

## THE ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM

IN HEALTH, SYPHILIS, PNEUMONIA, AND CERTAIN OTHER INFECTIONS,  
WITH THE BEARING OF GLOBULIN ON THE WASSERMANN  
REACTION \*

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In spite of numerous results found in literature, the albumin and globulin content of serum in health and disease has been reinvestigated by use of Robertson's<sup>1</sup> method, because of the simplicity and freedom from possible error of the new technic as compared with that of former methods. The small amount of serum used is another advantage and has made possible the use of two controls on each serum examined by the writer. The results which can be obtained are uniform, especially if the writer's suggestions about the technic, which will soon be published, are followed and his automatic pipet<sup>2</sup> is used to assure accuracy of measurements.

During the last seven months a number of normal serums have been examined. The average results differ slightly from those obtained by Robertson's method previously reported.<sup>3</sup> The total protein is lower than in the previous series, due to the fact that serums were taken, except in a few cases, from patients who were confined to bed as a result of a fracture or uncomplicated herniotomy, or from those who had been lying down for twenty-five or more minutes. The non-proteins are slightly higher, while the percentage of globulin in the total protein is moderately increased. The last four serums were taken from the same person at different times throughout a period of six months. The values are quite uniform (Table 3).

### LITERATURE

The literature contains many results for the normal value of serum proteins, most of which will be found in Table 1. Several of the average values were taken directly from the articles by Erben.<sup>4</sup> Com-

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\* From the Medical Service and Chemical Laboratory of the Massachusetts General Hospital.

1. Robertson, T. B.: *Jour. Biol. Chem.*, 1915, xxii, 233.

2. Rowe, A. H.: *Jour. Lab. and Clin. Med.*, 1916, i, 439.

3. Tranter and Rowe: *Jour. Am. Med. Assn.*, 1915, lxxv, 1433.

4. Erben, F.: *Zetchr. f. klin. Med.*, 1903, 1, 450; *ibid.*, 1900, xl, 266, 282; *ibid.*, 1902, xlvi, 302; *Ztschr. f. Heilk.*, 1905, xxvi, 245, 303, 449.

TABLE 1.—NORMAL VALUE OF SERUM PROTEINS AS FOUND IN THE LITERATURE

Investigator	Where Found	Date	Percentage of Total Protein
Berzelius.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1831	8
Marcett.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1831	8.7
Denis.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1838	8
Lecanu.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1837	7.8 to 8.1
Bostok.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1842	10
Nasse.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1836	7.2 to 9
Becquerel and Rodier.....	Gaz. méd. de Paris, 1844, Nos. 47, 48, 49, 50, 51; <i>Chemie pathologique</i> , 1854; <i>Untersuchungen über de Zusammensetzung des Blutes in gesunden und Kranken Zustande</i> , übersetzt von Eisenmann, Arlangen, 1845	1845	8
Otto.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1848	7.5 to 8
C. Schmidt.....	Charakteristik der epidem. Cholera, Leipzig, 1850, p. 30; reference from Joachim	1850	7.4 to 8.3
Leven.....	Malay's Jaresbericht, 1873, i, 115.....	1873	7.9
Hammarsten.....	Arch. f. d. ges. Physiol., 1878, xvii, 413; <i>Ergebn. d. Physiol.</i> , 1902, i, 348; <i>Lehrbuch der physiologischen Chemie</i> , 1914	1878	7 to 8.1
Hoffman.....	Arch. f. exper. Path., 1882, xvi, 133.....	1882	7.4 to 7.8
Limbeck and Pick.....	Prag. med. Wehnschr., 1893, xviii, 21, 133, 149, 165; <i>Deutsch. med. Wehnschr.</i> , xx, 563. Limbeck; <i>Grundriss einer klin. Path. d. Blutes</i> , Ed. 2., Jena, S. Fischer, 1896, p. 100	1893	6.5 to 7.4
Von Jaksch.....	Ztschr. f. klin. Med., 1893, xxiii, 187.....	1893	6 to 8.9
Lewinsky.....	Arch. f. d. ges. Physiol., 1903, c, 611.....	1903	6.7 to 7.6
Erben.....	Erben: Ztschr. f. klin. Med., 1903, l, 450; <i>ibid.</i> , 1900, xl, 266, 282; <i>ibid.</i> , 1912, xlvii, 302; Ztschr. f. Heilk., 1905, xxvi, 245, 303, 449	1905	8.5
Refractometric determinations by Reiss and others	<i>Ergbn. d. inn. Med. u. Kinderh.</i> , 1913, x, 531.....	1902 1912	7 to 9
Winternitz.....	Arch. f. Dermat. u. Syph., 1908, xciii, 65; <i>ibid.</i> , 1910, ci, 227	1908	8.2 to 8.7
Epstein.....	<i>Jour. Exper. Med.</i> , 1912, xvi, 719; <i>ibid.</i> , 1913, xvii, 444; <i>ibid.</i> , 1914, xx, 334	1912 1913	6.4 to 8.3
Tranter and Rowe.....	<i>Jour. Am. Med. Assn.</i> , 1915, lxxv, 1433.....	1915	6.7 to 8.7
Rowe.....	This article .....	1916	6.5 to 8.2

ment on these values from the literature will be found in the last portion of this review of the literature.

The best reviews of the literature on serum proteins in health and disease occur in the articles by Limbeck and Pick,<sup>5</sup> Joachim,<sup>6</sup> Von Jaksch,<sup>7</sup> Langstein and Mayer,<sup>8</sup> Erben,<sup>4</sup> and Winternitz.<sup>9</sup> Morawitz<sup>10</sup> discusses the methods for determining albumin and globulin and the results obtained up to 1908 in human serum, concluding that little of importance to pathology and physiology has come from the extensive work in this field. This opinion is due, probably, to the disparity of many of the former results.

