ing a comparison, that in man the histamine content of tissue is much greater than in the mouse, and that normally, sensitivity is also much greater. In the mouse, the tissue content is so low that the increased sensitivity can not be due to an additive amount of histamine released from the tissue. Whether or not the reaction is due to altered tissue susceptibility, vascular changes, a stress factor or some other factor must await further investigation. An explanation for the reaction in the mouse might help to elucidate the nature of this infection in man.

The resistance of the recovered mouse, to an intracerebral inoculation of *H. pertussis* provides further justification for the use of the latter route in testing the protective potency of pertussis vaccines in mice(6).

Summary. Mice inoculated intranasally with a culture of *H. pertussis* developed lung infections from which more than half of the mice survived. The average time of death ranged from 16 to 23 days. They developed a high degree of sensitivity to histamine comparable to that induced by pertussis vaccine, but it was slower in developing and it persisted much longer. A high percentage of the mice that died from histamine shock showed gross lung lesions and *H. pertussis* was recovered from the lungs of the majority. Most of the mice that survived this infection were resistant to an intracerebral inoculation of *H. pertussis*.

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Sulfhydryl Content of Normal Hemoglobin and Hemoglobin in in Sickle Cell Anemia.* (18683)

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Recent observations suggest that the abnormal physical and physiological properties of the erythrocytes in sickle cell anemia can be explained on the basis of abnormalities in their intracellular components. Evidence of a difference between the molecules of normal and of sickle cell hemoglobin has been obtained by electrophoresis(1). The increase in the viscosity of the whole blood of patients with sickle cell anemia which occurs on deoxygenation(2) has been reproduced in solutions of hemoglobin(3). The birefringence noted in sickled erythrocytes(4) is also a property of the tactoid aggregations which form when solutions of sickle cell hemoglobin are deoxygenated, and the tactoids themselves resemble in shape sickled erythrocytes(3).

Sickling *in vitro* of intact erythrocytes can be produced by a variety of reducing agents (5). Among these are cysteine, glutathione and BAL, which contain sulfhydryl (SH) groups(6). Sickling is inhibited by Hg^{++} and As^{+++} ions, iodoacetamide and o-chloromercuribenzoate, all of which are SH inhibitors(6,7). Thus, it appears that SH groups may be specifically concerned in the sickling phenomenon. It was of interest, therefore,

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TABLE I. Sulfhydryl (SH) Content of Normal, Sickle Cell Anemia, and Sickle Cell Trait Hemoglobins.

Hemoglobin	Normal				Sickle cell anemia					Sickle cell trait	
Patient	H.	K.	I.	Ru.W*	Ro.W.	Hz	Hr	Ho	C.	B.	S.
Moles SH/Mole hgb.	2.08	1.99	2.06	2.05	3.10	3.04	2.87	3.31	3.02	2.44	2.48
Mean SH/hgb.†		2.04 ± .04				3.07 ± .16				2.46 ± .03	

* Normal sister of patient Ro.W.
† Mean of group given with stand. dev.

to determine whether the reduced SH content of sickle cell hemoglobin differs from that of normal hemoglobin. In the present communication are presented the results of the determination of the SH content of the hemoglobin molecules of 4 normal individuals, 5 patients with sickle cell anemia, and 2 with sickle cell trait.

Methods. The hemoglobin solutions were prepared by collecting venous blood in Alsever's solution. The cells were then washed 3 times with 0.85% sodium chloride solution, and hemolyzed with distilled water. Sodium chloride was then added to a concentration of 2.0 g % and the solutions were allowed to stand overnight in the refrigerator. The stromal precipitate was removed by centrigugation of 4°C and 16,000 RPM for 1½-2 hours. Thereafter the hemoglobin solutions were dialyzed against 0.85% saline at 4°C until they were entirely free of nonprotein reducing substances, as determined by the method of Woodward and Fry for the estimation of glutathione(8). The hemoglobin concentrations of the solutions, as determined in an Evelyn photoelectric colorimeter, were brought to about 1.5 g % with saline solution. Aliquots of 0.2 ml were titrated by the amperometric method of Kolthoff and Harris(9), modified for the determination of protein-sulfhydryl by Benesch and Benesch(10) and Weissman, Schoenbach and Armistead(11). A further modification of this method was employed in that the titrations were carried out in 0.85% saline solution. The values obtained in this medium were found to be lower and more consistent than those obtained by titration in 20% methanol, the medium used by Weissman, *et al.* All solutions were titrated at least twice with 0.001 N $AgNO_3$. In several instances the same results were obtained by titration with 0.0005 N $AgNO_3$. A molecular weight of 68,000 was used in calculating the molarity of the solutions of both normal and abnormal hemoglobins. Inasmuch as hemoglobin comprises 95% of the dry weight of the entire erythrocyte(12) the SH groups contained in other nondialyzable materials within the erythrocytes were not considered to contribute significantly to the titration values.

