Adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) activity was detected in the flagella of ejaculated bovine spermatozoa. This activity provided sufficient ATP to produce normal motility in cells permeabilized with digitonin and treated with 0.5 mM MgADP. In the presence of ADP, adenylate kinase activity was inhibited by \( P^1, P^2\text{-di(adenosine 5')-pentaphosphate (ApP}) \), an adenylate kinase-specific inhibitor, and motility was stopped. ATP-supported motility was not affected by ApP. Mitochondrial adenylate kinase activity allowed AMP to stimulate respiration in permeabilized sperm. Adenylate kinase activity in tail fragments was most active in a pH range from 7.6 to 8.4, and a similar pH sensitivity was observed for this enzyme activity in a hypotonic extract of whole sperm. The apparent \( k_m \) of adenylate kinase activity in permeabilized tail fragments was about 1.0 mM ADP in the direction of ATP synthesis. The fluctuation of nucleotide concentrations in normal and metabolically stimulated sperm suggested that adenylate kinase was most active when the cell was highly motile, although adenylate kinase activity did not appear to be coupled strictly with motility.

Sperm motility is dependent on an adequate supply of ATP produced, depending on species and the availability of substrates, by glycolysis or oxidative phosphorylation (1). In cells relying exclusively on oxidative phosphorylation, mitochondrial ATP or a high energy equivalent must diffuse or be transported down the axoneme to the dynein ATPases, which produce motive force (2). In sea urchin sperm, a creatine phosphate shuttle apparently establishes a pool of ATP dedicated primarily to motility (3). At the junction of the mitochondria and tail, a creatine kinase isozyme forms phosphocreatine at the expense of mitochondrial ATP. This creatine phosphate diffuses down the tail where ATP is formed by another creatine kinase isozyme, presumably in close proximity to dynein ATPases. No such system has been described for bovine sperm, in which ATP apparently diffuses down the tail in quantities sufficient to provide energy for motility as well as ion pumps and other ATP-utilizing systems. Unlike sea urchin sperm, which exhibit full motility when introduced into sea water and continue at that rate until their energy supply is exhausted, mammalian sperm motility changes pattern and speed after capacitation and the acrosome reaction (4). In these cells, respiration is tightly coupled to motility (5); however, because mammalian sperm lack a system for transporting ATP down the tail in response to increased demand, changes in the speed of motility appear to be limited by the efficiency of diffusion.

We report here the presence of adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) in the bull sperm tail. When operating in the direction of ATP synthesis, adenylate kinase would allow the sperm to exploit the energy of two high energy phosphate bonds in each molecule of ATP. This capacity would double the energy available for use by the dynein ATPase without necessitating increased ATP diffusion.

**MATERIALS AND METHODS**

**Preparation of Bovine Sperm**—Bovine ejaculates were provided by American Breeders Service (De Forest, WI). Semen was centrifuged at 300 × g for 15 min, the seminal plasma was discarded, and the sperm were washed twice by centrifugation at 300 × g for 15 min in a medium containing 120 mM NaCl, 10 mM KCl, 1 mM MgCl\(_2\), and 10 mM MOPS, \( \text{pH} 7.4 \) (NK). The pelletled sperm were suspended at a concentration of 1-2 × 10\(^6\) cells/ml in NK and kept at 20 °C until use.

**Preparation of Tail Fragments**—Washed sperm suspended in 3 ml of NK at a concentration of 5 × 10\(^4\) cells/ml were disrupted using a Polytron (Brinkmann Instruments) at a power setting of nine for three five-s bursts separated by 1 min on ice. One-ml aliquots of disrupted sperm were centrifuged for 2 s at 15,600 × g in an Eppendorf centrifuge (model 5414, Brinkmann Instruments), the pellet was resuspended in 0.75 ml of NK, and the centrifugation was repeated. The supernatant fractions were layered on 2 ml of NK-buffered Percoll (1.065 g/ml) and centrifuged at 30,000 × g in an angle rotor for 15 min. A band at the buffer-Percoll interface, containing tail fragments with very little contamination by midpieces, heads, or whole sperm, was suspended in NK and centrifuged at 30,000 × g for 5 min, after which the pellet was suspended in minimal NK and kept at −70 °C until use.

