used immediately. At the level used in the experiment shown in Fig. 3 (10 mM), the compound crystallized out of solution within 15 min, after the mixing of sperm and activation media.

Changes in the intracellular level of cAMP after the addition of theophylline were followed by the radioimmunoassay procedure of Steiner et al. (1969). The assay kit was supplied by ICN Pharmaceuticals, Inc. At the time points indicated on Figs. 2 and 7, 0.3 ml of sperm suspension was withdrawn from the activation system, added to an equal volume of ice-cold 5 percent trichloroacetic acid, vortexed, and immediately frozen. Thawed sperm suspensions were extracted for cAMP according to the method described by Gilman (1970). Cyclic AMP levels shown in Figures 2 and 7 are the average of duplicate or triplicate determinations and are reported as pmoles of nucleotides/$10^9$ sperm. All sperm counts were made in triplicate with a haemocytometer.

The phosphodiesterase inhibitors SQ 20006 and 80002 (8-methylthio) cyclic $3',5'$-adenosine monophosphate) were gifts of E. R. Squibb and Sons, Inc. Dibutyryl cyclic AMP, crystallized bovine serum albumin, and theophylline were obtained from the Sigma

Chemical Co. Caffeine was a product of Mann Research Laboratories. All other reagents were of the highest quality available.

## RESULTS

In an earlier study (Hoskins et al., 1974) we observed that neither 5 mM theophylline nor 4 mM dibutyryl cAMP induced significant motion of any kind in washed bovine caput sperm. This is in marked contrast to results obtained with caudal sperm (immobilized by 1 hr incubation at $37°$) where these compounds at identical levels initiate vigorous motility (Hoskins, 1973). This disparity is evidently due to the fact that induction of any kind of flagellar activity in caput sperm requires much higher levels of theophylline (Fig. 1) than previously thought. Figure 1 shows that even at a level of 10 mM, scarcely any flagellar activity (less than 10 percent; no forward movement) is seen before 3 to 4 min and that peak activity does not occur until at least 10 min. Increasing levels of theophylline (up to 50 mM) cause increasingly more activity and bring about the maximal response of an earlier time. At a level of 50 mM, theophylline initiates a twitching motion in approximately 25 percent of the cells, with peak activity occurring within 3 to 4 min. The unusually high level of theophylline required to elicit this response can be appreciated if one realizes that the level approaches saturation and is 80 times the reported $K_i$ value of this compound for rat brain phosphodiesterase (O'Dea et al., 1970).
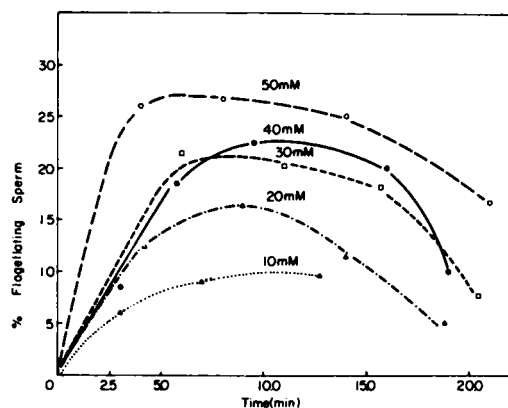


FIG. 1. Initiation of flagellar activity in bovine caput sperm by varying concentrations of theophylline. Sperm concentration in activation medium, 3.4 × $10^6$/ml. Other conditions described in Methods.
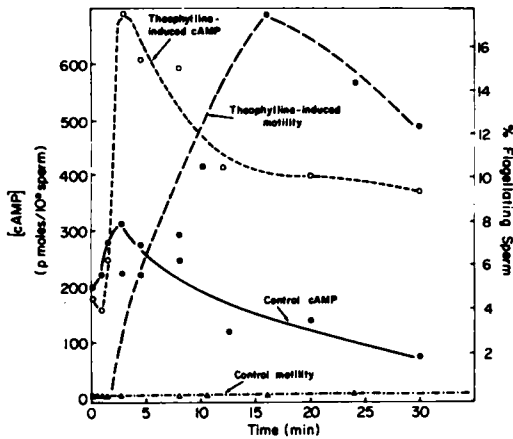
FIG. 2. Time relationship between the theophylline-induced change in cAMP concentration and flagellar activity. Theophylline concentration, 10 mM. Tubes used to follow motility and cAMP levels contained 3.0 X 10⁶ and 2.9 X 10⁸ caput sperm/ml, respectively.