The monograph of Becquerel and Rodier<sup>11</sup> is remarkable considering the early date of its production. It covers the chemistry of the blood in health and disease in a very thorough manner. In chlorosis and plethora it was interesting to note that the total proteins were normal, while in nephritis and cardiac disease with edema, endocarditis, typhoid, acute rheumatic fever, pneumonia, secondary anemias and pulmonary tuberculosis they were decreased.

C. Schmidt<sup>12</sup> found serum proteins increased in severe cholera and decreased in nephritis.

Hoppe-Seyler<sup>13</sup> found the serum proteins decreased in a woman with chyluria.

Leven<sup>14</sup> obtained 7.67 per cent. of protein in the blood serum from a patient with scorbutus.

Estelle<sup>15</sup> determined the albumin and globulin content in the blood serum of two nephritics, in one the albumin being 5.44 per cent. and the globulin 3.06 per cent., while in the other the values were 3.6 and 1.8 per cent., respectively.

Csatary<sup>16</sup> found increased serum globulin in nephritis.

5. Limbeck and Pick: *Prag med. Wchnschr.*, 1893, xviii, 21, 133, 149, 165; *Deutsch. med. Wchnschr.*, 1894, xx, 563. Limbeck: *Grundriss einer klin. Path. d. Blutes*, Ed. 2, Jena, S. Fischer, 1896, p. 100.

6. Joachim: *Arch. f. d. ges. Physiol.*, 1893, 588; *Wien. klin. Wchnschr.*, 1902, xv, 565.

7. Von Jaksch: *Ztschr. f. klin. med.*, 1893, xxiii, 187.

8. Langstein and Mayer: *Beit. z. chem. Phys. u. Path.*, 1904, v, 69.

9. Winternitz, R.: *Arch. f. Dermat. u. Syph.*, 1908, xciii, 65; *ibid.*, 1910, ci, 227.

10. Morawitz: *Blood Proteins*, Oppenheimer's *Handbuch der Biochemie des Menschen und der Tiere*, 1909, ii, Part 2, 70.

11. Becquerel and Rodier: *Gaz. méd. de Paris*, 1844, Nos. 47, 48, 49, 50, 51; *Chemie pathologique*, 1854; *Untersuchungen über die Zusammensetzung des Blutes in gesunden und kranken Zustände*, übersetzt von Eisenmann, Erlangen, 1845.

12. Schmidt, C.: *Charakteristik der epidem. Cholera*, Leipzig, 1850, p. 30, reference from Joachim.

13. Hoppe-Seyler: *Ztschr. f. physiol. Chem.*, xv, 179.

14. Leven, M.: *Maly's Jahresbericht*, 1873, i, 115.

15. Estelle: *Revue mens. de méd. et de chir.*, 1880, iv, 704.

16. Csatary: *Arch. f. klin. Med.*, 1891, xlvi, 358.

Nya-Viglezio<sup>17</sup> found increased globulin in pneumonia, angina pectoris, tetanus, nephritis, diabetes, the values varying from 4.8 to 3.6 per cent. for albumin and from 3.5 to 3 per cent for globulin.

Von Jaksch,<sup>7</sup> using serum obtained by cupping, showed a dilution of blood in acute illnesses and diseases of the heart, lungs, and kidneys. In leukemia he found 7.6 per cent protein, in pernicious anemia 7.4 per cent., in chlorosis 8.25 per cent., while in secondary anemias the protein was slightly reduced.

TABLE 2.—NORMAL VALUES FROM LITERATURE FOR ALBUMIN AND GLOBULIN WITH THE PERCENTAGE OF GLOBULIN IN THE TOTAL PROTEIN

Investigator	Where Found	Comment	Albumin	Globulin	Percentage of Globulin
Hammarsten..	Arch. f. d. ges. Physiol., 1878, xvii, 413; Ergebn. d. Physiol., 1902, i, 348; Lehrbuch der physiologischen Chemie, 1914	Average values of 4 sera obtained by cupping*	4.34	3.22	43
Patein.....	Jour. pharm. Chemie, 1899, x, 249.....	.....	4.89 4.63	2.86 2.77	37 37
Nya-Viglezio...	Archivio italiano di clinica medica, 1888, xxvii; reference from Joachim; Revista Clinica, 1887, xxvi, 673	.....	5.72	2.43	30
Limbeck and Pick	Prag. med. Wehnschr., 1893, xviii, 21, 133, 149, 165; Deutsch. med. Wehnschr., 1894, xx, 563. Limbeck: Grundriss einte klin. Path. d. Blutes, 1896, 100	Nervous diseases (six cases)	4.8	1.7	26
Lewinski.....	Arch. f. d. ges. Physiol., 1903, c, 611.....	.....	3.9	2.96	43
Winternitz.....	Arch. f. Dermat. u. Syph., 1908, xciii, 65; <i>ibid.</i> , 1910, ci, 227	.....	6.2	2	24
Epstein.....	Jour. Exper. Med., 1912, xvi, 719; <i>ibid.</i> , 1913, xvii, 444; <i>ibid.</i> , 1914, xx, 334	Average values of 7 cases (varicocele, orchitis, fistula, hemorrhoids)	4.4	2.7	38
Epstein.....	.....	One normal case.....	5.09	3.07	38
Hoffmann.....	Arch. f. exper. Path., 1882, xvi, 133.....	.....	5.04	2.72	35

\* All other values obtained from venous blood serum.

Limbeck and Pick<sup>5</sup> showed an increased globulin content in the serum of nineteen cases of acute infection, though the total proteins were much reduced. Five diabetics showed increased globulin, while three cases gave low globulin. In nephritis thirteen cases showed a low total protein. The results of these writers were undoubtedly too low and were criticized by Bleibtreu.<sup>18</sup> The latter was at fault, though, in using as his normal values those obtained from blood taken post mortem.

17. Nya-Viglezio: Archivio italiano di clinica medica, 1888, xxvii; reference from Joachim, Revista clinica, 1887, xxvi, 673.

18. Bleibtreu: Deutsch. med. Wehnschr., 1893, xix, 1167.

E. Freund<sup>19</sup> found that the percentage of globulin in nephritis varied from 25 to 33 per cent., while in pernicious anemia it was 33 per cent.