**Hypotonic Extraction of Whole Sperm**—Washed sperm were suspended at 5 × 10\(^6\) cells/ml in a hypotonic medium containing 2 mM MgSO\(_4\), 0.2 mM EDTA, and 10 mM MOPS, \( \text{pH} 7.4 \), for 1 min at 4 °C and then centrifuged at 30,000 × g for 15 min. The supernatant fraction was collected and kept on ice until use.

**Permeabilization of Sperm and Reactivation of Motility**—Digitonin (0.15 mM) was added to a reactivation medium containing 230 mM sucrose, 30 mM potassium lactate, 5 mM sodium lactate, 1 mM EDTA, 10 mM MOPS, \( \text{pH} 7.8 \), washed ejaculated sperm at 1-1.5 × 10\(^7\) cells/ml, and, in some cases, 2.5 mM CAMP. Sperm motility ceased by 0.5 min at 20 °C. After 1 min, a mixture of 1 mM MgSO\(_4\), 1 mM MgCl\(_2\), and either ATP (0.5-1.0 mM) or ADP (0.5 mM) was added. Sperm were incubated for 1 min after which motility was video-recorded.

**ATP Assay**—Washed sperm were prepared as in the permeabilization and reactivation procedures using MgADP, except that they were incubated at 30 °C. After 2 min, cold trichloroacetic acid was added (10%, final concentration). The acid extract was collected by centrifugation, and a mixture of fron and trictoxygen (4:1) was added (solvent extract, 2×), agitated vigorously for 15 s, and centrifuged at 15,600 × g for 15 s. The organic layer was removed, and the neutralized extract was used immediately or stored at −70 °C.

**Extract ATP** was analyzed in a modification of a luciferase assay used by Lemasters and Hackenbrook (6) containing (final concentra-

\( ^* \)This work was supported by Grant AM 10334 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
was qualitatively and quantitatively indistinguishable from directional patterns were not examined. Stuck by their heads to the glass slide or cover slip, speed and
titers were measured after incubation with 50 mM glycine, 10 mM NaAsO$_4$, 0.1 mM EDTA, 5.62 mM ATP, 0.5 mM MgCl$_2$, 0.22 mg/ml luciferin, and 0.01 units/ml luciferase at pH 7.8. The assays were
taken in and ATP standards analyzed concurrently. The ATP content of ADP preparations was also determined and subtracted.

These conditions were also appropriate for continuous monitoring of ATP production by sperm tail fractions or whole sperm (7). Tail fraction was added to an assay mixture, and then 10-22.5 μM ADP was added. Light emission was recorded on a chart recorder, and ATP content was determined by comparison with ATP standards. Digitonin, which was used in some assays, had no effect on the luciferase assay.

When ATP, ADP, and AMP were analyzed in the same extract, all three nucleotides were measured in coupled enzymatic assays. ATP was measured by change of absorbance at 340 nm in an assay mixture containing 50 mM Tris, pH 8.0, 5.25 mM MgCl$_2$, 0.22 mg/ml NAD, 8 units/ml glucose-6-phosphate dehydrogenase, 37.5 mM glucose, and 7 units/ml hexokinase. ADP and AMP were analyzed in a mixture containing 200 mM Tris, pH 7.6, 11.0 mM MgSO$_4$, 54.0 mM KCl, 0.65 mM EDTA, 0.045 mM ATP, 0.22 mg/ml NADH, 12.2 mM phosphoenolpyruvate, and 16.5 units/ml lactate dehydrogenase. ADP titers were measured after incubation with 4 units/ml pyruvate kinase, and AMP was subsequently measured following incubation with 3.6 units/ml myokinase.