The observation that 10–25 mM theophylline stimulated activity only after a pronounced lag phase provided the opportunity to study the temporal relationship between the initiation of activity and changes in the intracellular level of cAMP. That 10 mM theophylline causes an increase in the level of cAMP which precedes the change in activity is shown by the data given in Fig. 2. The figure shows that, after a 2- to 3-min lag phase, the intracellular level of cAMP increases 3- to 4-fold after the addition of 10 mM theophylline and precedes maximal stimulation of motility by about 10 min. The change in the level of cyclic AMP with time describes a bell-shaped curve similar to that seen during the induction of flagellar activity. This type of curve is shown when motility levels remain high for longer periods of time (see Fig. 7 and Garbers et al., 1973). Note in Fig. 7 that a small increase in cyclic AMP levels occurs on dilution of sperm in the absence of
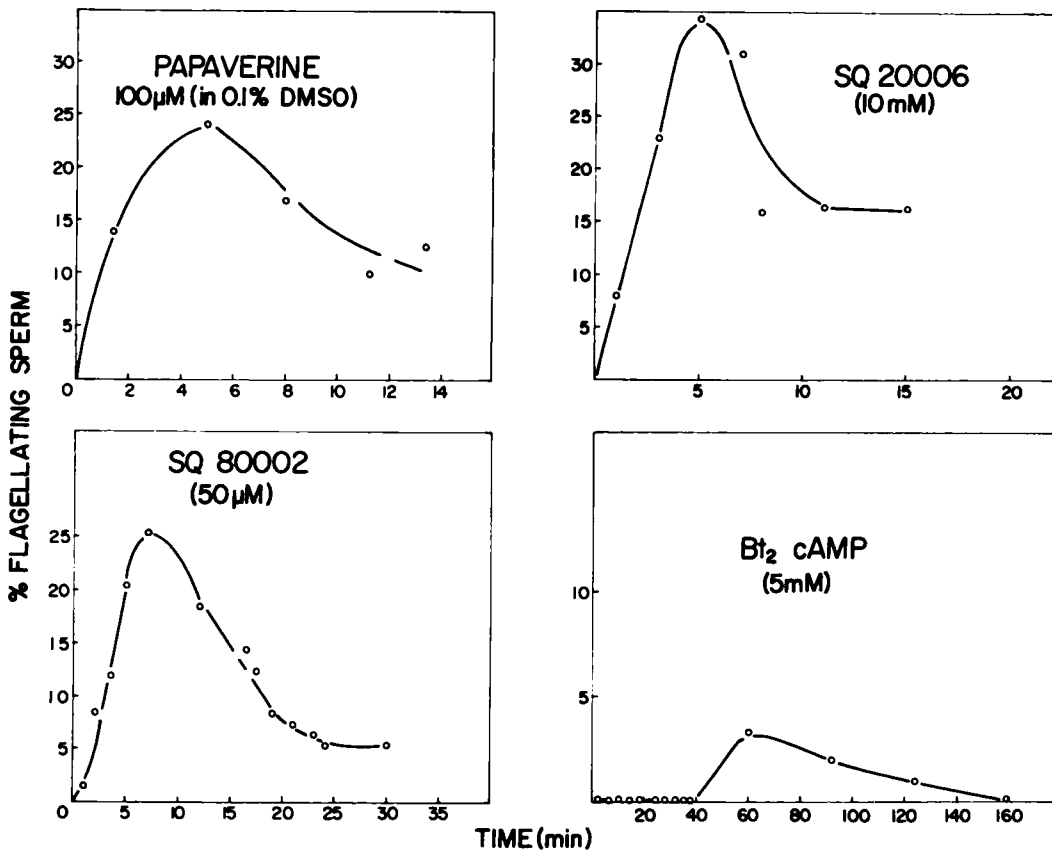


