

presented no demonstrable abnormalities other than the uterine bleeding.

The study of the results of the injections demonstrated that the effect on the blood was so slight as to be negligible. In most cases there was some drop in the hemoglobin after the administration of the hormone, the greatest difference being 13% and in only 1 case a rise of 4%. The other variations were too unimportant to require comment. It is interesting to note that the platelet count almost uniformly was lower after treatment. This is surprising, if the fact that a reduction in platelets is usually found associated with an increased tendency toward hemorrhage, is accepted. As has been reported elsewhere, we have not been able to obtain cessation of bleeding in cases of functional metorrhagia with large doses of Antuitrin-S. It is needless to add that the patients studied in the above series showed no improvement from the standpoint of the bleeding. The continued bleeding may even explain the uniformity in diminution of the hemoglobin.

From the above studies we concluded the injections of Antuitrin-S did not affect the formed blood elements.

## 7677 C

### Basic Amino Acids Yielded by Thyroglobulin.\*

ABRAHAM WHITE AND WILLIAM G. GORDON.†

*From the Department of Physiological Chemistry, Yale University, New Haven.*

The present analysis of thyroglobulin was undertaken in order to obtain information by modern isolation methods regarding its basic amino acid composition. The preparation‡ was made by the method of Barnes<sup>1</sup>; the low phosphorus content (Table I) indicates that little impurity of a nucleoprotein nature could have been present. The iodine content is slightly higher than that reported by Heidelberger and Palmer<sup>2</sup> but is considerably lower than Oswald<sup>3</sup>

---

\* Grateful acknowledgement is made to Dr. H. B. Vickery for aid in the preparation of this manuscript.

† National Research Council Fellow in Medicine, 1933-34.

‡ This sample of thyroglobulin was generously furnished by Dr. B. O. Barnes, of the Department of Physiology, University of Chicago; the protein was prepared from hog thyroids.

<sup>1</sup> Barnes, B. O., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 680.

<sup>2</sup> Heidelberger, M., and Palmer, W. W., *J. Biol. Chem.*, 1933, **101**, 433.

<sup>3</sup> Oswald, A., *Z. physiol. Chem.*, 1899, **27**, 14; 1901, **32**, 121.

or Eckstein<sup>4</sup> found in their preparations. Barnes has shown, however, that the iodine content of thyroglobulin preparations varies with the locality from which the glands are obtained.<sup>5</sup>

TABLE I.  
Some Analyses of Thyroglobulin.

|                               | Analysis I | Analysis II | Aver. |
|-------------------------------|------------|-------------|-------|
|                               | gm.        | gm.         | %     |
| Basic Amino Acids             |            |             |       |
| Protein taken                 | 37.7       | 29.9        |       |
| Histidine                     | 0.65       | 0.58        | 0.62  |
| S in diflavanate, theory 8.17 | 8.15       | 8.13        |       |
| Arginine                      | 8.06       | 8.37        | 8.22  |
| S in flavianate, theory 6.56  | 6.61       | 6.65        |       |
| Lysine                        | 1.98       | 1.88        | 1.93  |
| N in picrate, theory 18.67    | 18.59      | 18.58       |       |
| Total Nitrogen                |            |             | 15.58 |
| " Sulfur                      |            |             | 1.46  |
| " Iodine§                     |            |             | 0.75  |
| " Phosphorus                  |            |             | 0.08  |

§ We are indebted to Mr. F. Paquin, Jr., for the iodine analyses.

The analytical procedure followed for the estimation of the basic amino acids was that employed by Vickery and White<sup>6</sup> in a recent analysis of casein. The results of the determinations, expressed as percentages of the ash- and moisture-free protein, are shown in Table I together with the more important analytical data. The 2 analyses were conducted independently by the authors, and the close agreement indicates the accuracy with which it is possible to obtain duplicate results with the procedure as developed by Vickery and co-workers.

The results of the present analysis are expressed in Table II in terms of amino acid nitrogen as per cent of the total nitrogen. Eckstein's results<sup>4</sup> are included for purposes of comparison. The values for arginine are in good agreement, but there is no relation-

TABLE II.  
Basic Amino Acids of Thyroglobulin, Expressed as Nitrogen in % of Protein Nitrogen.

|             | Analysis I | Analysis II | Aver. | Results of Eckstein <sup>4</sup> |
|-------------|------------|-------------|-------|----------------------------------|
| Histidine N | 1.13       | 1.00        | 1.07  | 11.92<br>11.34                   |
| Arginine N  | 16.64      | 17.28       | 16.96 | 16.55<br>16.27                   |
| Lysine N    | 2.44       | 2.31        | 2.38  | 4.43<br>4.19                     |

<sup>4</sup> Eckstein, H. C., *J. Biol. Chem.*, 1926, **67**, 601.

<sup>5</sup> Barnes, B. O., personal communication.

