THE DETERMINATION OF SULFHYDRYL GROUPS IN SERUM*

I. METHODS AND RESULTS ON NORMAL SERA

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Compounds containing sulfhydryl groups have occupied a unique position in metabolic and chemical studies because of their intimate association with the problems of protein structure (1), enzyme function (2), and cellular proliferation (3). Kolthoff and Harris (4) described an amperometric method for the determination of mercaptans which Benesch and Benesch adapted for use with amino acids and proteins (5). We have employed this method to investigate the sulfhydryl content of normal human serum, serum albumin, and serum globulin.

The details of the methods and results obtained on sera secured from normal individuals are presented at this time.

EXPERIMENTAL

Experience with the method of Benesch and Benesch (5) showed that revisions of the procedure were necessary to insure reproducibility of results. Other changes were made to permit the simultaneous determination of sulfhydryl and protein content (by the biuret and Kjeldahl methods) in the fractions derived from serum by the Pillemer and Hutchinson method (6). Because the platinum electrodes may respond erratically after varying periods of use, the electrode is standardized daily by means of a stock solution of a known mercaptan. Although cysteine hydrochloride was originally employed, we now prefer n-dodecyl mercaptan because of its greater stability.

Reagents—

n-Dodecyl mercaptan.1 A stock standard is made by dissolving approximately 75 mg. in 10 ml. of absolute ethanol. 1 ml. of the stock solution

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1 We wish to thank Dr. P. Tawney of the United States Rubber Company for the gift of n-dodecyl and n-tetradecyl mercaptans. n-Dodecyl mercaptan may be
is diluted to 25 ml. with absolute ethanol for use as the working standard. This solution contains approximately 0.00150 mM of SH per ml. and will require less than 2 ml. of 0.001 N silver nitrate for standardization.

0.001 N silver nitrate. A liter of 0.1 N silver nitrate solution is prepared from Mallinckrodt silver nitrate, analytic reagent. The silver nitrate is standardized against recrystallized sodium chloride (7). A liter of 0.001 N is made by dilution and stored in the dark reservoir of an automatic burette.

Supporting electrolyte. 30 gm. of ammonium nitrate are added to a volumetric flask containing 125 ml. of concentrated ammonium hydroxide and 100 ml. of water. The solution is brought to room temperature and made up to 250 ml.

Dilute methanol. 416 ml. of absolute methanol are diluted to 2 liters with distilled water and stored in a dispensing burette reservoir.

Biuret reagents. (a) A 1 per cent solution of copper sulfate pentahydrate; (b) a 5 per cent solution of sodium potassium tartrate in 5 per cent sodium hydroxide.

Apparatus—The titration assembly consists of the following elements: (a) a General Electric portable type galvanometer, catalogue No. 32-C-226-G-7, whose sensitivity is 0.012 μA per scale division; (b) a mercury reference electrode and rotating platinum electrode (4); (c) a 2 foot length of Tygon tubing, 7 mm. outside diameter, filled with saturated potassium chloride solution. An 8 cm. length of 7 mm. glass tubing serves as the dipping end of the salt bridge. A tightly rolled coil of Whatman No. 1 filter paper is inserted into the open end of the bridge. This plug is replaced daily; (d) a reduction gear box, electrode holder, and drive shaft taken from a stirring apparatus (Arthur H. Thomas (1931), catalogue No. 9238). We have fitted the drive shaft with a rubber friction ring and used a constant speed alternating current motor with a friction plate to drive the stirrer. This is a Redmond, type T model No. 4028, 60 cycle, 110 volt motor. The motor is mounted on a base plate which can be moved laterally by rotating a knurled knob. This alters the position of the rubber drive ring on the friction drive plate mounted on the motor shaft, permitting a wide choice of speeds at constant torque. The rate of electrode revolution is not critical, provided the rate does not vary during a titration. We find that approximately 130 r.p.m. is a suitable rate; (e) an automatic 2 ml. burette (Scientific Glass, catalogue GG6-42, No. M570), with the reservoir painted black.

secured from the Paragon Division, Matheson Company, East Rutherford, New Jersey. It should be distilled in vacuo before use.
Methods

Electrode Standardization—1 ml. of dilute working standard is pipetted into a 100 ml. beaker containing 29 ml. of absolute ethanol and 1 ml. of supporting electrolyte. The electrode is started and the bridge opened. After a few minutes, the galvanometer comes to rest at its zero point, and the addition of silver nitrate is begun. After each addition of silver nitrate, one waits a few seconds for the galvanometer to register a steady state before taking the reading. This is important, since the first sign of poor electrode response is an aimless drift of the galvanometer. Fig. 1 shows typical standardization curves for n-dodecyl mercaptan in 90 per cent ethanol and methanol.