FIG. 3. Initiation of flagellar activity in caput sperm by various cAMP phosphodiesterase inhibitors and Bt₂cAMP. Tubes used to follow motility with papaverine, SQ80002, SQ20006, and Bt₂cAMP contained 2.4, 2.6, 5.2, and 4.0 X 10⁶ sperm/ml, respectively. Other conditions are described in Methods.

theophylline. This effect has been previously described in bovine caudal sperm by Cascieri et al. (1974) and has been ascribed to activation of adenyl cyclase by the simple act of dilution. The delay between the maximal stimulation of cAMP levels and the maximal response of flagellar activity in caput sperm in the presence of theophylline (Fig. 2) is much longer than that found in caudal sperm where maximal stimulation of both cAMP levels and motility occurs within 3—4 min after addition of the phosphodiesterase inhibitor caffeine (10 mM).

Sperm movement without forward progression is also induced by several other phosphodiesterase inhibitors and, to a very limited degree, by the cAMP analog $Bt_2$ cAMP (Fig. 3). One hundred $\mu M$ papaverine (6,7 dimethoxy-1-veratrylisoquinoline), 50 $\mu M$ SQ80002, and 10 mM SQ20006 all show time-activity responses similar to that induced by 50 mM theophylline (Fig. 1). That is, all responses are biphasic with respect to time and show peak activity after 5—10 min. For example, the levels at which papaverine and SQ20006 were added correspond roughly to 100 times their reported $K_i$ values for heart phosphodiesterase (O'Dea et al., 1970; Garbers et al., 1973) in order to better compare the efficacy of these com-

pounds with that of theophylline which was shown to give maximal response at 80 times its reported $K_i$ (Fig. 1). Of all the compounds tested, the cAMP analog $Bt_2$ cAMP at a level of 5 mM stimulated activity the least and required the longest incubation period before any motion was seen (Fig. 3). Extremely high concentrations of $Bt_2$ cAMP, comparable, for example, to 50 mM theophylline, have not been tested. The efficacy of $Bt_2$ cAMP was improved by adding 10 mM glucose to the activation medium (Fig. 4) but even here the long lag phase was not eliminated. In the experiment shown, the addition of glucose alone failed to initiate any sperm movement. Moreover, it augments the response of caput sperm to SQ80002 (Fig. 4) and to theophylline. In both cases, glucose causes more flagellar activity at an earlier time. Accordingly, in all subsequent experiments described in this report, 10 mM glucose was added to the activation medium.

We emphasize that none of the cAMP phosphodiesterase inhibitors used in our studies up to this point induced progressive motility in caput sperm under any set of conditions. Forward motility was successfully induced, however, by addition of bovine seminal plasma. The rationale for this experiment was provided
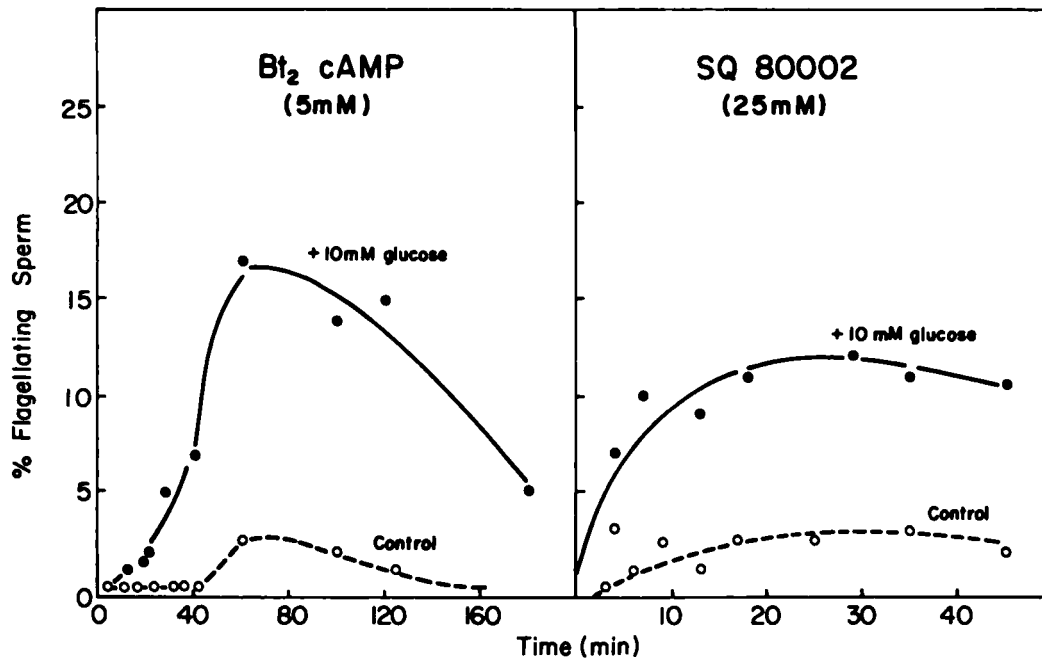


