Meadows, D. H., and Jardetzky, O. (1968), *Proc. Nat. Acad. Sci. U. S. 61*, 406.
Meadows, D. H., Markley, J. L., Cohen, J. S., and Jardetzky, O. (1967), *Proc. Nat. Acad. Sci. U. S. 61*, 1307.
Putter, I., Baretto, A., Markley, J. L., and Jardetzky, O. (1969), *Proc. Nat. Acad. Sci. U. S. 64*, 1396.
Richards, F., and Vithayathil, P. (1959), *J. Biol. Chem. 234*, 1459.
Schallenberg, E. F., and Calvin, M. (1955), *J. Amer. Chem. Soc. 77*, 2779.
Shulman, R. G., Wüthrich, K., Yamane, T., Antonini, E., and Brunori, M. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 623.
Weygand, R., and Geiger, R. (1956), *Chem. Ber. 89*, 647.
Wüthrich, K., Shulman, R. G., and Peisach, J. (1968), *Proc. Nat. Acad. Sci. U. S. 60*, 373.
Wyckoff, H. W., Hardman, K. D., Allewell, N. M., Inagami, T., Johnson, L. N., and Richards, F. M. (1967), *J. Biol. Chem. 242*, 3984.
Wyckoff, H. W., Tsernoglou, D., Hanson, A. W., Knox, J. R., Lu, B., and Richards, F. M. (1970), *J. Biol. Chem. 245*, 305.

Characterization of Adenyl Cyclase from the Testis of Chinook Salmon*

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ABSTRACT: Adenyl cyclase activity is found both in the tissue slice preparation and in the homogenate of *Oncorhynchus tschawytscha* testis. The enzyme activity in the homogenate proceeds maximally in the presence of 7 mM F^- and 14 mM Mg^{2+}. The requirement for Mg^{2+} could be replaced by Mn^{2+}. Unlike the mammalian adenyl cyclase, the enzyme from *Oncorhynchus tschawytscha* testis can be easily dissociated from low sedimenting cell fraction by homogenization in 0.01 M Tris-HCl (pH 7.5). Approximately 20% of the testicular enzyme activity appears in the soluble fraction. In the presence or absence of F^- and varying concentrations of Mg^{2+}, the testicular adenyl cyclase was not activated *in vitro* by added salmonid gonadotropin. A slice preparation of the testis, on the other hand, readily responded to added salmonid gonadotropin *in vitro* as evidenced by an increased incorporation of [^{14}C]ATP into cyclic [^{14}C]AMP.

Spermatogenesis in salmonid fish is a cystic, cyclical process where the diploid spermatogonial stem cell is differentiated to haploid spermatids and spermatozoa through a series of successive mitotic and meiotic divisions. A characteristic of salmonid spermatogenesis is the preponderance of one particular cell type at different stages of testis maturation which, in principle, allows biochemical characterization of various cellular processes without cell fractionation. From these considerations, we have chosen salmonid testis as a model system for the study of spermatogenesis (Schmidt *et al.*, 1965; Dixon and Smith, 1968). The endocrine control of spermatogenesis in salmonids and its dramatic response to gonadotropin has been established by the pioneer work of Robertson (1958). Another interesting aspect of this system is the replacement of histones by protamine (Miescher, 1874; Alfert, 1956; Ingles *et al.*, 1966) at a late stage of testes maturation. Further, serine residues of protamine from salmonid testis are phosphorylated both *in vivo* and *in vitro* and the latter phosphorylation is stimulated by adenosine 3′,5′-cyclic phosphate (Ingles and Dixon, 1967; Jergil and Dixon, 1970). Cyclic AMP[1] is now recognized as an intracellular messenger for several hormones in their respective target tissues; this subject has been amply reviewed (Sutherland *et al.*, 1965; Robinson *et al.*, 1968). The formation of cyclic AMP from ATP is catalyzed by membrane-bound adenyl cyclase which has been characterized from fat cells (Birnbaumer *et al.*, 1969), frog erythrocytes (Rosen and Rosen, 1969), and guinea pig ventricle (Drummond and Duncan, 1970). The purpose of the present investigation was to characterize the adenyl cyclase in salmonid (*Oncorhynchus tschawytscha*) testis and to study the nature of its control by salmon pituitary gonadotropin. It is hoped that such a study will be valuable in understanding the hormonal regulation of salmonid spermatogenesis at a molecular level.

