and fluoride, are considered from the point of view of available knowledge of the penetration of such substances into cells. Cyanide and sulfide, being salts of weak acids, penetrate the cells and probably affect the turnover rate of intracellular phosphate. The lack of effect with fluoride is consistent with its existence in the completely dissociated state. Iodoacetate, also completely ionized, may be effective at the cell surface, affecting the permeability of the cell to phosphate. The apparent rise in the rate of phosphate uptake in the presence of cysteine remains unexplained.

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The Sulfhydryl Content of Normal and Abnormal Human Sera.* (17569)

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The important role of the sulfhydryl group in metabolic processes has long been recognized although the precise manner in which it participates in these reactions is not clearly understood.(1,2) Kolthoff and Harris(3) have described an amperometric method for the determination of mercaptans and this technic has been adapted as a quantitative method for sulfhydryl groups in amino acids and proteins by Benesch and Benesch.(4) During the past 3 years, we have been conducting studies on the mechanism of action of various drugs and one of the determinations which has been of great interest has been that of the sulfhydryl content of the serum proteins. It was hoped that an indicator system could be developed which would aid not only in clarifying the metabolic abnormalities induced by the presence of a neoplasm but also measure the effect of chemotherapeutic agents. Although the objective of this study was to follow the progressive changes which occur in animals and patients with tumors, it also seemed desirable to investigate patients with other diseases in which changes in the serum proteins are known to occur.

Methods. Blood was obtained from patients in the fasting state and the serum carefully separated to avoid hemolysis. The fresh serum was then added to 21% methanol solution containing ammonium nitrate and ammonium hydroxide as supporting electrolytes. The current which flows between a mercury-mercuric iodide electrode and a rotating platinum electrode through the serum-methanol solution is measured with a sensitive galvanometer. As .001 N silver nitrate is added in small increments, a silver mercaptide is formed and the increment of current is small. When all the free SH groups have reacted, a slight excess of silver ion results in a relatively large increase in the diffusion current. By plotting graphically, the current flow observed against the added silver nitrate, one obtains two straight line curves whose intersection is the equivalence point.(3) The titrations are standardized with cysteine each day and the results are expressed as milligrams of cysteine per unit, *i.e.*, per 100 ml serum, gram protein nitrogen, gram albumin nitrogen, etc. It is not implied however, that the sulfhydryl determined in serum is cysteine. Proteins have been separated into albumin and globulin components by the method of Pillemer,(5) and the SH determined in each individually. Protein

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1. Hellerman, L., *Physiol. Rev.*, 1937, v17, 454.
2. Olcott, H. S., and Fraenkel-Conrat, H., *Chem. Rev.*, 1947, v41, 151.
3. Kolthoff, I. M., and Harris, W. E., *Ind. Eng. Chem. Ana. Ed.*, 1946, v18, 161.
4. Benesch, R., and Benesch, R. E., *Arch. Biochem.*, 1948, v19, 35.
5. Pillemer, L., and Hutchinson, M. C., *J. Biol. Chem.*, 1945, v158, 299.

TABLE I.
Summary of Sulfhydryl Determination in Human Sera.

Status of patients	No. of determinations	SH* per 100 ml serum	Total N† g per 100 ml	SH* per g N
Normal				
Male	9	6.53 ± .24	1.20 ± .06	5.46 ± .27
Female	10	6.36 ± .25	1.19 ± .03	5.36 ± .30
Abnormal				
Cancer	46	4.28 ± .23	1.13 ± .14	3.84 ± .48
Infection	13	5.03 ± .63	1.22 ± .12	4.17 ± .61
Degenerative disease‡	12	4.92 ± .95	1.25 ± .08	3.85 ± .74
Miscellaneous states§	12	2.70 ± .91	1.06 ± .15	2.76 ± .97
Leukemia	34	4.56 ± .65	—	—

* Expressed as mg of cysteine. Values are given as the mean ± one standard deviation (S.D.). When less than 20 observations are recorded, the S.D. has been multiplied by $\sqrt{\frac{n}{n-1}}$.

† No correction was made for serum N.P.N.

‡ Includes asthma, arteriosclerosis, hypertension, arthritis, etc.

§ Includes cirrhosis of liver, lupus erythematosus disseminata, chronic nephritis and hemachromatosis, in which reversal of the albumin-globulin ratio was present.

content was determined by a micro-Kjeldahl method and also by the color developed by a quantitative biuret reaction. The details of these methods will be presented at a later date.

Results. The values presented in the table show a distinct difference in the serum sulfhydryl content of normal individuals when compared with sera obtained from patients who were suffering from various disease states. The latter are lower in all cases. It should be noted that the mean deviations recorded are those of biological individuality and can not be attributed to technic. Duplicate sulfhydryl determinations on an individual serum showed less than a 2% variation while the nitrogen values agreed within one percent. The total serum protein as measured by nitrogen and biuret determinations revealed no evidence of hypoproteinemia in any of the groups. It is evident, as presented in the last column of the table, that the sulfhydryl determined per gram of nitrogen did not differ significantly from one another among the abnormal sera.