FIG. 4. The effect of glucose on the stimulation of flagellar activity by $Bt_2$ cAMP and SQ80002. Each activation tube contained 4.0 X $10^6$ sperm/ml.

by the studies of Eliasson and Lindholmer (1973) and Lindholmer (1974). The latter showed that the addition of 8 percent human seminal plasma to sperm from the caput epididymidis of several patients with obstructive azoospermia resulted in immediate forward progression that was rated as "moderate to good." It should be noted, however, that these pathologically retained cells, unlike physiologically normal bovine caput sperm, are partially motile (25—30 percent flagellation, no forward progression) before the addition of seminal plasma and move in a manner somewhat like that of sperm after ligation of the vas deferens (Mooney et al., 1972).

The induction of forward progression in immotile bovine caput sperm by a combination of 8 percent bovine seminal plasma (v/v) and 25 mM theophylline is shown in Fig. 5 which has been divided into parts A and B in order to distinguish between the increase in the number of cells showing any movement (A) and the increase in those showing forward progression (B). In A, the number of cells showing any movement is plotted against time after the addition of either 25 mM theophylline, 8 percent seminal plasma, or seminal plasma plus theophylline. Seminal plasma by itself does not initiate movement in these cells whereas theophylline elicits its typical biphasic response (cf. Fig. 1). The combination of theophylline and

seminal plasma, however, induces motion in nearly 50 percent of the sperm and maintains this activity for the 50 min duration of the experiment. The time points on the upper curve in Fig. 5 (and Fig. 8) shown by the double-ended horizontal arrows, shows the time span during which observations were made in three separate experiments. In Part B of the figure, only those cells showing forward progression have been counted. Note that neither theophylline nor seminal plasma alone induces any forward motion but that the combination of these two agents induces a vigorous motion in 80—90 percent of the moving sperm (compare with Part A) which resembles, in many respects, that shown by ejaculated sperm. Fig. 6 shows an artist's reproduction of a photomicrographic slide, taken with dark field illumination and a 5 sec exposure time, of the direction and velocity of motion of the activated caput sperm. The kind of motion made by those sperm that were stimulated to flagellar activity without forward progression is also shown. Note that all moving cells describe essentially straight paths. Most of them also rotate as they move, as evidenced by the periodic flashing of sperm heads. Sperm velocity calculated from the length of the tracks on the film, the dimensions of the field, and the film speed was $58 \pm 4$ (S.E.M.) $\mu$m/sec for the cells shown in the figure. This value compares with that of $97 \pm 6$ $\mu$m/sec reported
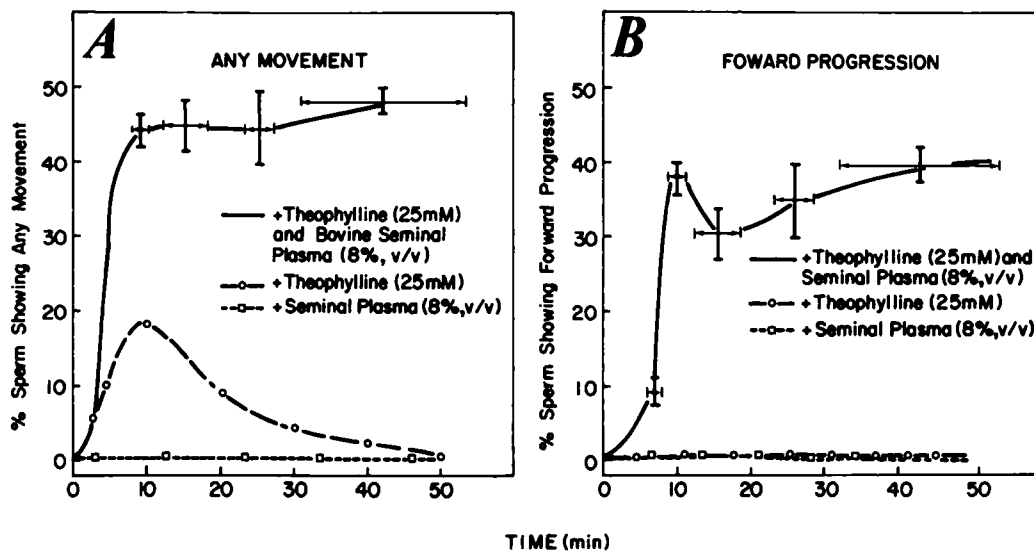