Serum Titrations—Serum and serum fractions are titrated in dilute methanol in the same manner as the standard. 1 ml. of serum or the appropriate amount of serum fraction (see below) is added to a mixture of dilute methanol and supporting electrolyte until the final volume is 31 ml. in all cases. The titration is then carried out as above (Fig. 2). After each titration, the electrode and salt bridge are rinsed with 0.14 M saline followed by distilled water, and blotted dry with filter paper.

![Figure 1](http://www.jbc.org/)
Preparation of Serum—25 to 30 ml. of blood from a fasting subject are drawn into dry tubes and allowed to clot at room temperature for 45 minutes. While the blood is clotting, the daily check of the electrode is carried out. The clots are rimmed with a glass rod and the tubes are centrifuged. The serum is poured into clean centrifuge tubes and again centrifuged for 5 minutes to remove any remaining cells. This procedure yields from 10 to 15 ml. of clear, non-hemolyzed serum. 6 ml. of serum are required for the serum fractionation, sulfhydryl, Kjeldahl, and biuret procedures. The remainder may be frozen and stored in a dry ice cabinet for electrophoretic, stability, and other studies.

The 6 ml. of serum are distributed as follows: Slightly more than 2 ml. are placed in a tube in a cold room at -1° for fractionation. While this sample is cooling, 1 ml. of serum is diluted to 25 ml. in a volumetric flask for use in the Kjeldahl and biuret determinations. Two 1 ml. samples are titrated for the determination of sulfhydryl in the whole serum.

Fractionation Procedure—2 ml. of serum which have been at -1° for at least 45 minutes are pipetted into a 15 ml. conical, graduated centrifuge
tube. The fractionation is conducted according to the method of Pillemer and Hutchinson (6) up to and including centrifugation at 3000 r.p.m. Following centrifugation, the albumin supernatant is carefully decanted into a graduated centrifuge tube and allowed to drain completely. The volume of this supernatant solution varies from 8.5 to 9.2 ml. Specimens from normal individuals have a range of 8.9 to 9.2 ml. Water is added to bring the volume to 14 ml. and the solution is well mixed, stoppered, and kept at $-1^\circ$. The solution is brought out temporarily to room temperature for the removal of aliquots for the Kjeldahl, sulfhydryl, and biuret determinations.

The globulin precipitate, which has been drained as dry as possible, is dissolved by the addition of sufficient 0.14 M sodium chloride so that the final volume is 3 ml. In the event of an unusually large globulin fraction, it is brought to 4 ml. The globulin does not go completely into solution, maintaining a cloudy appearance. It should be well stirred before withdrawing aliquots for analysis.

Following the completion of the fractionation, aliquots are removed for Kjeldahl determination. Two 0.5 ml. samples of albumin and two 0.2 ml. samples of globulin are taken. 1 ml. and 0.2 ml. Mohr pipettes graduated in 0.01 ml. divisions are used. At the same time, two 1 ml. samples of diluted whole serum (see "Preparation of serum") are taken.

While the Kjeldahl samples are digesting, the serum fractions are titrated for sulfhydryl. The albumin is determined by adding 4 ml. of the 14 ml. supernatant to a beaker containing 26 ml. of dilute methanol and 1 ml. of supporting electrolyte. For the globulin determination, 1 ml. aliquots are used if the final volume was 3 ml. or 1.5 ml. if the final volume was 4 ml. These are added to 29 ml. of dilute methanol plus 1 ml. of electrolyte with the electrode stirring. At this point, the globulin generally forms a clear solution. Both fractions are titrated in duplicate. The samples are kept at $-1^\circ$ between runs.

Following the sulfhydryl titrations, the biuret determinations are made. Duplicate samples are prepared as follows: 1 ml. of albumin and 7.0 ml. of water; 0.2 ml. of globulin and 7.8 ml. of water; 2.0 ml. of dilute serum and 6.0 ml. of water.

To these tubes are added, with rotary agitation, 1 ml. of the alkali and 1 ml. of copper sulfate. After standing at least 5 minutes, they are read at 560 $\lambda$ in a Coleman model No. 14 spectrophotometer, with round cuvettes having a light path of 15.7 mm.

