active principle concerned. These experiments with homologous implants afford important confirmation of our previously reported evidence as to the biological effect of thymus extract.

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THE SECRETION OF AN ANTIDIURETIC HYPOPHYSEAL HORMONE IN RESPONSE TO THE NEED FOR RENAL WATER CONSERVATION

EVIDENCE that the posterior lobe of the pituitary gland secretes a hormone concerned with the normal regulation of mammalian water metabolism is still incomplete. The classification of the posterior hypophysis as a gland of internal secretion is the result of two types of experimentation: first, the chemical isolation from the hypophysis of a substance possessing marked anti-diuretic activity in normal, unanesthetized hydrated animals, and, second, the occurrence of polyuria and polydypsia following degeneration or removal of the posterior pituitary and their relief by administration of pituitary extracts. From such evidence it has been deduced that the hypophysis secretes a hormone which enables the kidney to reabsorb water against an osmotic pressure in the renal tubule higher than that of the blood. No one, however, has demonstrated the presence in any body fluid of an anti-diuretic substance secreted by the posterior hypophysis in response to a physiological need.

The regulation of water retention and excretion in the mammalian organism is so well adjusted that the degree of body hydration is maintained within narrow limits. If renal water reabsorption is under hormonal control, an exceedingly labile relationship should exist between the secreting gland and the end organ to permit such accurate function. For such a labile system, two requirements are necessary: (1) the degree of hypophyseal activity must be readily adjusted to the body requirements for renal water reabsorption, and (2) the hormone must be easily inhibited or destroyed to permit diuresis following hydration. The postulate of a labile system does not seem to be fulfilled when one considers that the polyuria of diabetes insipidus can be relieved for many hours by the administration of a single unit of pitressin. That this is many times the effective dose, however, has been shown by Theobald,[1] who observed antidiuresis in one individual after the administration of 0.0005 units. That pitressin is readily destroyed by the body has been clearly demonstrated by Heller and Urban,[2] who proved its rapid inactivation by blood and tissues. They also observed that a portion of the injected pitressin is excreted in the urine. It would appear, therefore, that the kidney is extremely sensitive to minute amounts of this pituitary extract and the body is capable of rapidly eliminating amounts exceeding its physiological requirements.

The above facts suggest that the secretion of an antidiuretic substance is capable of effecting an accurate and sensitive control of renal water reabsorption. The demonstration that such a control actually exists and that it represents a true hormonal regulation of water reabsorption would be complete if it could be shown that the anti-diuretic substance is secreted in amounts varying with the need for water conservation. This necessitates the detection of this substance in body fluids and a method of quantitative estimation sufficiently sensitive to measure physiological variations in amounts.

The logical body fluid to examine for the presence of an anti-diuretic hormone would be the blood, were it not for the fact that extremely low concentrations are physiologically effective, and such minute amounts are readily inactivated. The observation that injected pituitrin passes through the glomerulus and is excreted in the urine, a medium in which the hormone is presumably more stable, offers another body fluid in which an anti-diuretic substance might be demonstrated. Heller and Urban failed to detect anti-diuretic activity in normal rat urine. If one increases the need for water reabsorption by dehydration, presumably this should result in greater hypophyseal activity and the urinary excretion of the anti-diuretic hormone in increasing amounts. The experiments to be described are based upon the above postulates.

Rats, dogs, monkeys and man were used as experimental subjects. Dehydration was accomplished either by the oral administration of 5 per cent. NaCl solution or by water deprivation over periods varying up to 72 hours. Urine was collected in vessels containing sufficient 1 per cent. acetic acid to make the final sample weakly acid. The urine was dialyzed through a Cellophane membrane to remove salts and urea which would interfere with the anti-diuretic assay. It was then concentrated *in vacuo* to a small volume and the antidiuretic activity determined by the rat method of Burn as described by Heller and Urban.[2] This method is based upon the rate of renal excretion of water in rats that have been hydrated to the extent of 5 per cent. of their body weight. Each sample was assayed collectively on four rats, the time for the excretion of 50 per cent. of the fluid administered being taken as the index of antidiuresis. A difference of five milliunits of pitressin can be detected by this method. The procedure of dialysis was controlled with known solutions of pituitrin, which showed no demonstrable loss

[1] G. W. Theobald, *Clinical Science*, 1: 225–239, 1934.
[2] H. Heller and F. F. Urban, *Jour. Physiol.*, 85: 502–518, 1935.

of activity over the three-hour period necessary to dialyze the urine free from chloride.

