

hormone lysine vasopressin (LVP) is attached at its receptor site through a covalent bond. The bond probably is formed through a disulfide displacement reaction<sup>2,3</sup> involving the hormone disulfide and the thiol groups on the receptor protein.<sup>4</sup> 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<sup>5</sup> 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.<sup>6</sup> 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 ( $H^3$ LVP) by solution in very dilute acetic acid and subsequent lyophilization. The process of solution and lyophilization was repeated three times. The firmly labeled  $H^3$ LVP was chromatographed twice on carboxymethylcellulose columns. A yield of about 0.02 mg. of  $H^3$ LVP was obtained with a specific activity of approximately 300 uc./mg. Paper electrophoresis showed a single radioactive component and anti-diuretic 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  $H^3$ LVP was injected through the external jugular vein. After 5 to 10 minutes, at the peak of anti-diuretic 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  $\times$  *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<sub>3</sub> solution saturated with cysteine at a *pH* 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.<sup>7</sup> Tritium activity was assayed in a Packard liquid scintillation counter using DPO (diphenyloxazole) as phosphor and POPOP (bis-phenyloxazolybenzene) 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

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(3) A. P. Ryle and F. Sanger, *Biochem. J.*, **60**, 535 (1955).

(4) E. J. Cafruny, E. Carhart and A. Farah, *Endocrinology*, **61**, 143 (1957).

(5) Kindly supplied by Parke, Davis & Co.

(6) D. N. Ward and R. Guilleman, *Proc. Soc. Exp. Biol. & Med.*, **96**, 568 (1957).

(7) M. Vaughan, D. Steinberg and J. Logan, *Science*, **126**, 446 (1957).

TABLE I  
RELEASE OF  $H^3$ LVP 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

(ionic, disulfide and thiol groups) and the stereo-configuration of these groups within the protein macromolecules.

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#### TRIETHYLBORANE AS AN ALKYLATING AGENT IN BOTH ORGANIC AND AQUEOUS MEDIA

Sir:

We wish to report the preparation of organo-metallic 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.),<sup>1</sup> 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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