

Stuart<sup>1</sup> and the quinine sulfate method of Williams.<sup>2</sup> Recently, Cook and Carroll<sup>3</sup> have reported the successful use of pyridine solutions.

We have been studying the concentration of vitamin B<sub>1</sub> for the last two years and have made an extensive study of the extraction from the adsorbate. In general, the published methods have extracted the vitamin, but in most cases the yields were not high. The quinine sulfate method of Williams probably gave the best yields. Early in our work we observed that pyridine solutions extracted some vitamin from the adsorbate, but the yields were far from satisfactory. In marked contrast, we found that the acid salts, such as the hydrochloride of pyridine, quinoline, aniline and certain other nitrogen bases gave very high yields of vitamin; yields of 90 per cent. or better were ordinarily obtained. Even solutions of ammonium chloride gave better yields of vitamin than solutions of either ammonia or hydrochloric acid.

Aqueous, aqueous alcoholic or alcoholic solutions of some of the acid salts of these bases have been extensively used in our studies. A more detailed description of the procedure of extraction will be contained in a later publication.

Our studies appear to show that the acid salts of certain nitrogen bases have the property of freeing the vitamin B<sub>1</sub> from the fuller's earth or Lloyd's reagent. These salt solutions have a much more marked effect than either the base or the acid alone. In fact, our evidence indicates that a part at least of the extractive power of the nitrogen base or hydrochloric acid is due to the formation of acid salts with either the acid or the nitrogen bases adsorbed with the vitamin on the fuller's earth.

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#### VITAMIN C IN AN ESTRIN PRODUCING OVARIAN TUMOR

It has been already indicated by the authors<sup>1</sup> that vitamin C seems to be related to the formation of progesterone in the corpus luteum, but not to the production of estrin in the ovary. Weight is added to this latter idea by an examination of a rare estrin-producing ovarian tumor which we were fortunate enough to obtain from an operation upon a 19-year-old girl.

The pathological and clinical aspects of this case are

<sup>1</sup> E. H. Stuart, R. J. Block and G. R. Cowgill, *Jour. Biol. Chem.*, 105: 463, 1934.

<sup>2</sup> R. R. Williams, R. E. Waterman and J. C. Keresztesy, *Jour. Am. Chem. Soc.*, 56: 1187, 1934.

<sup>3</sup> C. A. Cook and R. H. Carroll, *Ind. and Eng. Chem.*, 28: 741, 1936.

<sup>1</sup> G. R. Biskind and D. Glick, *Jour. Biol. Chem.*, 113: 27, 1936.

to be reported in detail by Drs. G. Y. Rusk, R. Rypins and A. Palmer. Hormone studies carried out by Dr. Allan Palmer<sup>2</sup> showed that the tumor contained the estrogenic equivalent of 11.7  $\gamma$  theelin per gm desiccated tissue, and 50.0  $\gamma$  per liter cyst fluid. The daily excretion of estrogenic substance in the urine was about 66.0  $\gamma$  per 24 hours before operation, and fell to normal limits after removal of the tumor. All estimations for gonadotropic hormones were negative.

The vitamin C concentration was determined by titration of a 2 per cent. metaphosphoric acid extract of the tissue with 2, 6-dichlorophenol-indophenol, and was found to be 0.20 mg per gm fresh tissue. Using a technique previously employed,<sup>3</sup> it was estimated that fibrous tissue composed 18 per cent. of the weight of the tumor; hence if the reasonable assumption is made that practically all the vitamin C is contained in the parenchymal cells, it may be calculated that there is 0.24 mg of vitamin C per gm of parenchymatous tissue.

From the cell-counting procedure<sup>4</sup> the number of parenchymal cells per mg was found to be  $712 \times 10^3$ .

It follows, then, that there is  $\frac{0.24}{712 \times 10^3}$  or  $0.34 \times 10^{-6} \gamma$  vitamin C per cell.

Up to the time of operation the patient was on a high vitamin C diet.

The comparatively low concentration of vitamin C in this tissue, which has been generating estrin in large amounts, would emphasize the previous suggestion that vitamin C is unrelated to estrin formation in the ovary.

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#### AN ARTHROPOD VECTOR FOR EQUINE ENCEPHALOMYELITIS, WESTERN STRAIN<sup>1</sup>

IN a forthcoming report<sup>2</sup> we have described experiments which suggest that the "gopher," *Citellus richardsonii* (Sabine), may serve in nature as a reservoir host for the virus of equine encephalomyelitis, Western strain. We record this supplementary note at the present time to permit workers in the field to use

<sup>2</sup> Hormone studies were made possible by a grant from the Christine Breon Research Fund, the Department of Obstetrics and Gynecology of the University of California Medical School.

<sup>3</sup> D. Glick and G. R. Biskind, *Jour. Biol. Chem.*, 114: 1, 1936.

<sup>4</sup> D. Glick and G. R. Biskind, *Jour. Biol. Chem.*, 110: 1, 1935.

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<sup>2</sup> Jerome T. Syverton and George Packer Berry. *Proc. Soc. Exp. Biol. and Med.* In press.