Adenylate Kinase and Nucleoside Diphosphokinase Assays—Adenylate kinase activity in sperm sonicate fractions was assayed in a medium containing 70 mM glycylglycine, pH 8.0, 10 mM glucose, 5 mM MgCl$_2$, 3 mM ADP, 1 mM NADP, 5 units/ml hexokinase, and 1 unit/ml glucose-6-phosphate dehydrogenase. Nucleoside diphosphokinase activity was monitored in the same medium with the addition of 3 mM dCTP to act as a substrate and 3 mM AMP to inhibit adenylate kinase. Enzyme activity was correlated with absorbance change at 340 nm.

Sperm Respiration—Oxygen uptake was analyzed polarographically in a Gilson Oxygraph (Gilson Medical Electronics, Middleton, WI) fitted with a Clarke-type oxygen electrode.

RESULTS
Reactivation of Motility in Ejaculated Bovine Sperm Permeabilized by Digitonin—Washed ejaculated sperm at 1 x 10$^8$ cells/ml became immotile within 0.5 min of exposure to 0.15 mM digitonin (0.184 mg/ml). Addition of 0.3-1.0 mM ATP in the presence of Mg$^{2+}$ resulted in poor quality motility (narrow tail arcs, nonrhythmic, jerky movement; Table I). With the addition of cAMP, both the number of motile cells and the quality of motility increased (Table I). The pattern of tail movement became rhythmic, arc amplitudes increased, and the cells generally appeared to move with increased vigor. Because, under these conditions, nearly all of the cells were stuck by their heads to the glass slide or cover slip, speed and directional patterns were not examined.

In the presence of cAMP, ADP stimulated motility that was qualitatively and quantitatively indistinguishable from that produced by MgATP (Table I). Although ADP preparations usually contain some contaminating ATP, this was less than 1.0% of the ADP concentration in these studies, far below the minimum necessary to reactivate full motility (0.2-0.3 mM, data not shown). Mammalian spermatozoa contain enzymes that could transform ADP into the ATP necessary for dynein activity, most likely adenylate kinase and nucleoside diphosphokinase (8). In order for nucleoside diphosphokinase to provide ATP from added ADP, another nucleoside triphosphate must donate a terminal phosphate. Inosine 5'-triphosphate, which was included in these preparations to act as a substrate for nonspecific phosphatases, could act as a phosphate donor. However, exclusion of ITP did not affect the stimulatory action of ADP (Table I). Another phosphate donor might be the structural guanine nucleotides bound to the axoneme (8). This possibility has not been investigated. No other added nucleotides, including GTP, GDP, GMP, AMP, or IDP, supported motility in permeabilized sperm.

Adenylate kinase is presumed to salvage adenine nucleotides by converting AMP to ADP at the expense of ATP. The resulting diphosphate is then rephosphorylated by the mitochondrial F$_1$-ATPase. However, the equilibrium constant of adenylate kinase approaches unity, indicating that the direction of catalyzed reaction is determined by substrate concentrations. Thus, ATP could easily be produced from excess ADP. Adenylate kinase is inhibited by Ap$_6$A (9), which is considered specific because it does not inhibit nucleoside diphosphokinase (10) or other phosphotransferases. Adding low concentrations of Ap$_6$A to permeabilized sperm had no effect on sperm reactivated by MgATP but inhibited motility in sperm reactivated by MgADP (Table I). In some trials, sperm exposed to ADP and Ap$_6$A recovered some motility after several minutes, albeit of lower quality (i.e. jerky, small amplitude strokes). This recovery of motility by Ap$_6$A-treated cells may indicate that the inhibitor was degraded by sperm phosphatases.