Materials and Methods

Collection of Testes. Testes were collected from *O. tschawytscha* during their spawning migration along the Fraser River, British Columbia. Soon after collection, the testes were frozen in solid CO_2 and transported to the laboratory where they were subsequently stored at −80°. In those experiments where fresh tissue was used (*e.g.*, activation of adenyl cyclase by gonadotropin), the testes were transported to the laboratory in ice. The average time lag between collection and experimentation was approximately 4 hr. The ratio of testes weight to the body weight ranged from 0.2 to 1.5%. The predominant germ cells in testes of this size are spermatogonia and spermatocytes.

Chemicals. Uniformly labeled [^{14}C]ATP was purchased from New England Nuclear Corp. Ethanol was removed

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[1] Abbreviations used are: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; cyclic AMP, adenosine 3′,5′-cyclic phosphate.

from the sample under a stream of nitrogen and the dried residue was dissolved in an aqueous solution of ATP to give a final specific activity of 16 μCi/μmole. Phosphoenolpyruvate, pyruvate kinase, and cyclic AMP were purchased from Calbiochem. Cyclic 3′,5′-nucleotide phosphodiesterase (rabbit brain) was a gift from Dr. George I. Drummond (Drummond and Perrott-Yee, 1961). All other chemicals used were conventional commercial products.

Salmon Pituitary Gonadotropin. Salmon pituitary glands were collected by the procedure described by Tsuyuki *et al.* (1964). Protein fraction corresponding to the gonadotropic hormone was isolated according to the published procedure (Light and Donaldson, 1967) except that the second Sephadex G-100 and the DEAE-cellulose chromatography step were omitted.

Preparation of the Enzyme. To 1 g of frozen testis tissue was added 5 ml of 0.01 M Tris-HCl (pH 7.5). The mixture was homogenized for 1 min at maximum speed using a TRI-R stirrer with a loose-fitting Teflon pestle and with ice cooling. The connective tissue was removed by straining through four layers of cheesecloth. This preparation was assayed within 10 min.

Preparation of Testis Slices. A portion of unfrozen testis was transferred to phosphate-buffered saline (pH 7.5) and thin slices of testes were prepared by cutting with scissors. A suitable aliquot (150–200 mg) was transferred onto a blotting paper to remove excess buffer; the tissue was then weighed on a balance and then transferred to the incubation tubes containing substrate and other additives.

Assay of Adenyl Cyclase in the Testis Homogenate. The assay of adenyl cyclase was performed as described by Drummond and Duncan (1970) with minor modifications. Unless stated otherwise, assay of the homogenate was performed in a final volume of 170 μl in a medium containing 35 mM Tris-HCl (pH 7.5), 2 mM cyclic AMP, 7 mM NaF, 14 mM $MgSO_4$, 8 mM theophylline, 18 mM phosphoenolpyruvate, 3 enzyme units of pyruvate kinase, 7 mM KCl, 0.4 mM [^{14}C]-Na_2ATP and, usually, 50 μl of enzyme preparation. The incubation was performed at 37° for appropriate time intervals and the reaction was stopped by immersing the incubation tube in a boiling-water bath for 3 min. The mixture was then clarified by centrifugation for 5 min at 7500 rpm in a Sorvall centrifuge fitted with an SS34 rotor. The supernatant (100 μl) was chromatographed on Whatman No. 40 paper, using descending technique in the 1 M ammonium acetate (pH 7.5)–ethanol (3:7) system for 20 hr at room temperature. Authentic standards (0.05 μmole) each of ATP, ADP, AMP, cyclic AMP, adenosine, and inosine were chromatographed alongside unknowns. After drying, the chromatograms were viewed in a Chromavue and ultraviolet-absorbing regions were marked. The region corresponding to cyclic AMP was cut and placed in 20 ml of scintillation fluid composed of 2,5-diphenyloxazole (4 g) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (50 mg) dissolved in toluene (1 l.). The radioactivity was determined in a Nuclear-Chicago, Mark I, liquid scintillation counter. The counting efficiency under these conditions was 59%.

