heated suspension was even more active than the unheated. Plasma diluted to the same extent as were the leucocytes in washing—namely, 1:200,000—had no reactivating effect; in an amount 40 times as great, a 1:5000 dilution, it partially restored the activity of ammonia-treated serum; 50% hemolysis was obtained in 2 hours at 37°C. Heated serum in a 1:400 dilution reactivated to about the same degree as the unheated leucocyte suspension; the limit of its activity was reached in about a 1:6400 dilution.

After heating for one-half hour at 55°C., the ammonia-inactivated complement was not reactivated by the leucocyte suspension; the latter, in 4 times the amount used in the other tests, had no hemolytic action alone.

Thus the leucocytes contain or convey complementary substances essential to the hemolytic activity of ammonia-treated serum. Further study may determine more clearly whether the leucocytes are active *per se,* or through the absorption of minute quantities of serum or plasma.

8174 C

Some Analyses of Thyroglobulin.

ABRAHAM WHITE.

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

In view of the physiological importance of thyroglobulin, it is somewhat surprising that little information has been made available concerning its amino acid composition. Until recently, the investigation of Eckstein[1] represented the only study, by more recent methods of protein analysis, of the distribution of the more important amino acids of thyroglobulin. The basic amino acids yielded on hydrolysis of this protein have been recently determined in this laboratory[2] by isolation procedures, and the results compared with those of Eckstein. Additional data have now been obtained regarding some of the other amino acids of thyroglobulin and are being presented inasmuch as further chemical investigation of this protein is not contemplated at the present time. A portion of these analyses supplement information already available in the literature;

1 Eckstein, H. C., *J. Biol. Chem.*, 1926, **67**, 601.

2 White, A., and Gordon, W. G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 354.

others represent the first determinations of certain of the amino acids of thyroglobulin.

The preparation used for this analytical work has been previously described.[2] Glutamic acid and aspartic acid were determined by the isolation procedure of Jones and Moeller.[3] The tyrosine and tryptophane determinations were conducted as described by Folin and Ciocalteau.[4] Proline was determined by utilizing the fractionation technique recently described by Jukes.[5] The percentage of proline is calculated from the weight of the analytically pure cadmium chloride salt, isolated as recommended by Kapfhammer and Eck.[6] Cystine analyses were conducted by 2 methods, the colorimetric procedure of Folin and Marenzi,[7] and the cysteine cuprous mercaptide method of Vickery and White.[8] Cystine values found by the 2 methods are in good agreement. The results of the various determinations, expressed as percentages of the ash- and moisture-free protein, are presented in Table I. Results already reported[2] are also included to provide the complete data which have been obtained for this protein in this laboratory.

TABLE I.
Some Analyses of Thyroglobulin.

	Analysis I %	Analysis II %	Average %	Results of Eckstein[1] %
Total Nitrogen			15.58	
" Sulfur			1.46	
" Iodine			0.75	
Glutamic Acid	6.61	6.52	6.56	
Aspartic "	1.76	1.43	1.59	
Tyrosine	3.15	3.18	3.17	5.45
Tryptophane	1.72	1.88	1.80	2.15
Cystine				1.55
Folin-Marenzi method	2.06	2.09	2.07	
Vickery-White "	2.00	2.05	2.03	
Proline	4.34	4.61	4.47	
Histidine			0.62	
Arginine			8.22	
Lysine			1.93	

The cystine, tyrosine and tryptophane values found in the present investigation do not agree well with those reported by Eckstein.[1] However, the latter investigator employed the methods described

[3] Jones, D. B., and Moeller, O., *J. Biol. Chem.*, 1928, **79**, 429.

[4] Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627.

[5] Jukes, T. H., *J. Biol. Chem.*, 1933, **103**, 425.

[6] Kapfhammer, J., and Eck, R., *Z. physiol. Chem.*, 1927, **170**, 294.

[7] Folin, O., and Marenzi, A. D., *J. Biol. Chem.*, 1929, **83**, 103.

[8] Vickery, H. B., and White, A., *J. Biol. Chem.*, 1932-33, **99**, 701.

earlier by Folin and Looney;[9] recent improvements in the procedures of estimating these amino acids have frequently given results which are at variance with those obtained by the original technique of Folin and Looney.† The glutamic acid, aspartic acid and proline values are, so far as it has been possible to determine from the literature, the first recorded quantitative determinations of the amounts of these amino acids yielded by thyroglobulin.

8175 P

Experimental Rabies in White Mice. Studies on Passive Immunization I.*

ANSON HOYT, ROY T. FISK, AND FREDERICK J. MOORE. (Introduced by John F. Kessel.)

From the Department of Bacteriology, School of Medicine, University of Southern California and the Los Angeles County Hospital, Los Angeles.

The subject of passive immunization against rabies has received relatively little attention, even though Fermi,[1] Pfeiler[2] and others have reported striking experimental demonstrations of the efficacy of anti-rabic serums. In the current report, which summarizes 12 protection tests performed in the past 2 years, the protective properties of such serums have been studied using white mice as experimental animals.

A fixed virus strain, obtained through the courtesy of the Cutter Laboratories, was passed through rabbits and preserved at ice-box temperature, either fresh or in 50% glycerine, for periods which did not exceed 2 weeks. Details of preparation varied, but in effect the virus was ground to an initial dilution of 1/20 in normal saline and centrifuged 10 minutes at 2200 R.P.M. The supernatant fluid was then filtered through "Whatman No. 1" paper and final dilutions of from 1/100 to 1/800 were made. The virus was injected intracerebrally, at first in amounts proportional to the body weights of the mice (0.005 cc./gm. or 0.001 cc./gm.), later in a constant dose of 0.02 cc.

The serums were prepared by hyperimmunizing rabbits and goats

[9] Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, **51**, 421.

† For examples and amplification of this statement, see references 4 and 7.

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[1] Fermi, C., *Cent. f. Bakt., Parasit. u. Infektskr.*, Orig., 1909, **52**, 576.

[2] Pfeiler, W., *Berliner Tierarzt. Wochenschr.*, 1913, **29**, 269.