"normal control" reaction in the eye first instilled. In 39 experiments, "curative" effect was observed only once.

The appended table enumerates the results obtained in 75 control experiments, as compared with 17 experiments in which sodium salicylate was administered, and 22 experiments in which quinine bisulphate was used. It also furnishes an adequate indication of the readability of the edematous reaction, and the faithfulness with which it may be reproduced. This method is being used to investigate a number of other drugs.

TABLE I.
The incidence of edema elicited by 15% mustard oil in mineral oil, and its suppression by sodium salicylate and quinine bisulphate.

| Reaction | Controls (75) | Sodium Salicylate (17) | Quinine Bisulphate (22) |
|---|---|---|---|
| (++++) max. | 55 | 0 | 0 |
| (+++) | 15 | 0 | 0 |
| (++) | 5 | 5 | 1 |
| (+) | 0 | 5 | 10 |
| No reaction | 0 | 7 | 11 |

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## Protein Sulfhydryl Groups.

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The importance of the sulfhydryl (SH) groups of proteins in cellular respiration has been clearly recognized (deRey Pailhade,[1] Heffter[2]) and the appearance of these groups in some proteins as the result of denaturation has been demonstrated (Arnold[3]). For the study of both cellular respiration and protein chemistry, therefore, a means of measuring protein sulfhydryl groups is desirable. Investigation of them has depended mainly on the color reaction they give with nitroprusside. For this test the groups must be in the reduced (SH) and not in the oxidized (S-S) form. Heffter[2] supposed that since many denatured proteins, the denatured serum

[1] deRey Pailhade, *Recherches Experimentales sur le philothion, etc.* Paris, Masson, 1891.

[2] Heffter, *Med. Naturwiss. Arch.*, 1908, **1**, 81.

[3] Arnold, *Z. Physiol. Chem.*, 1911, **70**, 300, 314.

proteins for example, did not give a color with nitroprusside, they contained disulfide (S-S) rather than sulfhydryl (SH) groups. He was indeed able to reduce the disulfide groups of denatured serum proteins with sodium sulfite so that they then gave a color reaction with nitroprusside. Protein disulfide groups have also been reduced by means of zinc and hydrochloric acid (Arnold[3]), by soluble sulfhydryl compounds (Hopkins[4]), and by cyanide (Walker[5]). On the other hand, protein sulfhydryl groups can be oxidized by sulfur (deRey Pailhade,[1] Heffter[2]), by disulfides (Hopkins[4]), and by other reagents. Since disulfide groups, as well as sulfhydryl, exist in proteins, and since these groups are interconvertible by oxidation and reduction, information will be incomplete until both the S-S and SH forms are measured.

*First Method.* We find that the color intensities given with the Folin-Marenzi[6] uric acid reagent by equivalent quantities of cysteine and cystine are precisely in the ratio of 2:1. This is contrary to what has been assumed (Folin and Looney[7]). If equal quantities of a denatured protein are completely reduced and completely oxidized and then hydrolyzed, the color intensities with the Folin-Marenzi reagent of the 2 hydrolysates are also in the ratio of 2:1. A sample of protein partly oxidized and partly reduced will yield a hydrolysate with a color intensity proportional to its state of oxidation-reduction. Measurements can be made to within 5-10%.

*Second Method.* The procedure is based on the fact that protein SH groups are oxidized by a suitable disulfide, the S-S groups of the latter thereby being converted into SH groups which can readily be estimated. The validity of this method depends upon proof that it is only SH groups of protein that react with a disulfide, that the reaction is in fact specific. Evidence of specificity has been secured by showing that in a number of completely reduced denatured proteins that number of SH groups measured is equal to twice the number of cystine molecules obtained on hydrolysis.

The 2 methods outlined yield results agreeing to within less than 10%.

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[4] Hopkins, *Biochem. J.*, 1925, **19**, 787.

[5] Walker, *Biochem. J.*, 1925, **19**, 1082.

[6] Folin and Marenzi, *J. Biol. Chem.*, 1929, **83**, 109.

[7] Folin and Looney, *J. Biol. Chem.*, 1922, **51**, 428.