FIG. 5. The induction of motility in bovine caput sperm by 25 mM theophylline and 8 percent seminal plasma. Part A shows the number of cells showing any flagellar activity. In Part B only those showing forward progression were counted. Sperm count, $2.0 \times 10^6$/ml.
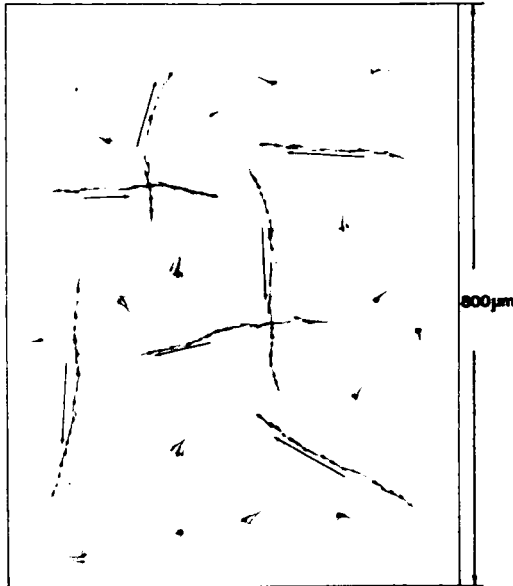
FIG. 6. Artist's representation of the direction and velocity of motion of caput sperm activated by 25 mM theophylline and 8 percent seminal plasma. Picture drawn from a photomicrographic slide taken with dark field illumination and an exposure time of 5 sec. The distance on the ordinate corresponds to the dimensions of the haemocytometer slide.
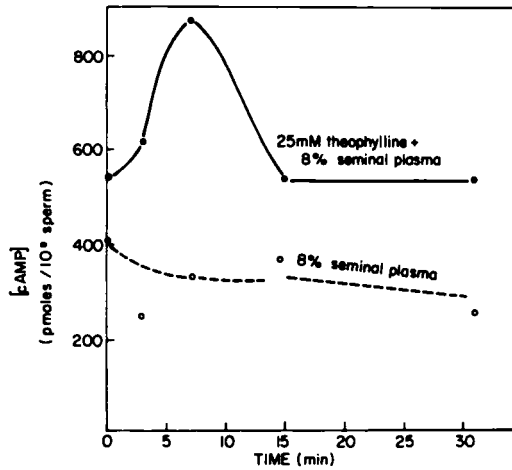


FIG. 7. The effect of seminal plasma (8 percent) and seminal plasma plus theophylline (25 mM) on the intracellular cAMP level in activated bovine caput sperm. Sperm concentration, $5.5 \times 10^8$/ml. Cyclic AMP was assayed according to the method described in Methods.

by Rikmenspoel (1964) for ejaculated bovine sperm.

Although we have not made a detailed study of the effect of varying levels of seminal plasma on motility, we have shown that progressive motility is reduced by either greater or lesser amounts of plasma. At levels of 4 percent and 12 percent, motility is considerably reduced and almost no forward progression is seen at levels as low as 1 percent or as high as 16 percent.

How seminal plasma induces forward motion in caput sperm is not known. It is clear, however, that it does not increase intracellular cAMP levels or alter the bell-shaped nature of the curve of cAMP levels with respect to time which was obtained with theophylline alone (Fig. 7).