<sup>6</sup> Vickery, H. B., and White, A., *J. Biol. Chem.*, 1933, **103**, 413.

ship whatsoever between the results he has reported for histidine and our own. The high histidine value obtained by Eckstein with the Van Slyke distribution method was checked by him by means of the bromination technique of Plimmer and Phillips.<sup>7</sup> The close agreement between the results of these 2 methods was taken as evidence for the validity of the histidine figure. It is well known, however, that the Van Slyke method may yield values for histidine considerably greater than those found by the isolation technique, particularly in the presence of considerable amounts of cystine. This is strikingly illustrated by the published analyses of wool. Marston,<sup>8</sup> employing the method of Van Slyke, gave 6.9% as the amount of histidine yielded by this keratin; the modified silver precipitation procedure, however, indicates the presence of only 0.66%,<sup>9</sup> a value which has been checked by Stewart and Rimington.<sup>10</sup>

Plimmer and Phillips developed their method for application to pure histidine solutions. They likewise applied it to gelatin, this protein being selected because it is lacking in tyrosine, tryptophane and cystine, amino acids which markedly interfere with the accuracy of the histidine determination. These investigators clearly state, however, that "further work will be necessary to show that the method is applicable to other proteins, and to ascertain the effect of the presence of cystine." Data at present available in this laboratory indicate that thyroglobulin yields a considerable proportion of cystine. It is therefore probable that the histidine methods employed by Eckstein might yield misleading results when applied to this protein.

The lysine figure calculated from the weight of isolated pure lysine picrate is considerably lower than that obtained by Eckstein using the Van Slyke method. The magnitude of this difference between the results of the 2 investigations is of the order generally observed in a comparison of results of the determination of this amino acid by the 2 analytical procedures. Expressed as per cent nitrogen of the total nitrogen, the lysine yielded by edestin and casein, for example, is 2.25%<sup>11</sup> and 7.72%<sup>6</sup> respectively, by the isolation technique; the corresponding values by Van Slyke's method are 3.86%<sup>12</sup> and 10.3%.<sup>13</sup>

---

<sup>7</sup> Plimmer, R. H. A., and Phillips, H., *Biochem. J.*, 1924, **18**, 312.

<sup>8</sup> Marston, H. R., Council Se. and Ind. Research, Commonwealth of Australia, 1928, Bull. 38.

<sup>9</sup> Vickery, H. B., and Block, R. J., *J. Biol. Chem.*, 1930, **86**, 107.

<sup>10</sup> Stewart, A. M., and Rimington, C., *Biochem. J.*, 1931, **25**, 2189.

<sup>11</sup> Vickery, H. B., and Leavenworth, C. S., *J. Biol. Chem.*, 1928, **76**, 707.

<sup>12</sup> Van Slyke, D. D., *J. Biol. Chem.*, 1911, **10**, 15.

<sup>13</sup> Van Slyke, D. D., *J. Biol. Chem.*, 1913, **16**, 531.

*Summary.* Analysis of the basic amino acids of thyroglobulin by direct isolation methods indicates that this protein yields 0.62% of histidine, 8.22% of arginine and 1.93% of lysine.

7678 P

### Quantitative Studies with the Friedman Test in Excessive Vomiting of Pregnancy.\*

FERDINAND J. SCHOENECK. (Introduced by Walter S. Root.)

*From the Department of Obstetrics, Syracuse University, College of Medicine.*

Using a modification of the technique of Friedman and Lapham,<sup>1</sup> involving intravenous injection of urine and the inspection of the ovaries after a period of 48 hours, an effort was made to determine the minimum amount of urine required to produce a positive reaction in cases of normal pregnancy. Observations were made between the fifth and the fourteenth weeks. All animals used were

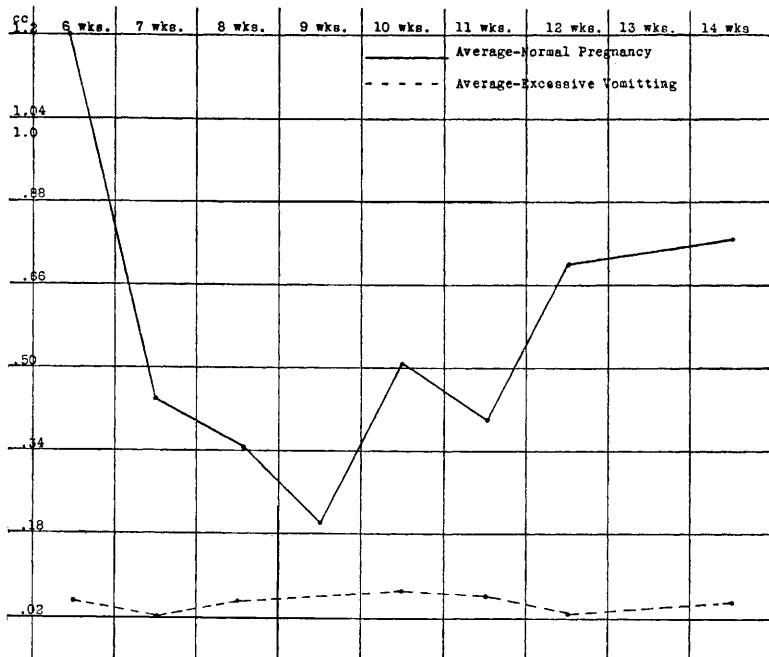


FIG. 1.

\* Aided by a grant from the Hendricks Research Fund.

<sup>1</sup> Friedman, M. H., and Lapham, M. E., *Am. J. Obst. and Gynec.*, 1931, **21**, 405.