Assay of Adenyl Cyclase Activity in Testis Slices. The composition of the medium used for the incubation of the testes slices was the same as that described for the homogenate. After stopping the incubation by boiling, the contents were transferred to a glass homogenizer fitted with a Teflon pestle (0.7 × 14.5 cm) and homogenized for 1 min using a TRI-R stirrer with cooling in ice. The homogenate was diluted by the addition of 200 μl of H_2O and the diluted homogenate was transferred to the original tube used for the incubation. The homogenizer cup was rinsed with 200 μl of water and the wash was transferred to the original incubation tube. The combined solutions were then clarified by centrifugation at 7500 rpm for 5 min in a Sorvall centrifuge (SS34 rotor) and the supernatant solution (300 μl) was chromatographed for separation of the products as described for the assay of the homogenate (see above section).

Results

Localization of Adenyl Cyclase Activity. The distribution of adenyl cyclase activity in various subcellular fractions obtained by differential centrifugation of testis homogenates is presented in Table I. Under these experimental conditions only 14% of the total enzymatic activity was sedimented in the nuclear fraction. Of the total enzyme activity, 25% was present in the mitochondrial fraction. High-speed centrifugation of the mitochondrial supernatant resulted in a 50% loss of the enzyme activity as evidenced by the recovery in the combined microsomal and soluble fractions when compared to the activity present in the mitochondrial supernatant. An interesting observation is that about 20% of the total adenyl cyclase activity appeared in the high-speed supernatant.

TABLE I: Subcellular Distribution of Adenyl Cyclase Activity in *O. tschawytscha* Testis Cell Fractions.[a]

Fraction	% of Total Act.
Whole homogenate	100
2200-rpm sediment	14
7200-rpm sediment	25
40,000-rpm sediment	25
40,000-rpm supernatant	21

[a] Testis tissue (5 g) was homogenized in 25 ml of 0.25 M sucrose solution buffered with 0.01 M Tris-HCl (pH 7.5) to yield the whole homogenate. This preparation was centrifuged at 2200 rpm (SS34 rotor) for 10 min in a Sorvall refrigerated centrifuge to sediment the nuclear fraction. This nuclear fraction was resuspended in the homogenizing medium (15 ml). The nuclear supernatant was centrifuged at 7200 rpm (SS34 rotor) for 25 min and the resulting sediment was washed once with the homogenizing medium by resuspending in 10 ml of the homogenizing medium followed by centrifugation at 12,500 rpm (SS34 rotor) for 15 min. The resulting pellet (mitochondria) was resuspended in 10 ml of the homogenizing medium. The supernatant from the 7200-rpm centrifugation was recentrifuged at 40,000 rpm for 90 min using the Beckman Model L ultracentrifuge. The microsomal pellet was suspended in 10 ml of the homogenizing medium. The supernatant fraction is the soluble enzyme. The enzyme preparation (50 μl) was assayed for the adenyl cyclase activity. Incubations were done at 37° for 30 min as described under Materials and Methods.

Requirements of the Adenyl Cyclase for Divalent Metal Ions. Figure 1 depicts the dependence of the enzyme activity of homogenate preparations on divalent metal ions in the

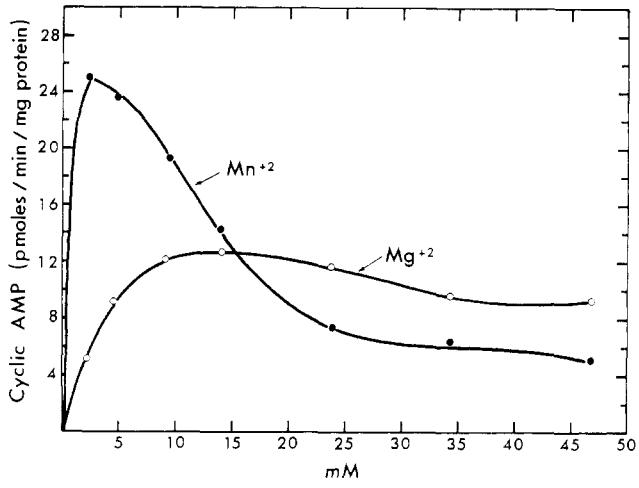

FIGURE 1: Effect of varying concentrations of Mg^{2+} and of Mn^{2+} on the adenyl cyclase activity of testis homogenate. Assay conditions are those described in the text except that Mg^{2+} or Mn^{2+} concentrations were varied. Incubations were performed at 37° for 30 min.