The results in rats may be briefly summarized by the citation of several characteristic experiments.

(1) The combined urine excretion of 12 animals, normally hydrated and given free access to water, collected over 24 hours and reduced to 10 cc, exhibited no anti-diuretic activity when injected into assay rats at the dose level of 1 cc/100 g.

(2) The combined six-hour excretion of 12 rats after the administration of 5 cc/100 g of 5 per cent. NaCl contained, in repeated experiments, from 100 to 200 milli-units of anti-diuretic substance, expressed as units of a pituitrin solution (Parke, Davis and Company, 20 pressor units per cc) possessing comparable anti-diuretic activity.

(3) The combined excretion over the subsequent 18 hours collected from 12 rats treated as above consistently contained 200 milli-units of anti-diuretic substance.

(4) Twelve rats dehydrated by withholding water for 24 hours excreted a total of 100 milli-units of anti-diuretic substance.

(5) The same rats dehydrated over an additional 24 hours excreted in that period 200 milli-units of anti-diuretic substance.

(6) Twenty rats dehydrated over a period of 72 hours excreted a total of 5 units of anti-diuretic substance.

Dogs proved more variable than rats in their response. The most striking result was obtained in a female collie. This animal during the first 24 hours of dehydration excreted 0.1 unit. After an additional 24 hours of dehydration, the urine contained approximately 5 units.

Anti-diuretic activity of the urine of monkeys and man has also been observed.

That the anti-diuretic substance found in the urine has its origin in the posterior pituitary gland and is probably identical in nature to the pharmacological preparations derived from that gland is based on the following evidence:

(1) Hypophysectomized rats,[3] dehydrated to the point of death (40 hours), fail to secrete this anti-diuretic substance in their urine. Control rats under identical experimental conditions always show appreciable amounts.

(2) Both the anti-diuretic substance of the urine and solutions of pituitrin (Parke, Davis and Company) of the same anti-diuretic strength are destroyed to the same degree by (a) 30-minute hydrolysis with 1 per cent. HCl, (b) 30-minute hydrolysis with 2N NaOH and (c) 30-minute reduction with 0.1N Na_2SO_3.

[3] Kindly prepared and examined post-mortem by Dr. Robert T. Hill, of the department of anatomy.

(3) The molecular size of both the anti-diuretic substance extracted from the pituitary and that excreted by the kidney is such that no appreciable loss in activity results from a 3-hour dialysis with a Cellophane membrane with a wall thickness of 0.00072 inches.

The authors interpret the above experiments as strong evidence that (1) an anti-diuretic substance is secreted by the posterior pituitary, (2) this substance is a true hormone, passes into the circulation and acts upon the kidney, (3) the pituitary hormone in its circulation through the kidney filters through the glomerulus and escapes into the urine, in which it is relatively stable and easily detectable, (4) the need for water conservation by the body is a stimulus for the secretion of this hormone.

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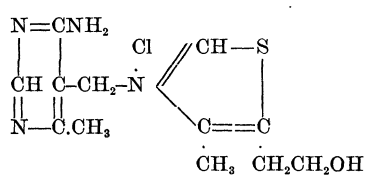
RESEARCHES ON PYRIMIDINES. CLIII. THE STRUCTURE OF VITAMIN B_1

STRUCTURE I has been proposed by the Japanese investigators Makino and Imai[1] for vitamin B_1. They are the first investigators to suggest a structure formulated on the basis that the thiazol cycle of the vitamin is joined to the pyrimidine moiety through an aliphatic methylene group (CH_2). A methyl group is represented as being substituted for hydrogen in position – 4 of the pyrimidine ring in the vitamin.



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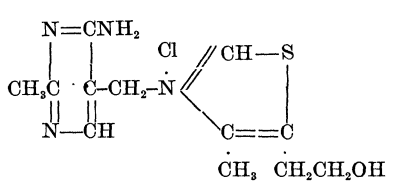

II

In a recent publication by R. R. Williams,[2] this investigator has presented new chemical evidence to support the postulation of the Japanese workers and has revised his vitamin B_1 formula previously proposed.[3] Williams now feels justified in proposing

[1] *Jour. physiolog. Chemie*, 239, Heft 1; Private communication, February 14, 1936.

[2] Communication to the editor of the *Jour. Am. Chem. Soc.*, dated May 23, 1936, Vol. 58: 1065, 1936.

[3] Williams, *Jour. Am. Chem. Soc.*, 57: 229, 1935.