for 96 μmoles periodate → iodate is 1.92 ml. of 0.1 *M* thiosulfate; found, 93 μmoles $NaIO_4$ (1.86 ml. 0.1 *M* thiosulfate) had been consumed and 93.5 μmoles of formaldehyde produced. This showed that attack was centered only on the glycerol portion of the GPI molecule.

Subsequently, 98.5 μmoles of GPI-cyclohexylamine was oxidized, as above, with 1.01 ml. of 0.1 *M* $NaIO_4$. The optical rotation of the glycolaldehydeinositol phosphate was $[\alpha]^{25}D$ −13.2 ± 0.3°; $[M]^{25}D$ −53.0 ± 1.2°.

These results strongly support the proposal that the phosphate is esterified to the 1 or 4 position on the myo-inositol and this asymmetric unit accounts for the major optical activity of the GPI. The above findings are in agreement with the recent excellent observations of Pizer and Ballou[4] on the nature of the inositol phosphates of soybean inositol lipides. Further, the data here show that the attachment of the phosphate to the glycerol is at the 3-position.

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ADENOSINE-5′-TRIPHOSPHATE REQUIREMENT FOR LUMINESCENCE IN CELL-FREE EXTRACTS OF RENILLA RENIFORMIS[1]

Sir:

A survey of the ATP[2] requirement for luminescence in extracts of a variety of luminous organisms was reported[3] recently in which a negative ATP response was obtained for the sea pansy, *Renilla reniformis*. Using similar techniques, negative results also were obtained in this laboratory with the same organism, but by varying the extraction procedure very active extracts that require ATP have been obtained from *R. reniformis*.

Using crude extracts, a 5–10-fold increase in light intensity was obtained by adding ATP. With ammonium sulfate fractions, 50–100 fold stimulations by ATP have been observed (Table 1). The system is specific for adenine-containing nucleotides since other nucleoside-5′-triphosphates such as GTP, CTP, and UTP are inactive. In addition to ATP, AMP and ADP also show activity, but the type of response to these compounds is different from that with ATP. Thus at concentrations corresponding to near saturating levels of ATP, AMP and ADP are only one-third as effective. At saturating levels, AMP and ADP are about 80% as effective as ATP in supporting luminescence. Adenosine is inactive in this system.

Since the extracts contain a phosphatase that rapidly converts ADP to AMP, the ADP effect can be explained in terms of its conversion to AMP. Adenylate kinase activity cannot be demonstrated. The AMP used in these experiments did not contain ATP or ADP as determined by ATP analysis using the firefly technique.[4,5] ADP was measured by coupling adenylate kinase to the firefly system. The AMP effect is not due to oxidative phosphorylation since AMP is effective in a phosphate-free system as well as in the presence of 2,4-dinitrophenol.

TABLE I

EFFECT OF ATP ON THE LUMINESCENCE OF *R. Reniformis* EXTRACTS

Conditions: 0.05 *M* potassium phosphate, *p*H 7.5 (1.1 ml.); reduced glutathione (2 μmoles); nucleoside-5′-triphosphate (0.5 μmole); enzyme (0.5 mg. protein); total volume, 1.4 ml. Divalent cations such as Mg^{++} or Mn^{++} have no effect on the complete system.

Additions	Relative light intensity
None	2
ATP	101
GTP, CTP, or UTP	2

In the presence of ATP, a relatively stable intermediate accumulates under anaerobic conditions. It does not appear to be an adenylate derivative since inorganic pyrophosphate ($10^{-3}M$, final concentration) or purified inorganic pyrophosphatase has no effect on the luminescence system. These observations are in direct contrast to the firefly system[6] which requires the intermediate formation of an enzyme bound adenylate derivative.

The effect of AMP on this system may be interpreted as a cyclic phenomenon involving either ATP or ADP formation, although the mechanism of action of these adenine-containing nucleotides is not understood.

In addition to ATP and the enzyme fraction, the requirements for *Renilla* luminescence include oxygen and a dialyzable heat-stable factor obtained from boiled *Renilla* extracts. Flushing with nitrogen abolishes the light, which is returned by flushing with oxygen. Using a resolved enzyme, 80-fold stimulations of luminescence can be observed upon the addition of boiled extract. Attempts to demonstrate a specific metal requirement have not succeeded.

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(2) Abbreviations: AMP, adenosine-5′-monophosphate; ADP, adenosine-5′-diphosphate; ATP. GTP, CTP, and UTP, the nucleoside-5′-triphosphates of adenosine, guanosine, cytidine, and uridine, respectively.

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(4) B. L. Strehler and J. R. Totter, *Arch. Biochem. and Biophys.*, **40**, 28 (1952).

(5) W. D. McElroy and B. L. Strehler, *Bacteriol. Revs.*, **18**, 177 (1954).

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ON THE MOLECULAR BONDING OF LYSINE VASOPRESSIN AT ITS RENAL RECEPTOR SITE[1]

Sir:

Although the elucidation of the structure and synthesis of vasopressin has been accomplished elegantly by du Vigneaud and collaborators the mechanism of the antidiuretic action of the hormone remains obscure. We have evidence that the

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