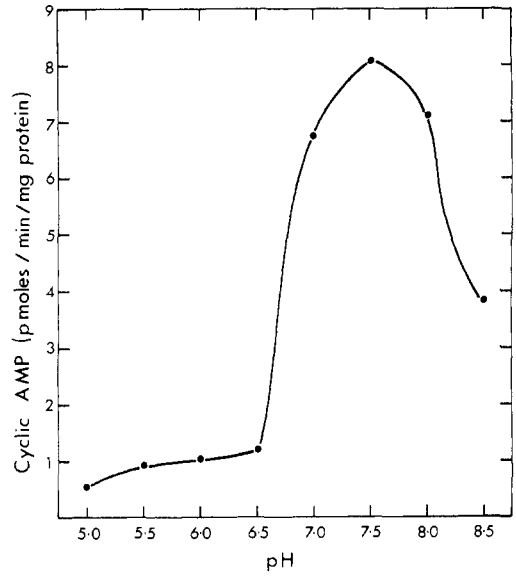

FIGURE 3: The pH optimum of *O. tschawytscha* testis homogenate adenyl cyclase. Assay was performed as described in the text except for variations in pH. The following buffers were used: ammonium acetate for pH 5.0–5.5, sodium phosphate for pH 6.0–7.0, Tris-HCl for pH 7.5–8.5. Concentrations of the buffer used in each assay were identical.

presence of optimal concentration of F^- ion. The enzyme activity could be demonstrated only in the presence of Mg^{2+} or Mn^{2+}; Ca^{2+} was without any effect. The maximal enzyme activity was found at a concentration of 14 mM Mg^{2+}. At a lower concentration of Mn^{2+} the extent of stimulation was 100% higher than that observed at the optimal concentration of Mg^{2+}. These findings are similar to those obtained by Drummond and Duncan (1970) in guinea pig ventricular adenyl cyclase.

Requirement for F^- Ion. The testicular enzyme has a marked requirement for F^- ion, in homogenate preparations (Figure 2). At optimal concentrations of divalent metal ions other anions such as Cl^-, Br^-, I^-, CN^-, SCN^-, or N^-_3 did not support the reaction. The stimulatory effect caused by F^- ion is concentration dependent. Optimal F^- concentration is 7.0 mM; a further increase in concentration up to 21.0 mM resulted in slight decrease as compared to the activity found at 7.0 mM.

pH Optimum. At optimal concentrations of F^- and Mg^{2+}, testis homogenate adenyl cyclase activity is maximal at pH 7.5 (Figure 3). At pH values from 5 to 6.3 there is very little enzyme activity. The activity increases sharply from 6.5 to 7.5.

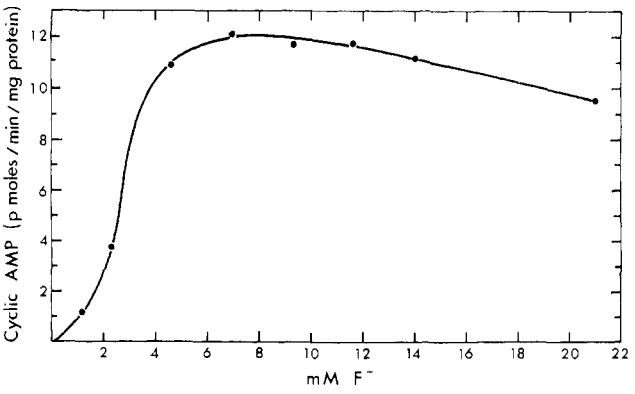

FIGURE 2: Effect of the addition of varying concentrations of F^- on the adenyl cyclase activity of testis homogenate. Incubations were performed under standard conditions with varying concentrations of F^- ion.