Eliasson and Lindholmer (1973) observed that 4 percent human serum albumin stimulates forward motion in "epididymal-like" washed human sperm, and Lindholmer (1974) reported that this same level of albumin stimulates forward progression in caput sperm from men with obstructive azoospermia. In the latter case the increased motility was described as weak to

moderate. Figure 8 shows that 4 percent bovine seminal plasma elicits a similar response in washed bovine caput sperm. Part A of Fig. 8 shows that the addition of bovine serum albumin to theophylline-treated sperm doubles the number of sperm with any movement and, like seminal plasma, maintains flagellar activity for the 45-min duration of the experiment. Part B of the figure shows that serum albumin also induces forward progression. In contrast to the results obtained with seminal plasma (Fig. 5), however, motility induction peaks at about 5 min and is lost almost completely by 30 min. Even at this peak time, less than half of all flagellating cells show forward progression. Finally, most of the stimulated cells do not move in a straight line but describe tight circular paths. In general, the movement induced by serum albumin appears much less coordinated than that induced by seminal plasma. Sperm heads move wildly from side to side and tails describe large amplitude beats. How varying the level of serum albumin affects motility or intracellular cAMP levels in the presence or absence of phosphodiesterase inhibitors has not been studied.

## DISCUSSION

In an earlier report, we showed that an increase in the intracellular level of cAMP accompanies the maturation of sperm in the
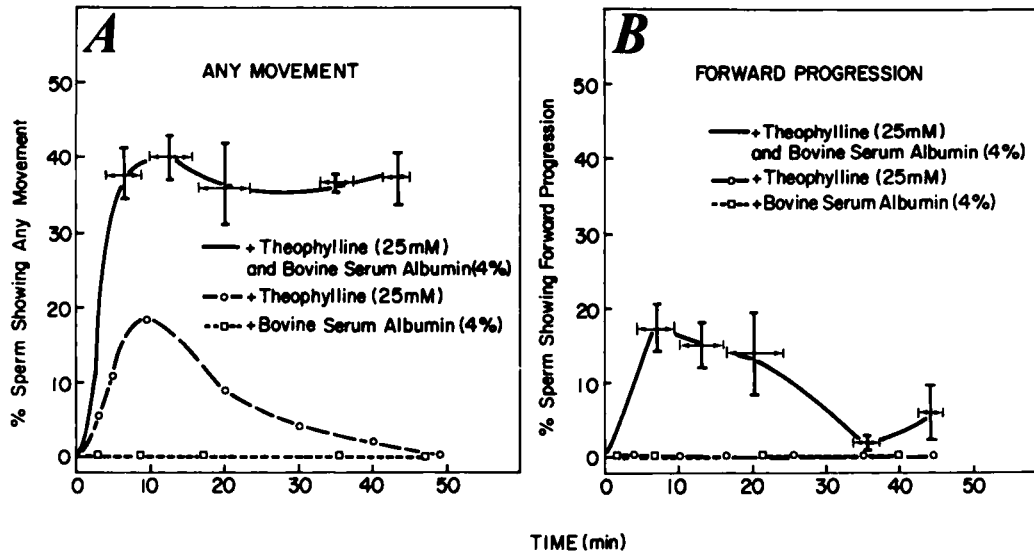
FIG. 8. The induction of motility in bovine caput sperm by 25 mM theophylline and 4 percent bovine serum albumin. Part A shows the number of cells showing any flagellar activity. In Part B only those showing forward progression were counted. Sperm count, 2.0 X 10⁶/ml.

bovine epididymis (Hoskins et al., 1974). Here we provide evidence that an increase in the level of cAMP, brought about by the addition of phosphodiesterase inhibitors, induces flagellar activity without forward progression in immotile sperm from the distal caput portion of the epididymis. An unusual feature of the induction is that high levels (e.g., 50 mM theophylline) of inhibitors are required to evoke a maximum response and that the response is preceded by a pronounced time lag which is unique to each inhibitor. The reason for this apparent insensitivity is unknown but may be due to either caput sperm membrane permeability barriers or unusual kinetic properties of the phosphodiesterase in situ.