Emmerich and Tsuboi<sup>20</sup> stated that the globulin in blood serum decreased to zero in animals immune to hog cholera and pneumonia. This statement was never confirmed.

Seng<sup>21</sup> found increased globulin in serum from animals immune to diphtheria.

Atkinson<sup>22</sup> showed that the antitoxic power of a serum was caused by the globulins.

V. Szontagh and Wellmann<sup>23</sup> found that with immunization the total proteins were slightly increased.

Butjagen<sup>24</sup> confirmed the former findings of V. Szontagh.

Joachim<sup>6</sup> in one article studied the protein of the blood serum, showing that globulin increased in many diseased conditions. In a second article the globulin content of ascitic fluids was determined and he also demonstrated that globulin increased at the cost of albumin when a horse was immunized to diphtheria, the albumin before immunization being 5 per cent. and afterwards dropping to 3.7 per cent.

Jakoby<sup>25</sup> found euglobulin increased in animals immunized to castor oil.

Moll<sup>26</sup> found in animals injected with various proteins, gelatin, and killed bacteria a marked increase in the globulin content and concluded that with an increase in immune bodies there goes an increase in globulin. He suggested that possibly some globulin arises from leukocytes, which suggestion first came from A. Schmidt.<sup>27</sup>

Glaessner<sup>28</sup> repeated the above work, showing a moderate increase in the globulins, but not to the extent found by Moll. He could demonstrate no constant relation between the globulin and immune body formation. He suggested that the globulin has a greater resistance to the proteolytic ferment trypsin than has albumin, to account for its relative increase in disease.

Langstein and Mayer<sup>8</sup> found the serum proteins increased in immunized and infected rabbits, the increase being largely in the glob-

19. Freund, E.: *Wien. klin. Rundschau*, 1895, ix, 49.

20. Emmerich and Tsuboi: *Verhandl. d. Cong. f. inn. Med.*, 1892, ii, 202.

21. Seng: *Ztschr. f. Hyg. u. Infekt.*, 1899, xxxi, 513.

22. Atkinson: *Jour. Exper. Med.*, 1900, v, 67.

23. V. Szontagh and Wellmann: *Deutsch. Med. Wehnschr.*, 1898, xxiv, 421.

24. Butjagen: *Hyg. Rundschau*, 1902, xii, 1193.

25. Jakoby: *Beitr. z. chem. Phys. u. Path.*, i, 59.

26. Moll: *Beit. z. chem. Phys. u. Path.*, 1903, iv, 563, 578.

27. Schmidt, A.: *Zur Blutlehre*, Leipzig, 1892. *Beiträger zur Blutlehre* Wiesbaden, 1895. Ref. from Hammarsten.

28. Glaessner: *Ztschr. f. exper. Path. u. Therap.*, 1905, ii, 154.

ulin fraction. They were unable to confirm the work of Emmerich and Tsuboi.

Müller<sup>29</sup> confirmed the findings of Langstein and Mayer.

TABLE 3.—ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM IN NORMAL CASES

Case No.	Age	Albumin	Globulin	Total Protein	Nonprotein	Percentage Globulin
1	27*	5.85	1.97	7.82	1.2	25
2	30	5.15	2.2	7.35	1.3	30
3	36*	5	2.3	7.3	1.3	32
4	21*	5.7	2	7.7	1.3	26
5	24*	6.4	1.2	7.6	1.1	16
6	30	5	2.4	7.4	1.2	32
7	32	5.8	2.2	8	1.1	27.5
8	48	5.8	2.1	7.9	1.2	27
9	19	6	2.2	8.2	1.2	27
10	25	5.7	2	7.7	1.3	26
11	48	5.1	2.2	7.3	1.2	30
12	28	4.8	2	6.8	1.3	29
13	23	5.6	1.8	7.4	1.2	24
14	19	4.6	1.9	6.5	1.25	29
15	48	4.6	2.1	6.7	1.25	31
16	25	5.9	1.6	7.5	1.3	21
17	26	5	1.7	6.7	1.3	25
18	29	5.4	1.4	6.8	1.3	21
19	3/18/16 26	6	1.5	7.5	1.3	20
20	1/26/16* 26	6.5	1.7	8.2	1.3	21
21	10/19/16* 26	6.7	1.5	8.2	1.3	18
22	10/11/16* 26	6	1.9	7.9	1.1	24
Averages.....		5.6	1.9	7.5	1.24	25.5
Former averages.....		6.2	1.74	7.94	1.1	22

\* These subjects had been walking about without doing any real work before blood was taken.

Lewinski<sup>30</sup> found the percentage of globulin a little higher in pregnant and a little lower in eclamptic women than in normal ones.

In a case of pernicious anemia Erben<sup>4</sup> found 5.2 per cent. total protein, of which 4.2 per cent. was albumin and 1 per cent. globulin.

29. Müller, Paul: Beitr. z. chem. Phys. u. Path., 1905, vi, 454.

30. Lewinski: Arch. f. d. ges. Physiol., 1903, c, 611.

In parenchymatous nephritis the albumin was reduced to below 1 per cent., but was not so reduced in amyloid. The globulin was slightly increased in pericarditis. In tuberculosis the globulin was moderately increased as compared with normal values of Erben. He offers the suggestion of Gottwalt<sup>31</sup> that the relative increase of globulin in infections is due to its greater resistance to the toxins. In a case of carcinoma a slight reduction in total protein of the serum occurred though the relation of albumin to globulin was normal. In diabetes the total protein was either slightly decreased or increased, which confirmed the findings in previous literature with the exception of those of Limbeck and Pick. In a case of chronic lead poisoning no definite changes in the serum protein occurred. In a case of typhoid no values for albumin and globulin were given.

The articles of Erben include extensive bibliographies on the entire chemistry of the blood. Normal values of all organic and inorganic substances investigated in the blood are given, together with a table of the average normal values from the literature before 1905.