Results. The results of the titrations in saline of the SH content of the hemoglobin of each individual are given in Table I. Normal hemoglobin gave a mean value of 2.0 SH groups per molecule, whereas the hemoglobin of patients with sickle cell anemia gave a mean value of 3.0 SH groups per molecule. Intermediate values were obtained in the hemoglobin of the patients with sickle cell trait. In no instance did duplicate determinations vary by more than 2.0% and the mean variation of duplicate determinations in all patients with 0.82%.

When titrations were carried out in 20% methanol in saline, the values for titratable SH of the hemoglobins of all 4 groups of individuals was 4 to 5 moles per mole of hemoglobin. The values obtained from normal hemoglobin titrated in this medium did not differ significantly from those obtained from sickle cell anemia or trait hemoglobins.

In saline solutions, the SH groups of both normal and abnormal hemoglobins were

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blocked stoichiometrically with p-chloromercuribenzoate. an agent with a high degree of specificity in binding SH groups(13).

Discussion. Although a significant difference has been demonstrated between the SH titration values of normal hemoglobin and that of patients with sickle cell anemia as determined amperometrically, the data do not make clear whether this is due to a difference in the total content of SH groups or to an alteration in their reactivity.

The possibility was entertained that the 5 to 15% reticulocytes present in the blood of patients with sickle cell anemia might alter the values obtained in the determination of the SH content of the hemoglobin. This was rendered unlikely by the findings in the patients with sickle cell trait, in whom reticulocyte counts were within normal limits. In addition, hemoglobin was obtained from two patients with pernicious anemia during the period of reticulocyte response subsequent to treatment with vitamin B_{12}. These, like normal hemoglobin, contained about 2 moles SH per mole of hemoglobin.

The finding of 2 moles of SH per mole of normal human hemoglobin agrees with that reported by Hughes, who employed methylmercury iodide as a SH-blocking agent (15). The SH content of the hemoglobin molecules in the 2 patients with sickle cell trait was greater than that of normal hemoglobin but less than that of sickle cell anemia hemoglobin. This is consistent with evidence that the hemoglobin in such patients is a mixture of normal and sickle cell anemia hemoglobins(16). It was estimated that the hemoglobins from the 2 patients with sickle cell trait who were studied contained approximately 48% and 44% of abnormal hemoglobin, respectively.

The present study confirms the finding that the SH titration value of hemoglobin, as determined amperometrically in 20% methanol, is 4 to 5 moles per mole of hemoglobin (11). Denaturing procedures may uncover previously unmeasurable SH groups(17). Slight denaturation of the hemoglobin molecules, produced by 20% methanol at room temperature, may account for the higher figure that is obtained in methanol as compared with saline. The data are not adequate to explain why the difference in the sulfhydryl content of normal and sickle cell anemia hemoglobin is not demonstrable in methanol.

The capacity of p-chloromercuribenzoate to block stoichiometrically the measured SH groups of both normal and abnormal hemoglobins suggests that when the amperometric determinations are carried out in 0.85% saline solution, the method is specific for SH groups.

Conclusions. 1. The titratable SH content of hemoglobin was determined amperometrically in saline, and found to approximate 2 moles SH per mole of normal hemoglobin and 3 moles SH per mole of hemoglobin from patients with sickle cell anemia. 2. The SH content of hemoglobin from two patients with sickle cell trait was greater than that of normal hemoglobin and less than that of sickle cell anemia hemoglobin suggesting that a mixture of the two types of hemoglobin was present in these patients.

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