Calculations—1 ml. of 0.001 N silver nitrate is equivalent to 1 $\mu$m (micromole) of SH. The results to be presented are given in terms of micromoles of SH per 100 ml. of serum or micromoles per gm. of nitrogen.
This method of presentation is independent of the sulfhydryl compound used as a standard and does not arbitrarily assign the sulfhydryl group to the cysteine molecule.

Total serum sulfhydryl is given by \((a)\).

\[
(a) \quad \text{Ml.} 0.001 \text{ N AgNO}_3 \times \frac{100}{\text{Ml. serum}} = \mu\text{M SH per 100 ml. serum}
\]

Albumin sulfhydryl is given by \((b)\).

\[
(b) \quad \text{Ml.} 0.001 \text{ N AgNO}_3 \times \frac{14}{4} \times \frac{100}{2} = \mu\text{M albumin SH per 100 ml. serum}
\]

Globulin sulfhydryl is given by \((c)\) or \((c')\).

\[
(c) \quad \text{Ml.} 0.001 \text{ N AgNO}_3 \times \frac{3}{1} \times \frac{100}{2} = \mu\text{M globulin SH per 100 ml. serum}
\]

\[
(c') \quad \text{Ml.} 0.001 \text{ N AgNO}_3 \times \frac{4}{1.5} \times \frac{100}{2} = \mu\text{M globulin SH per 100 ml. serum}
\]

The biuret values are

\[
(d) \quad D \times \frac{25}{2} \times \frac{100}{1} = \frac{D}{100 \text{ ml. serum}}
\]

\[
(e) \quad \text{D albumin} \times \frac{14}{2} \times \frac{100}{1} = \frac{\text{D albumin}}{100 \text{ ml. serum}}
\]

\[
(f) \quad \text{D globulin} \times \frac{3}{0.2} \times \frac{100}{1} = \frac{\text{D globulin}}{100 \text{ ml. serum}}
\]

\[
(f') \quad \text{D globulin} \times \frac{4}{0.2} \times \frac{100}{1} = \frac{\text{D globulin}}{100 \text{ ml. serum}}
\]

\(D\) = optical density.

The correlation values for peptide to nitrogen, \(D/(\text{mg. of N})\), and sulfhydryl to peptide, \(\text{SH}/D\), were derived by using the above values and the Kjeldahl nitrogen content for the appropriate fraction or whole serum. No correction was made for non-protein nitrogen in normal sera.

Electrophoretic analyses were performed in a modified Longsworth apparatus. All determinations were carried out in veronal buffer, pH 8.6, 0.1 ionic strength at 1°C.

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*The electrophoresis apparatus was modified by Dr. H. Sidney Newcomer of New York so as to fold the optical path on itself. This has been accomplished by the*
Results

Table I shows the distribution of sulfhydryl and nitrogen in the serum proteins of normal males and females. It is apparent that there is no significant difference between the sexes. These subjects were drawn from a university hospital staff population and have a restricted age distribution.

### Table I
Nitrogen and Sulfhydryl Content of Human Sera and Serum Fractions

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Total N as gm. per 100 ml. serum (c)</th>
<th>μM SH per 100 ml. serum (d)</th>
<th>μM SH per gm. N (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum Albu-</td>
<td>Glob-</td>
<td>Serum Albu-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>min</td>
<td>min</td>
<td>min</td>
</tr>
<tr>
<td>Normal males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. S.</td>
<td>37</td>
<td>1.23</td>
<td>0.60</td>
<td>0.43</td>
</tr>
<tr>
<td>N. W.</td>
<td>34</td>
<td>1.22</td>
<td>0.64</td>
<td>0.43</td>
</tr>
<tr>
<td>J. G.</td>
<td>24</td>
<td>1.15</td>
<td>0.71</td>
<td>0.43</td>
</tr>
<tr>
<td>J. C.</td>
<td>34</td>
<td>1.15</td>
<td>0.67</td>
<td>0.43</td>
</tr>
<tr>
<td>M. L.</td>
<td>29</td>
<td>1.25</td>
<td>0.70</td>
<td>0.50</td>
</tr>
<tr>
<td>L. S.</td>
<td>25</td>
<td>1.26</td>
<td>0.78</td>
<td>0.44</td>
</tr>
<tr>
<td>D. B.</td>
<td>21</td>
<td>1.26</td>
<td>0.73</td>
<td>0.50</td>
</tr>
<tr>
<td>J. B.</td>
<td>27</td>
<td>1.09</td>
<td>0.70</td>
<td>0.37</td>
</tr>
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<td>R. P.</td>
<td>28</td>
<td>1.18</td>
<td>0.75</td>
<td>0.38</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.20</td>
<td>0.71</td>
<td>0.45</td>
</tr>
<tr>
<td>σ</td>
<td></td>
<td>0.06</td>
<td>0.04</td>
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</table>