Production of ATP by Sperm Tails Using ADP as a Substrate—A sperm tail fraction, practically devoid of contaminating mitochondria, produced ATP when incubated with ADP (Table II). ATP synthesis was not affected by fluoride, oligomycin, or vanadate but was inhibited by Ap$_6$A. This tail fraction appeared to retain at least partial membrane integrity because the addition of digitonin increased the rate of ATP production (Fig. 1A). The ATP-sparing function of ITP is illustrated in Fig. 1A, indicating increased accumulation of ATP in the presence of ITP. ITP is not a substrate for the luciferin-luciferase reaction, and this nucleotide preparation contained a negligible amount of contaminating ATP (about 1%). In addition, ATP was not synthesized from IDP or GDP

Table I

| Reactivation conditions | % control | Quality |
|-------------------------|-----------|---------|
| ATP$^a$                  | 75.5 ± 15.6 (n = 3) | Slow, jerky, narrow arcs |
| ATP + cAMP              | 100       | Fast, broad arcs, rhythmic |
| ADP + cAMP              | 100.3 ± 6.8 | Indistinguishable from control |
| ATP + cAMP + Ap$_6$A    | 100.5 ± 4.4 | Indistinguishable from control |
| ADP + cAMP + Ap$_6$A    | 57.7 ± 10.4 | Slow, uncoordinated |
| ADP + cAMP without ITP  | 107.8 (n = 1) | Indistinguishable from control |

$^a$Sperm were added to medium at 20°C containing digitonin and, where indicated, cAMP and Ap$_6$A. After 1.5 min, ATP or ADP was added, incubated 1 min, and then motility was videorecorded for approximately 4 min.

Motility was analyzed by reviewing the characteristics of 400-600 cells. Motility of sperm with ATP + cAMP was used as 100% (actual values were 68.5 ± 9.9%, mean ± S.D. for four trials) and as control for qualitative comparisons.

$^b$ATP, 0.5 mM; ADP, 0.5 mM; cAMP, 5 μM; Ap$_6$A, 50 μM. 

Sperm Tail Adenylate Kinase
Adenylate kinase activity was determined by ATP production from added ADP in a reaction medium incubated at 30 °C for 5 min. ATP was measured by a luciferase assay. Activity was from sperm tail fractions from ejaculated sperm from three bulls.

| Treatment* | ATP | mmol/mg protein | % control |
|------------|-----|-----------------|-----------|
| ADP        | 412.3 ± 100 |
| ADP + Ap6A | 2.5 ± 2.1   | 0.6 ± 0.5       |
| ADP + NaF  | 378.7 ± 64.5| 92.9 ± 6.5      |
| ADP + oligomycin | 407.4 ± 65.9| 99.7 ± 6.4      |
| ADP + vanadate | 420.3 ± 61.1| 103.0 ± 5.3     |

*Inhibitor concentrations: Ap6A, 50 μM; NaF, 30 mM; oligomycin, 5 μM; vanadate, 5 μM.

Activity of Adenylate Kinase in Intact Sperm—As illustrated in Fig. 3A, the concentrations of adenosine phosphates were constant and roughly equal over 30 min in motile sperm; however, treatment with 2.5 mM caffeine (Fig. 3B), which stimulated motility and generated a 2-fold increase in respiration (11), drastically changed the adenosine phosphate distribution, decreasing ATP and increasing AMP. This shift in stimulated cells is consistent with the proposal that sperm tail adenylate kinase produces ATP from ADP, thus accumulating AMP. The equilibrium constant for these nucleotides at peak stimulation of sperm metabolism is about 1 (Table III), which corresponds to the reported Km of adenylate kinase. Thus, the measured reaction is not invalidated by contaminating enzymes that might alter the adenosine nucleotides. In sperm treated with 30 mM NaF, which blocked motility but did not affect oxidative phosphorylation (11), ATP titers were very high and remained stable for an extended period (Fig. 3C), while ADP and AMP titers were extremely low. Adding oligomycin to fluoride-inhibited sperm (Fig. 3D) caused a precipitous drop in ATP levels and increased titers of both ADP and AMP, suggesting that the sperm adenylate kinase activity was not coupled exclusively to motility but also provided ATP for other metabolic processes.

The sperm tail adenylate kinase had an apparent Km of 1.1 mM for ADP measured in situ (Fig. 4). Using a water volume of 14.3 μl for ejaculated bull sperm (12) and amounts of adenosine phosphates from Fig. 3A, we calculate the concentration of ADP in untreated and caffeine-stimulated sperm as 7.7 mM and 8.1 mM, respectively.