The data reported here support the idea that a change in the intrasperm level of cAMP is important to the development of motility in the bovine epididymis since vigorous motility can be induced in caput sperm by raising cAMP levels with theophylline in the presence of seminal plasma (Fig. 8A). The requirement for theophylline is absolute, seminal plasma alone having no effect on either motility or the level of cAMP.

Although we do not know how bovine seminal plasma stimulates forward motion in bovine caput sperm, seminal plasma has been known for some time to stimulate motility in

general. The impetus for part of the present study was provided by the recent report of Lindholmer (1974) on the beneficial effects of human seminal plasma in inducing forward progression in sperm from the caput epididymides of several patients with obstructive oligospermia. The earliest reports of this kind were those of Hotchkiss (1944) and Rozin (1961), who showed that poor sperm motility in human patients could be improved by replacing their seminal plasma with the plasma of men with normal sperm motility. Rozin (1960) also showed that the motility of oligospermic sperm could be improved by seminal plasma. Eliasson and Lindholmer (1973) suggested that a beneficial factor is present in prostatic secretions and a deleterious one in seminal vesicle secretions.

In his comprehensive review of sperm motility, Nelson (1967) pointed out that the significance of numerous observations on the effects of seminal plasma or other body fluids on sperm motility would be greatly enhanced if some of the beneficial factors could be isolated and their mechanism of action determined. Theophylline-treated sperm from the bovine caput epididymidis appears to be an ideal test system for isolating and characterizing such factors since these cells have no forward motion without seminal plasma and considerable for-

ward motion with it; hence the amount of factor causing the motion can be quantified. Accordingly, we plan to isolate and characterize the active factor(s) from bovine seminal plasma and then to determine if the factor is present in secretions from various areas of the bovine epididymis.

Whether an increase in the intrasperm content of cAMP is related to the induction of motility in all mammals is not known. We have, however, shown (D. D. Hoskins, unpublished) that flagellar activity without forward progression is also induced by 10 mM caffeine in caput sperm of the squirrel monkey *(Saimiri sciurea)* where the percent of flagellating cells is increased from less than 5 percent to more than 25 percent within 20 min at 37°. We have not yet tried to induce forward motion in monkey sperm with seminal plasma. Frenkel et al. (1973) appear to be the first to observe that phosphodiesterase inhibitors stimulate motility in immature sperm. They showed that the combination of 10 mM caffeine and 2 mM Bt$_2$cAMP increases the motility of guinea pig caput sperm from 10 percent to 60 percent in the presence of glucose (10 mM) and 10 percent to 30 percent in its absence. Neither changes in cAMP content nor the type of movement invoked were reported in this study nor do we know whether an additional factor, such as seminal plasma or serum albumin can improve forward progression in these cells.

We have shown here that the motility of sperm from the distal caput area of the bovine epididymis can be greatly stimulated in vitro by a number of specific cAMP-phosphodiesterase inhibitors and seminal plasma. These data raise several questions which seem best approached with a laboratory animal, such as the rabbit, in which the fertility profile of epididymal sperm is known. For example, since it is known (for review, see Orgebin-Crist, 1969) that the motility of rabbit caput sperm is increased, but not to a full and vigorous state, by retention for a week or more in the ligated epididymis, one wonders whether this increase in motility is related to an increase in intrasperm cAMP. Perhaps more importantly, one wonders whether it will be possible to induce vigorous motility in rabbit caput sperm in vitro by a combination of cAMP-phosphodiesterase inhibitors and seminal plasma and, if so, whether these activated cells are fertile.

## REFERENCES

Cascieri, M., Amann, R. P., and Hammerstedt, R. H. (1974). Alteration of metabolism in bovine pre- and post-epididymal sperm by cyclic AMP. Fed. Proc. 33, 1554 (abstract).

Casillas, E. R. (1973). Accumulation of carnitine by bovine spermatozoa during maturation in the epididymis. J. Biol. Chem. 248, 8227–8232.

Eliasson, R., and Lindholmer, Chr. (1973). Effects of Human Seminal Plasma on Sperm Survival and Transport. In: "Transport, survie, et pouvoir fecondont des spermatozoides." (E.S.E. Hafez & C. G. Thibalt, eds.). INSERM, Paris, 1974, pp. 219–230.