<table>
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<tr>
<th>Normal females</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
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<td>D. F.</td>
<td>23</td>
<td>1.21</td>
<td>0.71</td>
<td>0.46</td>
<td>55.6</td>
<td>47.2</td>
<td>9.9</td>
</tr>
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<td>B. A.</td>
<td>23</td>
<td>1.17</td>
<td>0.66</td>
<td>0.46</td>
<td>53.5</td>
<td>41.2</td>
<td>10.4</td>
</tr>
<tr>
<td>K. J.</td>
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<td>1.15</td>
<td>0.70</td>
<td>0.51</td>
<td>52.8</td>
<td>43.4</td>
<td>12.5</td>
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<tr>
<td>M. H.</td>
<td>28</td>
<td>1.24</td>
<td>0.68</td>
<td>0.51</td>
<td>48.8</td>
<td>40.1</td>
<td>10.7</td>
</tr>
<tr>
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<td>1.23</td>
<td>0.66</td>
<td>0.54</td>
<td>51.0</td>
<td>41.7</td>
<td>12.0</td>
</tr>
<tr>
<td>J. P.</td>
<td>31</td>
<td>1.18</td>
<td>0.73</td>
<td>0.40</td>
<td>55.8</td>
<td>47.4</td>
<td>10.4</td>
</tr>
<tr>
<td>A. M.</td>
<td>24</td>
<td>1.20</td>
<td>0.67</td>
<td>0.49</td>
<td>53.1</td>
<td>45.0</td>
<td>11.6</td>
</tr>
<tr>
<td>H. P.</td>
<td>27</td>
<td>1.18</td>
<td>0.71</td>
<td>0.42</td>
<td>50.8</td>
<td>45.0</td>
<td>9.9</td>
</tr>
<tr>
<td>B. B.</td>
<td>23</td>
<td>1.19</td>
<td>0.69</td>
<td>0.45</td>
<td>52.5</td>
<td>45.3</td>
<td>10.5</td>
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<tr>
<td>C. L.</td>
<td>24</td>
<td>1.13</td>
<td>0.65</td>
<td>0.44</td>
<td>52.0</td>
<td>42.7</td>
<td>10.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.18</td>
<td>0.69</td>
<td>0.47</td>
<td>52.6</td>
<td>43.0</td>
<td>10.8</td>
</tr>
<tr>
<td>σ</td>
<td></td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>2.1</td>
<td>2.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

σ = standard deviation $\times \sqrt{n/(n-1)}$. 

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The ratio of albumin nitrogen to globulin nitrogen is the same as that reported for normal sera by Pillemer and Hutchinson (6).

From the data presented in Column d, Table I, it may be seen that albumin SH represents 80 per cent of the total serum SH. It is known that alterations in the albumin and globulin contents of serum occur in many pathological states (8). Thus it might be anticipated that the SH values of pathological sera would be found altered. This is indeed the case, as will be shown in a subsequent paper.4

Evidence concerning a difference in the composition of the two fractions is tabulated under Column e. There is approximately 2½ times as much SH per gm. of albumin nitrogen as there is per gm. of globulin nitrogen. While sufficient data are not available to evaluate this observation completely, some information may be deduced from reported analyses of purified proteins (9). If we assume a molecular weight of 69,000 and 16.0 per cent of nitrogen, there would be 11,040 gm. of nitrogen per mole of human serum albumin. Our value of 64 μM of SH per gm. of serum albumin nitrogen results in a value of 0.71 mole of SH per mole of albumin. Hughes has isolated a crystalline mercury derivative of human albumin as the compound albumin-Hg-albumin (10). His data indicate that 0.67 mole of SH per mole of serum albumin is present. It would appear that the SH groups titrated amperometrically are the same as those yielding the crystalline mercury salt. Brand has reported 4 moles of cysteine per mole of human serum albumin (9). In view of the findings of Olcott and Fraenkel-Conrat that tryptophan reacts with cystine to give cysteine, it is not certain what value can be attached to the reported cysteine contents, determined on acid hydrolysates (11).