Effect of Incubation Temperature. The enzymic activity increased sharply from 0° to an optimum level of 37°. At 45° the enzyme was inactive. When the enzyme preparation was stored at 0°, 25% of the activity was lost in the first 30 min; at the end of 12-hr storage at 0°, only 25% of the original activity was retained. For this reason, testis homogenates were assayed within 10-min preparation.

Identity of Cyclic AMP. The cyclic [^{14}C]AMP isolated from the incubation of [^{14}C]ATP with testis homogenate was characterized enzymatically. Treatment with the nucleoside 3′,5′-cyclic phosphate phosphodiesterase from rabbit brain (Drummond and Perrott-Yee, 1961) resulted in complete hydrolysis of the cyclic [^{14}C]AMP. The bulk of the radioactivity in the product was associated with AMP although about 10% of the label appeared in adenosine. This is probably due to contamination of the phosphodiesterase with 5′-nucleotidase (Drummond and Perrott-Yee, 1961). Thus a small amount of ultraviolet-absorbing material appeared in the region corresponding to adenosine in a control incubation of authentic cyclic AMP with the phosphodiesterase.

Breakdown of Cyclic AMP by Endogenous 3′,5′-Nucleotide Phosphodiesterase. Under the standard assay conditions, no breakdown of cyclic [^{14}C]AMP to 5′-AMP was detected. However, when theophylline and unlabeled cyclic AMP were not included in the assay mixture, no cyclic [^{14}C]AMP was accumulated at the end of the incubation period (Table II). Addition of 7 mM theophylline alone resulted in the accumulation up to 30% of the cyclic [^{14}C]AMP as compared to the control incubation containing unlabeled cyclic AMP. Saturation of the system with unlabeled cyclic AMP (2 mM) prevented the breakdown even in the absence of theophylline. Similar findings have been previously reported by Bär and Hechter (1969) and by Drummond and Duncan (1970) using other systems.

TABLE II: Effect of Theophylline, Cyclic AMP, and F^- on the Conversion of [^{14}C]ATP into Cyclic [^{14}C]AMP.

Additions	Cyclic [^{14}C]AMP Formed (pmoles/min mg of Protein)
Complete system[a]	15.3
−Theophylline	17.3
−Cyclic AMP	5.3
−Theophylline and cyclic AMP)	0.23
−(F^- and cyclic AMP)	0.14

[a] Complete system refers to the standard assay mixture. This and the assay procedure are described in the text.

TABLE III: *In Vitro* Effect of Salmon Pituitary Gonadotropin on the Adenyl Cyclase Activity in Testis Slices.[a]

Fluoride Concn (mM)	Mg^{2+} (mM)	Hormone (mg)	Incubn Period (min)	Cyclic [^{14}C]AMP (pmoles/mg of Tissue)
0	14		20	0.95
0	14	1.0	20	2.0
2	4		10	0.31
2	4	0.5	10	0.96
2	4		20	1.1
2	4	0.5	20	2.8
2	4		20	1.4
2	4	1.0	20	4.2

[a] Experimental procedure is described in the text.

In Vitro Effect of Salmon Pituitary Gonadotropin on Testicular Adenyl Cyclase. Under a variety of experimental conditions it was not possible to stimulate the testicular adenyl cyclase in a homogenate preparation by added salmon gonadotropin. Alterations of Mg^{2+} concentration and the period of incubation in the presence of salmon gonadotropin did not produce any detectable adenyl cyclase activity. Thus in the homogenate preparation, enzyme activity was detectable only in the presence of F^-. However, when a sliced preparation of the testis was incubated in the absence of F^-, there was detectable adenyl cyclase activity. Further, the activity of this preparation was significantly higher in the presence of added gonadotropin as compared to the incubation in the absence of gonadotropin (Table III). The *in vitro* stimulatory effect of gonadotropin on cyclic [^{14}C]AMP formation from [^{14}C]ATP was observed even in the presence of low levels of F^- and Mg^{2+} (Table III). These concentrations of F^- and Mg^{2+} cause suboptimal stimulation of cyclic [^{14}C]AMP formation from ATP in a homogenate preparation. Cyclase activity was observed after 10- and 20-min incubations. The slower formation of cyclic AMP at 10-min incubation time would indicate that less substrate is available at shorter incubation time due to, perhaps, permeability barriers. The response to gonadotropin seems to be concentration dependent (Table IV). A control incubation with albumin did not have any stimulatory effect on the adenyl cyclase system. It is not possible to comment on the physiological significance of the response to gonadotropin because the salmonid hormone and its physiology have yet to be characterized.