Discussion. The results presented indicate that clinical abnormality can be detected by reduction in the serum sulfhydryl content, although our experience with this determination, on whole sera, does not permit utilization of this method for specific diagnosis as *e.g.*, the presence or absence of cancer. Studies in progress have revealed that 80 percent of the total normal serum sulfhydryl is in the albumin fraction. A reduction in the amount of albumin might be expected to result in lower sulfhydryl values per unit volume of serum. This *quantitative* factor does contribute to the lower sulfhydryl of abnormal sera. A *qualitative* factor, as yet unexplained, is also present, in that the sulfhydryl per gram of albumin nitrogen is below normal. The same qualitative change occurs in the globulin fraction despite the quantitative increase of globulin which often occurs. These findings indicate an altered protein synthesis in certain diseases which results in fewer sulfhydryl groups available for this titration.

The data of Brand(6) show 4 mols of cysteine per mol of human albumin and 9 mols per mol of gamma globulin. The values we have found for normal serum albumin average 0.68 mol available cysteine per mol of albumin. Hughes(7) has reported the isolation of a crystalline mercury salt of human albumin, from which the mercury could be removed by dialysis against 0.001 M cysteine. His recovery would correspond to 0.66 mol of cysteine per mol of total albumin. Thus it appears that we are titrating the same groups in human serum albumin. Desoxyribonucleic acid, cre-

6. Brand, E., *Ann. N. Y. Acad. Sci.*, 1946, v47, 187.

7. Hughes, W. L., Jr., *J. Am. Chem. Soc.*, 1947, v69, 1836.

atine, or formaldehyde when added to serum did not affect the sulfhydryl value previously determined.

The enzymes which are associated with anabolic processes, the cathepsins, are known to be activated by sulhydryl compounds. Altered protein synthesis and sulfhydryl content may be related to such intracellular enzyme systems. Huggins, *et al.*,(8) have studied changes in thermal coagulability, in the presence of iodoacetate, as a measure of serum protein alterations in disease. Iodoacetate is a well known reagent for sulhydryl groups. The iodoacetate indices determined by Huggins, *et al.*, for normal and abnormal sera are of the same order of magnitude as the sulfhydryl content determined with the amperometric method. Although we do not consider the determination of serum sulfhydryl to be a specific diagnostic test for cancer, it may serve as an additional objective indicator in following animals and patients receiving chemotherapy.

Summary. The sulfhydryl content of serum proteins has been quantitatively determined through an adaptation of an amperometric titration. Among normal adults, the values noted, expressed as milligrams of cysteine, were uniform, reproducible and averaged 6.5 ± 0.2 mg per 100 ml serum or 5.5±0.3 mg per gram serum nitrogen. The albumin component of serum contained 80% of the total sulfhydryl. The addition of desoxyribonucleic acid, creatine or formaldehyde did not change the sulfhydryl content of the serum. The addition of an SH reagent such as para-chloro-mercuri-benzoate abolished all reactant groups measured with this method.

Sera obtained from patients with various diseases showed a significant reduction in the sulfhydryl available for this titration. This quantitative reduction in sulfhydryl was still evident when corrected for differences in the albumin and globulin components and thus represented a qualitative change in the serum proteins. At the present time, the alteration in the sulfhydryl content of serum or its components is not characteristic enough to permit specific differentiation among the neoplastic, metabolic, or infectious diseases. The possible application of serial serum sulfhydryl determinations in animals and patients may be of value as an objective indicator of response and for study of the mechanism of action of chemotherapeutic and other agents.

8. Huggins, C., Miller, G. M., and Jensen, E. V., *Cancer Res.*, 1949, v9, 177.

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Surface Fixation. A New Method of Detecting Certain Immunological Reactions. (17570)

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The study of rapid agglutination tests for the diagnosis of a number of infections has shown that carefully prepared and standardized antigens are of considerable help to the practitioner as well as the epidemiologist. The use of a suspension of formalinized and blue-stained Proteus X-19 in slide agglutination tests with whole blood(1) has been a satisfactory preliminary method for the diagnosis of typhus fever. This encouraged us to apply the same method to brucellosis. We have had excellent results. Both tests are performed on a slide. A 3 mm wire loop of antigen is mixed with whole blood taken with a 2 mm loop. The mixture is rotated in order to favor the margination of the agglutinated antigen. In about 1 or 2 minutes a thick blue ring forms and surrounds the red cells which have separated from the antigen. In negative reactions the mixture remains a uniform greenish color often surrounded by a reddish ring formed by marginated red cells. The forma-

1. Castaneda, M. R., Silva, R., and Monnier, A., *Rev. Med. del Hospital General*, 1940, v8, 382.