Vadala,<sup>32</sup> in two cases of anemia from ankylostomiasis, obtained the average value of 7.8 per cent. for serum albumin and 2.4 per cent. for globulin.

Grenet,<sup>33</sup> Gilbert and Chiray<sup>34</sup> found serum proteins decreased in liver insufficiency with and without ascites.

Winternitz<sup>9</sup> found serum globulin moderately increased in syphilis.

Epstein<sup>35</sup> obtained a marked increase in globulin with a normal or very subnormal amount of total serum protein in cardiac diseases associated with decompensation and serous effusions, pulmonary and respiratory affections of inflammatory or noninflammatory origin (pneumonia, emphysema, polycythemia), diabetes mellitus and parenchymatous nephritis. Globulin was normal or decreased in achylia gastrica, tuberculosis, diabetes insipidus and chronic interstitial nephritis. In another paper he found localized infections of the kidney gave an increase in globulin, while the cases of prostatic hypertrophy and minor surgical cases gave no such increase.

The methods for determination of albumin and globulin in the past may be summarized as follows: (1) by diluting the serum ten times, precipitating out the globulin by adding salt, or obtaining the globulin by dialysis; (2) by precipitating the globulin by acetic or carbonic acid; (3) by salting out the globulin by neutral salts: (a) Ham-

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31. Gottwalt: (Reference given by Limbeck incorrect.) Correct reference, *Ztschr. f. phys. Chem.*, 1880, iv, 423, 427.

32. Vadala: *Clinica med. ital.*, 1907, vi; reference from *Biol. Centralbl.*, 1907, vi, No. 2480.

33. Grenet, H.: *Compt. rend. Soc. de biol.*, 1907, lxiii, 552.

34. Gilbert and Chiray: *Compt. rend. Soc. de biol.*, 1907, lxiii, 487.

35. Epstein: *Jour. Exper. Med.*, 1912, xvi, 719; *ibid.*, 1913, xvii, 444; *ibid.*, 1914, xx, 334.

mersten's method utilizes magnesium sulphate after which the precipitate is washed, dried and weighed, or the nitrogen in the precipitate is estimated by the Kjeldahl<sup>36</sup> method and the protein obtained by multi-

TABLE 4.—ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM IN CASES OF SYPHILIS \*

Case	Clinical Diagnosis	Wassermann	Treatment	Serum Examination				
				Albumin	Globulin	Total Protein	Non-protein	Globulin %
1	Chaneroid	Negative	None	6.4	1.2	7.6	1.1	16
2	Early secondary	Spirochetes demonstrated	None	5.3	2.3	7.6	1.1	30
3	Early malignant	+++	None	4.6	3.3	7.9	1.35	42
4	Early secondary	+++	None	5.4	2	7.4	1.3	27
5	Early secondary	+++	None	6.1	2.2	8.3	1.35	27
6	Tertiary	+++	Irregular for 2 yrs.	4.8	2.6	7.4	1.2	35
7	Early secondary	+++	2 Hg injections	5.8	2.2	8	1.3	27.5
8	Early secondary	+++	None	3.7	3.6	7.3	1.2	49
9	Tertiary	+++	2 doses salv.	4.7	2.6	7.3	1.25	36
10	Early secondary	+++	1 dose salv. and Hg pills	4.2	2.7	6.9	1.2	40
11	Tertiary	++	1 dose	4.6	2	6.6	1.2	30
12	Gastric ulcer (no evidence of lues)	Faintly +	None	5.7	1.2	6.9	1.1	17
13	Tertiary aneurism	+++	None	4.6	2.5	7.1	1.5	35
14	Secondary	+++	None	5.2	2.9	8.1	1.3	36
15	Late—6 yrs.	+	None	4.5	2.9	7.4	1.4	39
16	Early secondary	+++	1 dose salv. and Hg pills	5.5	2.4	7.9	1.4	30
17	Secondary	+++	Hg pills for 1 week	5.3	2.6	7.9	1.3	33
18	Late secondary	+++	Slight	5.4	1.9	7.3	1.4	26
19	Late secondary	+++	Salv. and Hg	5.6	2.5	8.1	1.4	31
Averages.....				5	2.5	7.5	1.3	33.7

\* Cases 1 and 12 were not used to obtain average values. All but three of these cases came from the South Medical department. Dr. C. Morton Smith furnished the diagnosis.

plying the nitrogen content by 6.25, as advised by König and Kisch.<sup>37</sup> The latter technic was described by Hoppe-Seyler.<sup>38</sup> (b) Hofmeister,<sup>39</sup> Pohl,<sup>40</sup> Kauder,<sup>41</sup> and Reye<sup>42</sup> used ammonium sulphate. Otherwise the method is the same as the former one.

36. Kjeldahl: Ztschr. f. anal. Chem., 1883, xxii, 366.

37. König and Kisch: Ztschr. f. anal. Chem., 1889, xxviii, 193.

38. Hoppe-Seyler: Handbuch der phys. u. path. chem. Analyse, 1893.

39. Hofmeister: Ztschr. f. phys. Chem., xx, 319.

40. Pohl: Arch. f. exper. Path. u. Pharmakol., 1886, xx, 426.

41. Kauder: Arch. f. exper. Path. u. Pharmakol., 1886, xx, 411.

42. Reye: Inaug. Diss., Strassburg, 1898.



There has been great divergence of opinion about the accuracy of these methods, well explained by Nellis Foster.<sup>43</sup> Howell<sup>44</sup> states that the precipitate of globulin by ammonium sulphate is unsatisfactory because of the changed properties of the globulin after precipitation. Robertson's method, in utilizing ammonium sulphate, thus carries with it some unavoidable objections. Still these objections are the same in all instances and thus deductions can safely be drawn from values obtained from pathologic cases. But in doing away with the evident sources of error arising from the complicated technic of the older methods and in utilizing small amounts of blood, Robertson's method is undoubtedly the most satisfactory one yet suggested. An experience of one year with the technic has shown me that uniform results are obtained, which, though they are much lower in globulin than former results, are thought to be dependable. The cause of this difference from former results will be investigated later.