Discussion

The experiments reported here point out several interesting features of salmon testis adenyl cyclase. Firstly, the enzyme is dissociated from the low-speed sediment by homogenization. Approximately 50% of the dissociated activity appears in the mitochondrial supernatant. Although there is significant loss in the enzymic activity upon centrifugation at 105,000*g*, 20% of the total enzyme activity appears in the high-speed supernatant. This system, therefore, appears to be attractive in terms of solubilization of the enzyme. The dissociation of the enzyme by homogenization occurs with both frozen and unfrozen tissue.

In this system, the stimulation of adenyl cyclase caused by F^- can be very clearly separated from the hormonal stimulation. In a whole homogenate preparation, addition of F^- caused a dramatic stimulation of adenyl cyclase activity whereas gonadotropin produced no effect whatsoever on the enzyme activity. On the other hand, a slice preparation readily responded to added gonadotropin without having a pronounced effect by added F^-, at least at lower concentration. The ability of the slice preparation to convert [^{14}C]-ATP into cyclic [^{14}C]AMP in absence of F^- and the lack of formation of cyclic AMP from ATP in the testis homogenate in the absence of added F^- underscores the loss of an essential factor during the homogenization process. A lack of hormonal response in a solubilized preparation has been reported in cat myocardial adenyl cyclase (Levy and Epstein, 1968). Activation of adenyl cyclase by a variety of hormones has been reported in several other tissues (Klainer *et al.*, 1970; Chase and Aurbach, 1968; Weiss and Costa, 1968; Rosen and Rosen, 1969; Williams *et al.*, 1968; Bar and Hechter, 1969; Birnbaumer *et al.*, 1969; Butcher *et al.*, 1965; Levy and Epstein, 1968; Murad *et al.*, 1969; Marsh, 1970; Dorrington and Bagget, 1969).

Although the experiments reported here were performed in the presence of saturating amounts of F^- and 8.0 mM theophylline to prevent degradation of cyclic AMP formed

TABLE IV: *In Vitro* Effect of Varying Concentrations of Salmon Pituitary Gonadotropin on the Formation of Cyclic [^{14}C]AMP from [^{14}C]ATP.[a]

Addition	Concn (mg)	Cyclic [^{14}C]AMP (pmoles/mg of Tissue)
Salmon pituitary gonadotropin	0	1.83
	0.05	1.67
	0.1	2.03
	0.5	2.38
	1.0	3.38
	5.0	4.40
Bovine serum Albumin	1.0	1.75

[a] Incubation was carried out in the presence of 2 mM F^-, 4 mM Mg^{2+}, and other usual additions at 37° for 20 min.

in vitro by cyclic 3′,5′-nucleotide phosphodiesterase, the possibility of inhibition of the cyclic diesterase by gonadotropin, thereby protecting the cyclic AMP formed during the incubation, cannot be ruled out. An accurate assessment is complicated by the probable permeability barrier preventing the entry of cyclic AMP into the cell. Clarification of this point will be possible only when the stimulatory effect could be demonstrated in a cell-free system. Recent experiments by Marsh (1970), however, indicate that luteinizing hormone stimulates cyclic AMP production in bovine corpus luteum by stimulating adenyl cyclase rather than by the inhibition of cyclic diesterase.