The globulin fraction of normal human serum includes α-, β-, and γ-globulins in approximately equal concentration. The average molecular weight of the human serum globulins has been taken as 170,000 (12). If one assumes an average nitrogen content of 15 per cent, then the 24 μM of SH per gm. of globulin nitrogen determined would be equivalent to 0.6 mole of SH per average globulin molecule. This value approximates that found for albumin, but its distribution among the different globulins is not known.

The biuret reaction may be employed as a measure of the number of peptide linkages in the serum proteins. Table II presents some interesting observations with regard to the relationship of peptide bonds to sulfhydryl groups in the serum proteins. It may be seen (a) that the ratio

3 We have determined the SH in fetal serum. The values for micromoles of SH per 100 ml. of serum are lower than in adults. However, μM SH/D is the same as in adult serum.

of peptide bonds per mg. of nitrogen is nearly alike for serum albumin and globulin. Since the albumin fraction contains the non-protein nitrogen, one would expect the slightly lower biuret extinction found per unit of nitrogen. In contrast to this similarity, (b) the albumin fraction is shown to contain 3 times as many sulfhydryl groups as the globulin fraction when these fractions are compared with reference to their peptide content.

A comparison of the albumin-globulin ratios (c) shows that the biuret values agree closely with the electrophoretic determinations. With one exception, the ratios of the biuret values are all lower than the Kjeldahl,
a reflection of the small non-protein nitrogen content of the albumin fraction, which increases the Kjeldahl ratio.

The effect of storage on the SH content was studied on nineteen serum samples. Serum stored at 0° showed a 6 per cent reduction after 24 hours, while at -78° no appreciable change was noted up to 4 months. Dialysis at 0° had little effect when compared to stored controls.

Effect of Certain Compounds of Biochemical Interest on Sulfhydryl Determination

When n-propylthiouracil was added to a serum whose SH content had been determined, it was recovered almost quantitatively. Ennor and Stocken have reported that sulfhydryl compounds interfere with the determination of creatine by the Barritt reaction (13). There was no change in the SH content of serum to which an equimolar amount of creatine was added. The addition of 0.1 to 1.0 mg. of sodium deoxyribonucleate to 1 ml. of serum also had no effect. Penicillin G gave a blank titration and, when added to serum, did not change the SH content. The observations of Benesch and Benesch (5) that p-chloromercuribenzoate completely abolished the SH of serum samples were confirmed.

DL-Cysteine as well as n-dodecylmercaptan could not be titrated in dilute alcoholic solutions or when added to serum. L-Cystine, titrated in 90 per cent ethanol, showed no evidence of an SH group.

Sulfhydryl Content of Other Body Fluids

Plasma—Samples of serum and heparinized plasma were prepared from the same blood samples. The sulfhydryl values per ml. agreed within experimental error.

Saliva—1, 5, and 7 ml. samples of saliva were titrated, but no sulfhydryl groups were detected. The biuret determinations on these salivas indicated approximately 1.3 mg. of protein per ml.

Ascitic and Pleural Fluid—Ascitic and pleural fluid contain titratable SH. The SH/D values found in three samples were 0.145, 0.140, and 0.097. The serum value corresponding to the last value was 0.122. One ascitic fluid, which showed no SH, had so little protein that the sulfhydryl titration was considered unreliable.

Urine—A specimen of protein-free urine titrated as a blank.

DISCUSSION

The studies presented herein have been concerned with the protein sulfhydryl groups of serum. The concordance of values secured in sera be-

* We wish to thank Dr. L. Hellerman for the gift of highly purified p-chloromercuribenzoate.
fore and after dialysis supports this assertion. These results are remarkable in view of the much higher content of sulfhydryl groups in the formed elements of the blood. The glutathione content of human erythrocytes is 35 mg. per 100 ml. of whole blood equivalent to 114 \( \mu \)M of SH (14). Thioneine has been reported present in human erythrocytes in amounts averaging 15 mg. per 100 ml. of whole blood or 65 \( \mu \)M of SH (15). Waelsch and Rittenberg have estimated that half of the glycine or of the glutamic acid of liver glutathione of rats and rabbits is replaced in less than 4 hours (17, 18). This is one of the most rapid turnover rates ever reported. These intracellular sulfhydryl compounds should be in equilibrium with serum non-protein sulfhydryl, yet our findings give no indication of such an equilibrium. It may be significant that Eagles and Vars were unable to detect thioneine added to blood or solutions of proteins. These authors observed that the thioneine seemed to be bound to protein (19). Also, it should be noted that both thioneine and glutathione have been isolated from cells only by very drastic chemical methods (16).

