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.

The author is indebted to Dr. John M. Teal, University of Georgia Marine Institute, Sapelo Island, for supplying the animals used in these experiments.

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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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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

(1) This work was supported by the U. S. Atomic Energy Commission.

hormone lysine vasopressin (LVP) is attached at its receptor site through a covalent bond. The bond probably is formed through a disulfide displacement reaction[2,3] involving the hormone disulfide and the thiol groups on the receptor protein.[4] This concept also may apply to other disulfide hormones such as oxytocin and insulin.

For the experiments LVP was partially purified from mixed beef and hog pituitary powder[5] by counter-current distribution between aqueous 0.09 *M* *p*-toluenesulfonic acid and 1-butanol with fourteen transfers. Further purification was carried out on a carboxymethylcellulose column according to the method of Ward and Guilleman.[6] Approximately 20 mg. of LVP was exposed to 1.1 curies of tritium gas for 8 days at room temperature and 2/3 atmospheric pressure. Exchangeable tritium was removed from the tritiated lysine vasopressin (H^3LVP) by solution in very dilute acetic acid and subsequent lyophilization. The process of solution and lyophilization was repeated three times. The firmly labeled H^3LVP was chromatographed twice on carboxymethylcellulose columns. A yield of about 0.02 mg. of H^3LVP was obtained with a specific activity of approximately 300 uc./mg. Paper electrophoresis showed a single radioactive component and antidiuretic assays on the rat demonstrated it to be biologically active.

Rats were hydrated, anesthetized with dilute ethanol and both kidneys were dissected free of all attachments except for the renal blood vessels. Approximately 3 milliunits of H^3LVP was injected through the external jugular vein. After 5 to 10 minutes, at the peak of antidiuretic activity, both kidneys were perfused via the renal artery with saline containing thiol blocking agents, *p*-chloromercuribenzoate and N-ethylmaleimide, to bind free sulfhydryl groups not involved in the hormone reaction. When both kidneys were freed of blood they were excised, homogenized and centrifuged at 1500 × *g*. The sediment was washed with acetone, alcohol and saline until the washings were free of radioactivity. Portions of the kidney protein (2 g.) were then shaken overnight in a 0.15 *M* $NaHCO_3$ solution saturated with cysteine at a *p*H of 8 along with controls containing bicarbonate only. The supernates containing amino acids and polypeptides were dried below 50° and dissolved in a hyamine solution.[7] Tritium activity was assayed in a Packard liquid scintillation counter using DPO (diphenyloxazole) as phosphor and POPOP (bis-phenyloxazolylbenzene) as a spectrum shifter. Correction for quenching was made with an internal standard. The results were shown in Table I.

These results suggest that the hormone interacts with the kidney receptor protein and is bound to it through a disulfide bond. The specificity of the reaction of the hormone with its receptor protein must depend on the interaction of reactive groups (ionic, disulfide and thiol groups) and the stereoconfiguration of these groups within the protein macromolecules.

TABLE I

RELEASE OF H^3LVP FROM VARIOUS TISSUES BY TREATMENT WITH CYSTEINE

Tissue	Average corrected tritium c.p.m.
Kidney treated with cysteine	96.7
Kidney control	28.2
Muscle treated with cysteine	8
Muscle control	1–2

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TRIETHYLBORANE AS AN ALKYLATING AGENT IN BOTH ORGANIC AND AQUEOUS MEDIA

Sir:

We wish to report the preparation of organometallic derivatives by ethylation reactions with triethylborane in both ethereal and aqueous solutions. The applicability of water as the medium for alkylation of metal salts is unique. The usual alkylating agents such as Grignard and organoaluminum derivatives decompose on contact with moisture and must, therefore, be employed under anhydrous conditions.

Mercuric oxide (0.05 mole) suspended in water containing 0.15 mole of sodium hydroxide was heated to 75° and treated with 0.05 mole of triethylborane. The reaction apparently was complete in 10 minutes as the mercuric oxide dissolved and a heavy oil settled to the bottom of the flask when the stirrer was stopped. Distillation of the oil gave a 95% yield of diethylmercury, b.p. 67–68° (19 mm.),[1] which was identified further by comparison of its infrared spectrum with that of an authentic sample.

In 1,2-dimethoxyethane, 0.02 mole of mercuric acetate and 0.04 mole of triethylborane were refluxed for 2 hours. Addition of aqueous sodium hydroxide caused the separation of a heavy liquid. Distillation gave a 66% yield of diethylmercury.

The result obtained in water was even more surprising than that in the ether. In addition to showing that trialkylboranes can be employed under conditions which preclude the use of moisture-sensitive reagents, the product can be isolated in a nearly pure state by simple physical separation. Furthermore, in water, two of the ethyl groups from triethylborane were used to ethylate the mercury atom. Under the proper conditions complete utilization of the alkyl groups on boron may be realized.

The reactions of triethylborane with compounds of other metallic and metalloidal elements are currently being explored. The work also is being extended to other organoboranes.

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