The results given in literature for the total proteins have been more uniform than those for albumin and globulin. The refractometer was introduced by Strubell in 1900 to determine quantitatively the serum proteins, the method being perfected by Reiss in 1902. Since then many data dealing with total protein in human serum have been added to literature, the chief workers having been Achard, Ascher, Bohme, Chiray, Chajes, Engel, Koranyi, Bence, Luthje, Oppenheimer, Schwenker, Strauss, Widal and Vaucher. References to all this work done with the refractometer are given in the comprehensive article recently published by Reiss.<sup>45</sup> The results are in most points like those arrived at by former methods.

In 1913 Schorer<sup>46</sup> studied Reiss' refractometric method and concluded that the changed relation between albumin and globulin in disease gives rise to a definite error in estimating the total protein. This was because the refractive index of albumin is less than that of globulin as determined by Reiss, Robertson, and confirmed by Schorer. That the total protein as estimated by the Kjeldahl-Kolben method, or by precipitation by alcohol and weighing, agreed within a few tenths of 1 per cent. with the refractometric estimation was the result of work done by Reiss,<sup>47</sup> Widal and Laudat, and Tuffier and Maute.<sup>48</sup> Chiray and Démanche<sup>49</sup> and also Schorer<sup>50</sup> had been unable to obtain such

43. Foster, N.: *THE ARCHIVES INT. MED.*, 1912, x, 415.

44. Howell: *Am. Jour. Physiol.*, 1906, xvii, 280.

45. Reiss: *Ergebn. d. inn. Med. u. Kinderh.*, 1913, x, 531.

46. Schorer: *Cor.-Bl. f. schweiz Aerzte*, 1913, xlvii, 1523.

47. Reiss: *Ztschr. f. Electrochemie*, 1908, xiv, 613.

48. Tuffier and Maute: *Tribune med.*, Paris, 1905.

49. Chiray and Démanche: *Compt. rend. Soc. de biol.*, 1907, lxiii, 235.

50. Schorer: *Ueber refraktometrische Pepsin bestimmungen*, Diss., Bern, 1908, p. 22.

agreement of results, there being a difference of 1.8 per cent. in one of their results.

Using Reiss' table for the determination of total serum protein, Schorer shows plainly the source of error involved in the former's method.

The protein content of an assumed serum may be supposed to be 8 per cent. If Hammarsten's relation between albumin and globulin is used, then for one part of globulin there is one and a half parts of albumin and 8 per cent. protein would contain 3.2 per cent. globulin and 4.8 per cent albumin.

Refractive index of 1% albumin = .00184 or 4.8% = ...	0.00883
Refractive index of 1% globulin = .00230 or 3.2% = ...	0.00736
<hr/>	
Refractive index of total protein = .....	0.01619
Refractive index of distilled water = .....	1.33320
Refractive index of nonprotein = .....	0.00277
<hr/>	

Hence the refractive index of whole serum = ..... 1.35216

But the refractive index of a serum containing 8% protein according to Reiss' table = 1.3490. Error = 0.0026 or 1.4% protein.

If my average values given in this article for albumin, globulin and nonprotein are substituted for those of Reiss in the above calculations, one obtains an error of about 0.5 per cent. protein, if Reiss' table is used to determine the total protein directly from the refractive index. This error would increase as the globulin and nonproteins increased in disease.

Besides this error there is one due to the too large value for non-proteins adopted by Reiss. This has already been explained in a former paper.† The slight increase in the nonproteins found in the normals in this communication over the value in the former series of normals decreases the percentage of error to approximately 0.4 per cent. instead of 0.5 per cent., which results from the use of the constant value of 0.00277 for nonproteins as advised by Reiss.

It is evident, therefore, that there are two sources of error in the method of Reiss for the estimation of total protein in serum, both of which are removed by Robertson's method. In pathologic cases in which it is known that the nonproteins are not decidedly increased the average value for them given in this paper can be used. If this is done an error of 0.2 per cent. in the albumin content may occur. For clinical work such an error could be allowed, since the technic is greatly shortened if the nonproteins are not estimated in a given serum.

Reiss<sup>51</sup> in his last article acknowledges the correctness of Schorer's criticism, but says that his method is of use in forming a serum pro-

† Footnote 63.

51. Reiss: Deutsch. Arch. f. klin. Med., 1915, cvii, 175.

tein curve for a given patient, by estimating the protein each day, which yields valuable information, especially when combined with the curve of the body weight. He admits that his method is useless in uremia, because of the increase in nonproteins and globulins which occurs in this condition. With Robertson's method, on the contrary, it is entirely possible to make correct determinations of the total protein, albumin, globulin, and nonproteins of uremic serum. This has been done and the results will be published in a separate paper, in which the albumin and globulin content of serums in chronic diseases will be considered.

#### SYPHILIS

The serum proteins in syphilis have met with considerable attention. Jolles and Oppenheim<sup>52</sup> found no difference in the protein content of normal and syphilitic serum. Klausner<sup>53</sup> was able to demonstrate an increase in the globulin in syphilitic serum by obtaining an abnormal precipitate when distilled water was added to such a serum. Sachs and Altmann<sup>54</sup> thought the Wassermann reaction was due to increased globulin, obtaining their precipitate with weak alcohol. Elias, Neubauer, Porges and Salomon<sup>55</sup> thought the active substance causing the Wassermann reaction belonged to the globulins and that they were present in greater amount in syphilitic than in normal serums. Noguchi<sup>56</sup> found globulin was increased in syphilis and thought it ran so nearly parallel to the Wassermann test that he, for a time, suggested it as a test for syphilis. His estimations were made by direct weighing of the moist globulin precipitate after centrifugalization at five thousand revolutions per minute for a definite time. Gay and Fitzgerald<sup>57</sup> were unable to obtain as definite results as did Noguchi and had better success with the quantitative estimation of euglobulin as a test for syphilis, than with the entire globulin fraction. Muller and Hough<sup>58</sup> found that syphilitic serums showed an increase in the euglobulin, but could establish no relation between the increase in globulin and the Wassermann reaction. Winternitz,<sup>9</sup> by using the method of Hofmeister, Pohl, and Reye, found an increase in the euglobulins in a short series of syphilitics as well as an increase in the total globulins. Moreover, by using the refractometer he demonstrated an increase in the fibrinogen in syphilis. To estimate fibrinogen, he took the difference between the reading of the plasma obtained