It is believed that the relationship between the ratio of biuret or peptide values to the nitrogen of the serum proteins \( D/(\text{mg. of N}) \) and the ratio of the sulfhydryl to peptide bond content of these proteins, micromoles SH/\( D \), is of importance in evaluating the nature of the serum proteins. It would appear that there is no great difference between albumin and globulin in their number of peptide bonds per mg. of nitrogen. By contrast, the large difference in the number of sulfhydryl groups in these protein fractions on the basis of their peptide content is most striking. Luetscher found two components in human serum albumin by electrophoresis at pH 4. The faster of these was present to the extent of 67 per cent, and decreased markedly in the pathological sera examined (20). The mercaptalbumin isolated by Hughes contains 1 mole of SH per mole of protein (10). The 0.71 mole of SH per mole of albumin protein which we have found may represent a statistical average; i.e., a mixture of albumins only one of which possesses a sulfhydryl group. It is possible that all newly formed albumin contains 1 mole of SH, which is lost with age. The hypothesis may be advanced that the lowered SH found in

4 The contribution of the leucocytes and platelets has not been measured (14–16). Benedict and Gottschall drew attention to the correlation between the hemoglobin and glutathione contents of the human bloods analyzed by them.

5 Studies on hemolyzed human erythrocytes have shown that there are about 1175 \( \mu \)M of intracellular SH per 100 ml. of whole blood. Of this, less than 6 per cent or 65 \( \mu \)M of SH are dialyzable. This latter amount is far below the total for glutathione and thioneine. Our results indicate that there are 4 to 5 moles of SH per mole of hemoglobin. Further work on this problem is in progress.

6 In a personal communication, Dr. Hughes has informed the authors that this theory is under test at Harvard.
pathological sera (21) represents an accelerated aging process, a retarded synthesis of new protein, or a rearrangement of molecular architecture. The globulin fraction will require separation into its component units before its sulfhydryl status becomes clear.

The manner in which sulfhydryl groups exist in proteins is unknown. In order to account for the appearance of SH groups, previously non-detectable, upon denaturation of crystalline ovalbumin, Harris postulated that there may be several forms of sulfur linkage other than S—S in native proteins (22). These postulated forms were thiopeptides, thioesters, and thiolactones. Walker showed that S—S linkages were not present in native ovalbumin (23). Mastin and Schryver studied the formation of an acidic denatured egg albumin and noted an increase in the number of free acid groups without a corresponding increase in amino groups. At the same time, free SH groups appeared. They considered this evidence for the existence of a thioester linkage in proteins (24). On the basis of very careful amino acid analyses, Chibnall has also postulated the existence of such protein linkages (1).

Greenstein found that, although guanidinium salts and urea produced SH groups in proteins, they had no such effect on a series of simple dithio compounds. He concluded that the sulfur in proteins which appeared as SH did not come from S—S (25). In our study, cystine in alcoholic solution did not titrate amperometrically.

It seems reasonable to infer that the "masked" sulfhydryl groups in proteins are actually linked in a chemical union which can be described in classical organic terms. It remains to be determined which mode or modes of linkage are most probable.

We wish to thank Dr. W. Mansfield Clark and Dr. Leslie Hellerman for helpful criticism and Mr. Jerry Reik for his aid in the performance of some of the electrophoretic analyses.

SUMMARY

1. Methods and details are given for the amperometric titration of the sulfhydryl group in human serum, serum albumin, and the serum globulin fraction.

2. The sulfhydryl contents of normal serum, serum albumin, and the serum globulin are presented.

3. The relationship of the sulfhydryl group to the number of peptide bonds has been determined. It is shown that normal serum albumin and the normal serum globulin fraction each have a characteristic ratio of sulfhydryl to peptide bond content.

4. Normal human serum albumin has 0.71 mole of sulfhydryl per mole
of protein. The implications of this finding with respect to the composition of albumin are discussed.

5. It is shown that the dithio linkage of cystine cannot be titrated amperometrically. The nature of sulfur linkages in proteins is discussed in the light of this and other observations.

BIBLIOGRAPHY

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