Stimulation of Respiration by AMP—In washed bull sperm that had been permeabilized by filipin, AMP was as effective as ADP at stimulating State 3 respiration (Table IV), indicating that adenylate kinase was converting AMP into ADP at the mitochondria. Addition of Ap6A blocked stimulation by AMP but did not affect stimulation by ADP. To preclude the possibility that tail adenylate kinases had migrated out of permeabilized flagella, filipin-treated sperm were centrifuged and resuspended in new media. These cells retained their responsiveness to AMP, indicating that the adenylate kinase activity was mitochondrial and not flagellar.

**DISCUSSION**

Adenylate kinase is a ubiquitous enzyme that, by virtue of an equilibrium constant approaching 1, can produce either ADP or stoichiometric amounts of ATP and AMP depending on the concentrations of the three nucleotides. Between the
inner and outer mitochondrial membranes, adenylate kinase probably uses ATP to salvage AMP generated by cellular metabolism (13). However, in all compartments, the direction of adenylate kinase activity is determined by substrate concentrations (13, 14), and where ATP is being used rapidly, the reaction is in the direction of ATP production from ADP.

Adenylate kinase activity has been detected in a variety of cilia and flagella including algal flagella (15), Tetrahymena cilia (16, 17), and sea urchin sperm flagella (3, 8, 18, 19). The function and location of this enzyme in vivo have not been determined, although Brokaw and Gibbons (18) noted that adenylate kinase activity was recovered with the tail fraction of homogenized sea urchin sperm. The ability of ADP to support motility in Triton-treated cell models was variable (18, 20), but there appeared to be a correlation between the presence of the cell membrane and the ability of ADP to stimulate motility.

Adenylate kinase activity has also been implied previously in bull sperm flagella in novel experiments reported by Lindemann and Rikmenspoel (21). ADP added to punctured or dissected bull sperm supported slow, but apparently normal, motility. Although no attempt was made to identify the responsible activity, the authors' conclusion that adenylate kinase was present in bovine sperm has been borne out by the evidence reported here.

In the flagella of bull sperm relying on oxidative phosphorylation, dynein and other ATPases consume ATP that is produced only in the midpiece and must diffuse down the tail to power motility. We suggest that, in the sperm tail, adenylate kinase forms ATP in response to high ADP concentrations that develop when motility is stimulated after capacitation or prior to fertilization (4). AMP formed by the reaction diffuses back to the mitochondria to be rephosphorylated to ADP by mitochondrial adenylate kinase. Because this mitochondrial phosphorylation takes place at the expense of ATP, the net energy expenditure of a system using a tail adenylate kinase would be equal to a conventional system. The advantage of the tail adenylate kinase pathway is that one diffusing nucleotide could supply energy from two high energy phosphate bonds instead of just one. A theoretical treatment by Raff and Blum (26) lends support to this proposal with the conclusion that adenylate kinase could increase the effective length of a cilium relying on ATP diffusion from mitochondria.

Several possibilities exist for the distribution and related function of the enzyme in sperm tails. The simplest proposal...
TABLE III

| K = [ADP]^2/[ATP][AMP]. | Time | Control* | Caffeine* |
|--------------------------|------|----------|-----------|
| min                      |      |          |           |
| 0.5                      | 0.78 | 0.56     |           |
| 2.5                      | 0.75 | 1.11     |           |
| 5.0                      | 0.91 |          |           |
| 10.0                     | 0.78 | 1.01     |           |
| 15.0                     | 0.84 | 1.19     |           |
| 20.0                     | 0.97 | 1.10     |           |
| 30.0                     | 0.96 | 0.95     |           |

*Control = no addition.
* Caffeine = 2.5 mM.