Frenkel, G., Peterson, R. N., and Freund, M. (1973). The role of adenine nucleotides and the effect of caffeine and dibutyryl cyclic AMP on the metabolism of guinea pig epididymal spermatozoa. Proc. Soc. Exptl. Biol. Med. 144, 420–425.

Garbers, D. L., First, N. L., Gorman, G. K., and Lardy, H. A. (1973). The effects of cyclic nucleotide phosphodiesterase inhibitors on ejaculated porcine spermatozoan metabolism and fertility. Biol. Reprod. 8, 599–606.

Garbers, D. L., First, N. L., and Lardy, H. A. (1973). The stimulation of bovine epididymal sperm metabolism by cyclic nucleotide phosphodiesterase inhibitors. Biol. Reprod. 8, 589–598.

Garbers, D. L., Lust, W. D., First, N. L., and Lardy, H. A. (1971). Effects of phosphodiesterase inhibitors and cyclic nucleotides on sperm respiration and motility. Biochemistry 10, 1825–1831.

Gilman, A. G. (1970). A protein binding assay for adenosine 3′,5′-cyclic monophosphate. Proc. Nat. Acad. Sci. 67, 305–311.

Hoskins, D. D. (1973). Adenine nucleotide mediation of fructolysis and motility in bovine epididymal spermatozoa. J. Biol. Chem. 248, 1135–1140.

Hoskins, D. D., Munsterman, D., and Hall, M. L. (1975). The control of bovine sperm glycolysis during epididymal transit. Biol. Reprod., in press.

Hoskins, D. D., Stephens, D. T., and Hall, M. L. (1974). Cyclic adenosine 3′,5′-monophosphate and protein kinase levels in developing bovine spermatozoa. J. Reprod. Fertil. 37, 131–133.

Hotchkiss, R. Sh. (1944). "Fertility in Man." Lippincott, Philadelphia, 216 pp.

Lindholmer, Ch. (1974). The importance of seminal plasma for human sperm motility. Biol. Reprod. 10, 533–542.

Mooney, J. K., Jr., Horan, A. H., and Lattimer, J. K. (1972). Motility of spermatozoa in human epididymis. J. Urol. 108, 443–445.

Nelson, L. (1967). Sperm Motility. In: "Fertilization, Vol. 1, Comparative Morphology, Biochemistry, and Immunology." (C. B. Metz & A. Monroy, eds.) Academic Press, New York, pp. 27–97.

O'Dea, R. R., Haddox, M. K., and Goldberg, N. D. (1970). Kinetic analysis of a soluble rat brain cyclic nucleotide phosphodiesterase. Fed. Proc. 29,

473 (abstract).

Orgebin-Crist, M.-C. (1969). Studies on the function of the epididymis. Biol. Reprod. Suppl. 1, 155–175.

Rikmenspoel, R. (1962). Biophysical Approaches to Measurement of Sperm Motility. *In*: "Spermatozoan Motility." (D. W. Bishop, ed.) Amer. Assoc. for the Adv. Sci., Washington, D. C., pp. 31–54.

Rozin, S. (1960). Studies on seminal plasma: I. The role of seminal plasma in the motility of spermatozoa. Fertil. Steril. 11, 277–285.

Rozin, S. (1961). Studies on seminal plasma. Int. J. Fertil. 6, 169–174.

Steiner, A. L., Kipnis, D. M., Utiger, R., and Parker, C. (1969). Radioimmunoassay for the measurement of adenosine 3',5'-cyclic phosphate. Proc. Nat. Acad. Sci. 64, 367–373.

## RECOMMENDED REVIEWS

Hoskins, D. D., and Casillas, E. R. (1974). Hormones, Second-Messengers, and the Mammalian Spermatozoon. *Chapter In*: "Advances in Sex Hormone Research." (R. L. Singhal & J. A. Thomas, eds.), Vol. 1. University Park Press, Baltimore, pp. 283–324.

Orgebin-Crist, M.-C. (1969). Studies on the function of the epididymis. Biol. Reprod. Suppl. 1, 155–175.