The requirement for high concentration of Mg^{2+} for optimal activity is similar to that found for washed particles of guinea pig ventricle (Drummond and Duncan, 1970). At optimal concentration of Mg^{2+} (14.0 mM) the K_m for ATP is 0.05 mM which is comparable to heart ventricle (Drummond and Duncan, 1970), fat cell ghost (Bär and Hechter, 1969), and frog erythrocytes (Rosen and Rosen, 1969). The stimulatory effect of Mn^{2+} ion is more pronounced than that of Mg^{2+}. The optimum concentration of Mn^{2+} (2.3 mM) is lower than that of Mg^{2+} (14.0 mM). The extent of reaction at optimal concentration of Mn^{2+} is 100% higher than Mg^{2+}.

The enzyme activity is completely lost on storage at $-20°$ suggesting extreme lability of the enzyme.

The occurrence of gonadotropin-sensitive adenyl cyclase in *O. tschawytscha* testis implies the involvement of cyclic AMP in salmonid spermatogenesis. Further experiments are needed to define the role(s) of this cyclic nucleotide in the regulation of cellular processes in salmonid testis.

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References

Alfert, M. (1956), *J. Biophys. Biochem. Cytol. 2*, 109.

Bär, H. P., and Hechter, O. (1969), *Biochem. Biophys. Res. Commun. 35*, 681.

Birnbaumer, L., Pohl, S. L., and Rodbell, M. (1969), *J. Biol. Chem. 244*, 3468.

Butcher, R. W., Ho, R. J., Meng, H. C., and Sutherland, E. W. (1965), *J. Biol. Chem. 240*, 4515.

Chase, L. R., and Aurbach, G. D. (1968), *Science 159*, 545.

Dixon, G. H., and Smith, M. (1968), *Progr. Nucl. Acid. Res. Mol. Biol. 8*, 9.

Dorrington, J. H., and Bagget, B. (1969), *Endocrinology 84*.

Drummond, G. I., and Duncan, L. (1970), *J. Biol. Chem. 245*, 976.

Drummond, G. I., and Perrott-Yee, S. (1961), *J. Biol. Chem. 236*, 1126.

Ingles, J., and Dixon, G. H. (1967), *Proc. Nat. Acad. Sci. U. S. 58*, 1011.

Ingles, J., Trevithick, J. R., Smith, M., and Dixon, G. H. (1966), *Biochem. Biophys. Res. Commun. 22*, 627.

Jergil, B., and Dixon, G. H. (1970), *J. Biol. Chem. 245*, 425.

Klainer, L. M., Chi, Y. M., Friedberg, S. L., and Levy, G. S. (1970), *Biochem. Biophys. Res. Commun. 38*, 86.

Levy, G. S., and Epstein, S. E. (1968), *Biochem. Biophys. Res. Commun. 33*, 990.

Light, P., and Donaldson, E. M. (1967), *Biol. Reprod. 1*, 307.

Marsh, J. M. (1970), *J. Biol. Chem. 245*, 1596.

Miescher, F. (1874), *Ber. Deut. Chem. Ges. A 7*, 376.

Murad, F., Strauch, B. S., and Vaughan, M. (1969), *Biochim. Biophys. Acta 177*, 591.

Robertson, O. H. (1958), *U. S. Fish Widl. Serv. Fish. Circ. 127*, 9.

Robinson, G. A., Butcher, R. W., and Sutherland, E. W. (1968), *Annu. Rev. Biochem. 37*, 149.

Rosen, O. M., and Rosen, S. M. (1969), *Arch. Biochem. Biophys. 131*, 449.

Schmidt, P. J., Mitchell, B. S., Smith, M., and Tsuyuki, H. (1965), *Gen. Comp. Endocrinol. 5*, 197.

Sutherland, E. W., Ye, I., and Butcher, R. W. (1965), *Recent. Progr. Hormone Res. 21*, 623.

Tsuyuki, H., Schmidt, P. J., and Smith, M. (1964), *J. Fish. Res. Bd. Can. 21*, 635.

Weiss, B., and Costa, E. (1968), *J. Pharmacol. Exp. Ther. 161*, 310.

Williams, R. H., Walsh, S. A., Hepp, D. K., and Ensinck, I. W. (1968), *Metabolism 18*, 653.