Fig. 4. Lineweaver-Burk plot of adenylate kinase activity in sperm tail fragments. Sperm tail fragments were incubated at 30 °C in the medium described in Fig. 2 with varying amounts of ADP. After 2.5 min, cold trichloroacetic acid was added, and ATP was extracted and measured in a luciferase assay as under “Materials and Methods.” Least squares linear regression analysis of ATP production versus substrate supply indicated an apparent $k_m$ of approximately 0.94 mM in the direction of ATP synthesis.

It is obvious that bovine sperm have a large amount of adenylate kinase activity, but separating the activity in the mitochondria (midpiece) and tail is difficult because the midpiece is an integral part of the tail. Several pieces of data presented here do, however, suggest that adenylate kinase is located both in sperm tails and mitochondria. The primary evidence for tail adenylate kinase is that permeabilized sperm become motile in the presence of ADP and cAMP. It is well established that dyneins use ATP exclusively (22, 23), so the added ADP must be converted to ATP before it can be used for motility. In permeabilized sperm treated with 30 mM sodium fluoride, which inhibited sperm motility (11) and blocked nucleotide-consuming phosphatases but did not inhibit adenylate kinase, added ADP would produce enough ATP to make the medium concentration about 12 μM. If adenylate kinase were located exclusively in the mitochondria, ATP produced there would have to diffuse through the medium to the tail to stimulate motility. However, these studies also indicate that bovine sperm require about 0.25 mM ATP to achieve full motility. Thus, in order for ADP to produce motility, it must be converted to ATP in the tail itself. In order for our hypothesis to be valid, mitochondrial adenylate kinase must rephosphorylate AMP returned from the mitochondria or to reduce adenylate kinase activity to salvage AMP at the mitochondria or to reduce ATP to make the medium concentration about 12 μM. If adenylate kinase were located exclusively in the mitochondria, ATP produced there would have to diffuse through the medium to the tail to stimulate motility. However, these studies also indicate that bovine sperm require about 0.25 mM ATP to achieve full motility. Thus, in order for ADP to produce motility, it must be converted to ATP in the tail itself.

Additional energy at times of increased demand. The possibility also exists that adenylate kinase may have been released and was redeposited in the vicinity of the mitochondria is lessened by the fact that the AMP effect persisted after the permeabilized cells were washed, which would probably remove any free tail enzyme. Although theoretical calculations indicate that diffusion of mitochondrial ATP should be sufficient to produce maximal motility in both sea urchin and bull sperm (24, 25), empirical observations of sea urchin motility (3, 27) indicate that ATP diffusion would not support full motility, particularly in the distal portions of the flagellum. Sea urchin sperm rely on a facilitated diffusion of high energy phosphates in the form of phosphocreatine to supply adequate energy to all portions of the tail. Although sea urchin sperm also contain adenylate kinase, its activity is insufficient to support full motility in the absence of the phosphocreatine shuttle (27). It may be postulated that sea urchin sperm retain adenylate kinase activity to salvage AMP at the mitochondria or to reduce

**TABLE IV**

| Respiration stimulated by ADP and AMP |
|--------------------------------------|
| Sperm treatment | Nucleotide added | Respiration |
|                |                  | State 4 | State 3 | % control |
| Intact cells   | ADP              | 2.43   | 2.33   |          |
|                | AMP              | 1.98   | 2.18   |          |
| Filipino       | ADP              | 2.51   | 8.08   | 100      |
|                | AMP              | 2.51   | 8.08   | 100      |
| Filipino-wash  | ADP              | 1.35   | 7.50   | 100      |
|                | AMP              | 1.26   | 7.36   | 93.1     |
| Filipino-wash  | ADP              | 1.70   | 11.87  | 100      |
|                | ADP + ApA        | 1.53   | 9.33   | 65.7     |
| Filipino-wash  | AMP              | 1.53   | 7.00   | 100      |
|                | AMP + ApA        | 1.48   | 2.12   | 11.7     |

