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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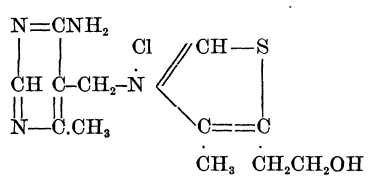
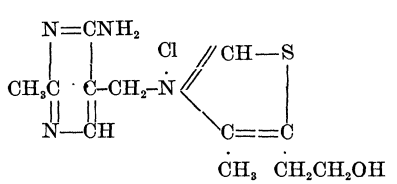
RESEARCHES ON PYRIMIDINES. CLIII. THE STRUCTURE OF VITAMIN B_1

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.



I



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.

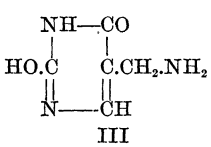
[2] Communication to the editor of the *Jour. Am. Chem. Soc.*, dated May 23, 1936, Vol. 58: 1065, 1936.

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

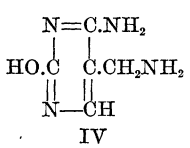
structure II for this vitamin, which differs from the Makino and Imai formulation in one respect only; in the position assigned to the methyl group substituted in the pyrimidine ring. In the Japanese formula I this group is substituted in position – 4, while in formula II the methyl radical occupies position – 2 of the pyrimidine cycle.

These conclusions are very important and add new interest to experimental work which has been in progress in the Yale Laboratory for several months. We are dealing here with a new and most interesting postulation, in so far as our knowledge of pyrimidine chemistry is concerned, and that is the fact that a side chain substitution characterizes the structure of the pyrimidine moiety of the vitamin molecule. We have at present very limited knowledge of the chemistry of such pyrimidine constructions, and evidence has been accumulated in pyrimidine researches already carried out in the Yale Laboratory showing that pyrimidine derivatives of this type are characterized by unique chemical properties. The study of several of these representatives of physiological interest is now in progress.

Predicating the probable physiological importance of several of these pyrimidine derivatives of the uracil type, the senior author started new work on this subject in 1934 and decided to undertake at first the development of a practical method for synthesizing the hitherto unknown amino derivative of thymine, namely, "Thyminyl-amine" III. This pyrimidine may be considered as an oxidized form of the pyrimidine cycle functioning in vitamin B_1. Its structural relationship to the proposed vitamin formulas, I or II, is clearly revealed when its constitution is expressed in its enolic form III. The corresponding 6-amino-pyrimidine expressed by formula IV is a derivative of 5-methylcytosine.



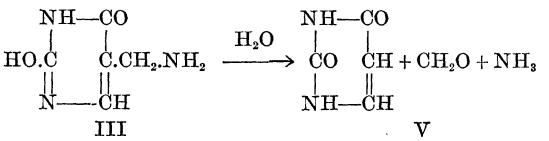
III



IV

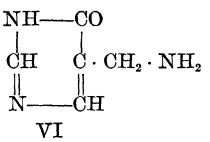
Due to a serious illness the senior author was unable to carry out his complete program of synthesis during 1935, and he turned over a part of the work to an assistant, Miss Anne Litzinger, who has accomplished successfully the synthesis of the desired thyminyl-amine III. A description of this synthesis will be presented for publication in a future number of the *Journal of the American Chemical Society*. Work dealing with the synthesis of the amino derivative of 5-methylcytosine IV is now in progress.

While the complete details of Miss Litzinger's investigation will not be reported in full until later, we do wish to make known at this time a characteristic chemical behavior of thyminyl-amine III when an aqueous solution of this pyrimidine is heated. Under such conditions the amine is broken down smoothly with formation of uracil V, formaldehyde and ammonia. This hydrolytic degradation is expressed by the equation below:



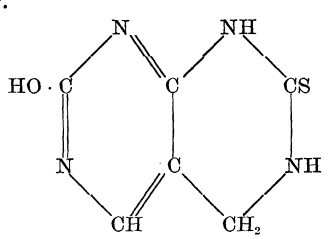
III V

In the form of its salts the pyrimidine base interacts normally as a primary amine and its derivatives crystallize well and are easily purified. The question whether a corresponding aliphatic amine of the type represented by formula VI will undergo a similar change by hydrolysis will soon be decided in this laboratory.



VI

Starting with the methyl-cytosine derivative IV it will be possible for us to synthesize a cyclic thiourea leading to the formation of a *thiodipyrimidine*, VII. Such a construction is of immediate biochemical interest on account of its possible structural relationship to *thiochrome*.



VII

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BOOKS RECEIVED

EDSER, EDWIN, The late. *Heat for Advanced Students.* Revised edition by N. M. BLIGH. Pp. x + 487. 204 figures. Macmillan. $1.75.

MACDOUGALL, FRANK H. *Physical Chemistry.* Pp. ix + 721. 95 figures. Macmillan. $4.00.

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PEATTIE, DONALD C. *Green Laurels; The Lives and Achievements of the Great Naturalists.* Pp. xxiii + 368. 32 plates. Simon and Schuster. $3.75.

ROEHL, KATHERINE McC., and H. H. NEWMAN. *A Laboratory Manual for General Zoölogy.* Pp. vii + 99. 2 figures. Macmillan. $1.00.

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