52. Jolles and Oppenheim: *Ztschr. f. Heilk.*, 1903, xxiv, 105.

53. Klausner: *Wien. klin. Wchnschr.*, 1908, xxi, 214, 363.

54. Sachs and Altmann: *Berl. klin. Wchnschr.*, 1908, xiv, 522.

55. Elias, Neubauer, Porges and Salomon: *Wien, klin. Wchnschr.*, 1908, xxi, 748, 831 .

56. Noguchi: *Jour. Exper. Med.*, 1909, xi, 84.

57. Gay and Fitzgerald: *Boston Med. and Surg. Jour.*, 1909, clx, 157.

58. Muller and Hough: *Wien. klin. Wchnschr.*, 1911, xxiv, 167.

by the use of hirudin and the reading of the serum. This difference he showed by quantitative chemistry was almost entirely due to fibrinogen.

In the series of nineteen cases in Table 4 the average value for globulin is definitely increased over the normal value, while that for nonprotein is very slightly increased. In Case 1, in which the clinical diagnosis of the early lesion was chancre, the percentage of globulin was found to be normal and the nonspecific nature of the lesion was confirmed by three subsequently negative Wassermann reactions. Cases 3, 6, and 8 were particularly severe and their globulin values

TABLE 5.—ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM IN PNEUMONIA

Case No.	Date	Diagnosis and Day of Disease	Wassermann	Serum Examination				
				Albumin	Globulin	Total Protein	Non-protein	Globulin %
1	1/19	Pneumonia, third day.....	Neg.*	3.6	2.7	6.3	1.4	43
2	1/10	Pneumonia, chronic endocarditis	Neg.	...	...	...	...	..
2	1/17	.....	Wk.*	3.8	1.4	5.2	1.4	27
3	1/18	Pneumonia, eighth day.....	Neg.*	3.7	2.3	6	1.5	38
4	1/24	Bronchopneumonia, ninth	Wk.	3.1	2.5	5.6	1.5	45
	2/ 3	.....	Neg.	...	...	...	...	..
5	1/20	Pneumonia, fourth day.....	Mod.	...	...	...	...	..
	1/24	.....	Wk.*	3.9	2.4	6.3	1.6	38
6	1/28	Pneumonia unresolved with arthritis, thirtieth day	Neg.	3.6	3.5	7.1	1.2	49
7	1/28	Pneumonia, third day.....	Neg.	4.7	2	6.7	1.3	30
8	1/31	Pneumonia, delayed resolution tenth day	Neg.*	3.1	3.1	6.2	1.3	50
Averages.....				3.7	2.5	6.2	1.4	40

\* Wassermann reactions were done on a sample of the same serum used for estimation of serum proteins.

were found to be among the highest in the series. Unlike other infections considered in this paper, there was no definite decrease in the total proteins.

#### PNEUMONIA

The literature reviewed in the first part of this paper shows a general agreement over the fact that globulin is increased in pneumonia. Sandelowsky,<sup>59</sup> moreover, has pointed out that the total serum protein falls during the fever and rises gradually during convalescence. An

59. Sandelowsky: *Deutsch. Arch. f. klin. Med.*, 1909, xcvi, 445; *ibid.*, 1910, c, 324.

investigation of the cause of this fall convinced the latter that two types of cases existed:

First, we have those in which the weight remained stationary or increased during fever, decreasing after the fever, while the serum proteins decreased during the fever due to an increase in the water content of the body. Along with this increase in fluid there is a retention of salts, which keeps the osmotic pressure constant and helps to explain the diminished salt in the urine. It was found that only small amounts of salt were contained in the stool and sputum of pneumonic patients and that the supposition that pneumonic exudate contained more salt than normal was not true.

TABLE 6.—ALBUMIN AND GLOBULIN CONTENT OF HUMAN BLOOD SERUM IN INFECTIONS OTHER THAN SYPHILIS AND PNEUMONIA

Case No.	Diagnosis	Wassermann	Serum Examination				
			Albumin	Globulin	Total Protein	Non-protein	Globulin %
1	Lung abscess.....	Neg.*	4.2	3.9	8.1	1.2	48
2	Septic knee.....	Neg.	3.8	3.4	7.2	1.5	47
3	Pyelitis.....	Neg.	4.7	2.2	6.9	1.4	32
4	Infected compound fracture.....	Neg.	4.5	2.1	6.6	1.3	33
5	Recent empyema; acute nephritis....	Neg.	3.6	3.4	7	1.6	49
6	Acute mastoiditis; acute nephritis...	Neg.	3.1	2.1	5.2	1.5	40
7	Erythema nodosum.....	Weak+	4	4	8	1.45	50
8	Acute and chronic endocarditis; secondary anemia	Neg.	3.7	2.7	6.4	1.2	42
9	Malignant endocarditis.....	Neg.	3.8	3.4	7.2	1.5	47
10	Chronic endocarditis; acute arthritis	Neg.	5.7	3.1	8.8	1.3	35
11	Chronic sepsis.....	Neg.	4.4	2.7	7.1	1.3	38
12	Acute tonsillitis.....	Neg.	5.8	2.2	8	1.2	27.5
13	Chronic bronchitis.....	Neg.	5.7	2	7.7	1.3	26
14	Tuberculosis of pleura.....	Neg.	4.8	2.9	7.7	1.3	38
15	Typhoid, tenth day; Widal +.....	Neg.	5.4	1.9	7.3	1.3	26

\* Wassermann reaction was done on a sample of the serum used for estimation of serum proteins.