*P. K. Schoff, unpublished observations.
ADP titers that might inhibit dynein ATPase in the tail. Although sea urchin sperm have both creatine and adenylate kinases, bovine sperm have virtually no creatine kinase (28). These authors also list several other sperm types (rabbit, mouse, human rooster, and frog) as well as the yeast Saccharomyces cerevisiae and the flagellated algae Chlamydomonas reinhardtii that have neither creatine nor arginine kinase. Their proposal is that reduced energy demand or the availability of energy from multiple substrates may eliminate the need for a facilitated diffusion of high energy phosphates in the sperm listed above. Analysis of adenylate kinase activity in these sperm and other flagellated cells might support an alternative explanation that, in flagella lacking creatine or arginine phosphate, adenylate kinase activity replaces phosphagen shuttles by increasing the supply of utilizable phosphoryl groups to dynein ATPase.

REFERENCES
1. Lardy, H. A. & Phillips, P. H. (1941) Am. J. Physiol. 133, 602-609
2. Gibbons, I. R. (1981) J. Cell Biol. 91, 1076-1089
3. Tombes, R. M. & Shapiro, B. M. (1988) Cell 41, 325-334
4. Yanagimachi, R. (1981) in Fertilization and Embryonic Development in vitro (Mastroianni, L., Jr. & Biggers, J. D., eds) pp. 81-182. Plenum Publishing Corp., New York
5. Lardy, H. A. & Phillips, P. H. (1941) Am. J. Physiol. 134, 542-548
6. Lemasters, J. J. & Hackenbrock, C. R. (1987) Methods Enzymol. 57, 36-50
7. Lundin, A., Rickardsson, A. & Thore, A. (1976) Anal. Biochem. 75, 611-620
8. Yangiasawa, T., Hagesawa, S. & Mohri, H. (1968) Exp. Cell Res. 52, 86-100
9. Lieshohn, G. E. & Seccmski, I. I. (1973) J. Biol. Chem. 248, 1121-1123
10. Feldhaus, P., Frohlich, T., Goody, R. S., Isakov, M. & Schirmer, R. H. (1975) Eur. J. Biochem. 57, 197-204
11. Schoff, P. K. & Lardy, H. A. (1981) Biol. Reprod. 37, 1037-1046
12. Hammarstedt, R. H., Amann, R. P., Rucinsky, T., Morse, P. D., Lepock, J., Snipes, W. & Keith, A. D. (1976) Biol. Reprod. 14, 381-397
13. Noda, L. (1973) in The Enzymes (Boyer, P. D., ed) pp. 279-305. Academic Press, New York
14. Bridger, W. A. & Henderson, J. P. (1983) Cell ATP, John Wiley & Sons, New York
15. Brokaw, C. J. (1981) Exp. Cell Res. 22, 151-162
16. Gibbons, I. R. (1966) J. Biol. Chem. 241, 5590-5596
17. Raff, E. C. & Blum, J. J. (1966) J. Cell Biol. 31, 445-453
18. Brokaw, C. J. & Gibbons, I. R. (1973) J. Cell Sci. 13, 1-10
19. Anderson, S. A. & Purich, D. L. (1982) J. Biol. Chem. 257, 6656-6658
20. Okuno, M. & Brokaw, C. J. (1979) J. Cell Sci. 38, 105-123
21. Lindemann, C. B. & Rikmenspoel, R. (1972) Exp. Cell Res. 73, 255-259
22. Gibbons, B. H. & Gibbons, I. R. (1972) J. Cell Biol. 54, 75-97
23. Shimizu, T. (1987) J. Biochem. (Tokyo) 102, 125-145
24. Nevo, A. C. & Rikmenspoel, R. (1970) J. Theor. Biol. 26, 11-18
25. Adam, D. E. & Wei, J. (1975) J. Theor. Biol. 49, 125-145
26. Raff, E. C. & Blum, J. J. (1968) J. Theor. Biol. 18, 53-71
27. Tombes, R. M., Browkaw, C. J. & Shapiro, B. M. (1987) Biophys. J. 52, 75-86
28. Tombes, R. M. & Shapiro, B. M. (1989) J. Exp. Zool., in press