Second, we have those in which weight dropped during fever and rose afterwards, while the serum proteins were reduced during the febrile period. Here the consumption of tissues must be accepted as the main cause for the reduced proteins.

The cause for this water and salt retention was thought to be kidney damage, caused mainly by toxins and not caused by fever in itself. Sandelowsky in a later article showed that by producing fever artifi-

cially in dogs the serum proteins were reduced, due to an increase in water in the blood, which he considered possibly a biologic reaction provided against overheating of the body.

Achard, Touraine and Saint-Girons<sup>60</sup> found that in pneumonia, as well as in typhoid, paratyphoid, acute rheumatic fever, phlegmonous sore throat, and streptococcic septicemia, the serum concentration falls until the fever ceases, in some cases, especially in typhoid, to a point higher than normal, after which it drops to the normal level. The most pronounced decrease occurred with typhoid and pneumonia, while only a small decrease came in slight infections.

The results of the examinations in pneumonic serum, as given in Table 5, show a more definite increase in the percentage of globulin than syphilis. The total proteins are decreased moderately, due probably to edema, while the nonproteins are slightly increased. Comment on the accompanying Wassermann reactions will be made in the last section of this paper.

#### INFECTIONS OTHER THAN SYPHILIS AND PNEUMONIA

The summary of literature under pneumonia applies in large part to the infections to be considered in this section. Though no serum from scarlet fever was examined, it is interesting to include a note about the work of Oppenheimer and Reiss,<sup>61</sup> who showed that the serum proteins decreased, while the body weight increased, during the febrile period. A salt retention was found in this disease, as well as in pneumonia. An attempt was made to determine the presence of nephritis, before the usual signs and symptoms appeared, by following the serum proteins, but without any definite success. Kalser and Löwy<sup>62</sup> found that serum proteins increased after the febrile period, but since they followed no case during the entire febrile period, their conclusion that the serum in scarlet fever is not diluted during the febrile period is not justified.

Most of the cases in Table 6 were of rather medium severity or of considerable duration. It is seen that the total proteins are higher than in cases of pneumonia, though the percentage of globulin is as high. The nonproteins are slightly increased.

Case 5 was one of empyema with little fever and an acute nephritis, with edema confined to the groins. The blood serum is not diluted, though the globulins are markedly increased. If these results are compared to those in Case 6, which was similar except that general edema was present, due to greater kidney damage, as shown by the high

60. Achard, Touraine, and Saint-Girons: *Arch. de med. expér. et d'anat. path.*, 1912, xxiv, 647.

61. Oppenheimer and Reiss: *Deutsch. Arch. f. klin. Med.*, 1909, xcvi, 464.

62. Kalser and Löwy: *Deutsch. Arch. f. klin. Med.*, 1914, cxiv, 82.

TABLE 7.—EFFECT OF THE INCREASE OF GLOBULIN BY VENOUS STASIS ON THE WASSERMANN REACTION

Case No.	Diagnosis	Normal Serum					Serum after Venous Stasis					
		Albumin	Globulin	Total Protein	Nonprotein	Percentage Globulin	Albumin	Globulin	Total Protein	Nonprotein	Percentage Globulin	Wassermann
1	Obstructive jaundice.....	3.8	2.5	6.3	1.25	40	4.7	3.4	8.1	1.3	42	Mod.*
2	Enlarged inguinal glands ?	4.7	2.4	7.1	1.35	34	7	3.4	10.4	1.45	33	Neg.†
3	Septic knee.....	3.8	3.4	7.2	1.5	47	5.2	4.2	9.4	1.5	45	Neg.*
4	Aortitis (syphilitic ?); arteriosclerosis; bronchopneumonia; secondary anemia	4.4	3.7	8.1	1.3	46	5	4	9	1.3	44	Neg.*
5	Normal.....	5.4	1.7	7.1	1.2	24	5.8	2.1	7.9	1.2	27	Neg.*
6	Chronic nephritis.....	3.9	2.4	6.3	1.8	38	5.3	3.4	8.7	1.8	39	Neg.*

\*Wassermann reactions were done on a sample of the same serum used for estimation of serum proteins.

† Five days before this examination Wassermann was weak positive. Patient was given mercury and iodid after that time.

value for nonproteins as well as a high nonprotein nitrogen, the effect of increased fluids in the blood is well demonstrated.

Case 12 was one of ordinary tonsillitis of about three days' duration. The values are normal. Case 14 shows a moderate increase in the percentage of globulin, which agrees with the findings of Erben referred to in the first part of this paper. Because of the season of year, only one typhoid serum was examined. The low globulin content is interesting and, if confirmed, will suggest the possibility that increased leukocytes have something to do with increased globulin, as suggested by A. Schmidt, to which suggestion Hammarsten called attention, or that the globulin has a greater resistance to destruction by toxins than has albumin, as suggested by Gottwald.<sup>63</sup>

#### RELATION OF SERUM GLOBULINS TO THE WASSERMANN REACTION

Literature, as reviewed in the section on syphilis, shows the association that serum globulin has been assumed to have with the Wassermann reaction. But attempts to use the globulin content as a test for syphilis have proved fruitless.

The data which are given in Tables 7 and 8 emphasize the fact that the Wassermann reaction does not depend on a quantitative increase in the serum globulins.

In Tables 4, 5 and 6 the results of the Wassermann reaction, as done in Dr. Wright's laboratory at the Massachusetts General Hospital, are given. The main point of interest is that serums showing a strongly positive test contained on the average considerably less globulin in relation to the total protein than did some showing a negative reaction.

In Tables 5 and 6 several weakly positive Wassermann reactions occurred without any history or any manifestations of syphilis. They were probably examples of spurious reactions, which are known to occur in severe infections. In Case 4 of Table 5 the weak positive became negative during convalescence.

In a recent article the writer<sup>63</sup> has shown that with venous stasis the serum globulin and albumin are proportionately increased. In Table 7 venous stasis has been used to determine whether an actual increase in the globulin content of a serum would increase or cause a positive Wassermann reaction. Wassermann reactions were done on samples of the same serums used in determining the serum proteins. The results show that an absolute increase of 1 per cent. of globulin does not change the results of the fixation test.

Minot and Sellards<sup>64</sup> have shown that negative serums exert an antagonistic action on the Wassermann reaction. The amount of this

63. Rowe, A. H.: *Jour. Lab. and Clin. Med.*, 1916, i, 485.

64. Minot and Sellards: *Jour. Med. Research*, 1916, xxxiv, 131.



antagonistic reaction can be determined by combining a definite amount of the negative serum with the minimal amount of a positive serum which causes complete fixation, after which a Wassermann test is done. When no antagonistic action is present in the negative serum, no hemolysis occurs, and when such an action is present, hemolysis takes place, up to an absolutely complete degree when the inhibitory power of the negative serum is great. The possibility of studying

TABLE 8.—RELATION OF GLOBULIN TO THE INHIBITORY POWER OF SERUMS TOWARD THE WASSERMANN REACTION \*

Case No.	Diagnosis	Globulin	Percentage Globulin	Hemolysis
1	Syphilis.....	1.4	18	0
2	Syphilis.....	2.3	34	0
3	Late syphilis.....	4.8	45	0
4	Syphilis.....	2.1	27	0
5	Chronic bronchitis (history of 5 miscarriages)	1.9	29	0
6	Endocarditis; arthritis.....	3.1	35	0
7	Cholecystitis; syphilis (?).....	2.3	33	T
8	Colloid goiter.....	1.8	28	T
9	Goiter hyperthyroidism.....	1.1	16	T
Averages.....		2.3	29.4	...
10	Chronic cholecystitis.....	2.5	35	P
11	Normal.....	1.9	29	P
12	Diabetes.....	2.3	29	P
13	Phthisis; pleurisy.....	2.9	38	AC
14	Cardiac.....	1.9	30	AC
15	Goiter; hyperthyroidism.....	2	27	C+++
Averages.....		2.25	31.3	...

\* All serums in this table were negative to the routine Wassermann reaction. In this table 0 signifies no hemolysis; T, trace of hemolysis; P, partial hemolysis; AC, almost complete hemolysis; C, complete hemolysis.

doubtful negative reactions with this method is evident. Friedemann<sup>65</sup> was able to obtain a positive Wassermann reaction with normal serum globulin, while normal serum albumin prevented fixation. He thought that in normal serum the albumin protects against fixation, while in syphilitic serum the albumin has no such power. Zinsser,<sup>66</sup> in discussing the Wassermann reaction, reviews the work of Schmidt, who

65. Friedemann: *Ztschr. f. Hyg. u. Infektionskrankh.*, 1910, lxvii, 279.

66. Zinsser: *Harvey Lectures, 1914-1915*, 178: *Infection and Resistance*, 1915.

states that globulin in syphilitic serum is increased quantitatively and is changed qualitatively and that it probably unites directly with the extract colloids in the antigen of the Wassermann test.

The writer, at the suggestion of Drs. Minot and Sellards, has estimated the globulin in serums on which they had tested the inhibitory power toward the Wassermann reaction. The minimal amount of a positive serum (0.005 c.c. in these cases) which caused complete fixation was added to 0.1 c.c. of each serum tested and a Wassermann reaction was done on each specimen with the results shown in Table 8. It was hoped that a diminution in globulin would occur in serums which inhibited the Wassermann reaction, whereas a high globulin content would be found in those in which no inhibitory power was present.

The results in Table 8 show an average value of 29.4 per cent. for the globulin in serums causing a trace or no hemolysis, while the average of the values in serums with partial, almost complete, and complete hemolysis is slightly higher, being 31.3 per cent. More estimations on serums causing complete hemolysis must be made before any definite conclusions can be drawn as to whether all serums showing complete hemolysis will have low globulin content.

The data given in the last section of this article lead to the conclusion that the Wassermann reaction is not due to a quantitative increase in the serum globulins.

#### SUMMARY

A series of normal cases shows that serum albumin varies between 4.6 and 6.7 per cent., that globulin varies between 1.2 and 2.3 per cent., that total proteins vary between 6.5 and 8.2 per cent., that nonproteins vary between 1.1 and 1.3 per cent., while the percentage of globulin in the total protein varies from 16 to 32 per cent. The average value for albumin was found to be 5.6 per cent. for globulin, 1.9 per cent., for total protein 7.5 per cent., for nonprotein 1.24 per cent. and for globulin 25.5 per cent.

The normal values for total protein, albumin and globulin from the entire literature are given, while the literature on these proteins in health and disease as well as on methods of their estimations is summarized.

The former use of the refractometer in medical research is discussed, while the error in Reiss' method for the determination of total proteins is pointed out. It is shown that Robertson's microrefractometric method for the determination of total protein is free from evident error and for the estimation of albumin and globulin is the most satisfactory method yet proposed.

In syphilis the globulin is definitely increased, while the total protein remains about normal.

In pneumonia the globulin is increased more in relation to the total protein than in syphilis, while the total protein is reduced, due, probably, in large measure to a dilution of blood serum by water retention, which occurs in fever.

In many chronic septic conditions, in mild infections and typhoid, the total protein is not decreased, as it is in pneumonia. Globulin seems definitely increased in all infections, except in acute tonsillitis, typhoid, and in certain mild infections, such as chronic bronchitis. The marked dilution of serum which occurs with anasarca is shown in two cases of acute infection associated with acute nephritis, which were investigated.

The evidence presented shows that the Wassermann reaction is not due to a quantitative increase in the serum globulins.

I wish to express my sincere gratitude to Drs. Edsall and Dennis for advice given during this research. I also appreciate the opportunity of using the ward cases on the services of Drs. Lee, Lord, Pratt, and William Smith, and the cases in the outpatient departments of Drs. Richard Cabot and C. Morton Smith.