As an incidental finding we observed that unusually large quantities of a greenish yellow pigment, giving the Prussian Blue reaction, were deposited in the spleens of all adrenalectomized animals, treated or untreated. Similar depositions have also been observed in the spleens of hypophysectomized animals. The decrease in number of eosinophiles in the hypophysis which usually follows adrenalectomy was not influenced by the salt treatment.

From these experiments we conclude that the dioestrus following removal of the adrenals is probably not the result of the absence of any specific sex hormone found in the adrenal glands, but rather of the general ill-health of the animal.

7667 P

Oxidation of Cystine Sulfur to Sulfate by Ferric Chloride.

ROBERT H. SIFFERD AND VINCENT DU VIGNEAUD.

From the Department of Biochemistry, School of Medicine, George Washington University, Washington.

The oxidation of thiol-imidazole sulfur to sulfate by $FeCl_3$ is frequently used in the conversion of thiol-imidazole compounds to imidazoles. One would assume from the literature that cystine sulfur is not similarly oxidized by this reagent.[1] This suggested the utilization of this reaction as a means of testing for the possible presence of a thiol-imidazole compound in insulin. So far only about 70% of the sulfur of insulin has been accounted for. The highly specific Sullivan reaction indicates the presence of about 8.4% cystine, whereas if all the sulfur of insulin (3.2%) were present as cystine, 12.0% should be present.

To test the feasibility of this approach experiments with cystine were first undertaken. These experiments showed contrary to our expectations that cystine sulfur was quite readily oxidized to sulfate by $FeCl_3$. Twenty mg. of cystine heated with 2.5 cc. of 6.5% $FeCl_3$ for 1 hour in the boiling water bath gave a positive test for sulfate. Under the same conditions no sulfate was formed from cysteic acid.

Other experiments on cystine were conducted in which the compound was heated in $FeCl_3$ (6.5%) with HCl varying in concentration from N/10 to 10%. All samples showed appreciable amounts of sulfate after being heated for an hour. The amount of oxidation

---

[1] Barger, G., and Ewins, A. J., *J. Chem. Soc.*, 1911, **99**, 2336.

decreased in a roughly proportional manner with the increase in acid content, yet we found no conditions under which no sulfate was obtained.

To determine the amount of sulfate formed a 5 gm. sample of *l*-cystine was suspended in 625 cc. of a 6.5% $FeCl_3$ solution and was heated on a boiling water bath for a total of 21 hours. Samples were removed at intervals for sulfate determination. The oxidation followed roughly an exponential curve of low coefficient. At 21 hours approximately 60% of the sulfur was accounted for as sulfate.

The failure of cysteic acid to yield sulfate under the above conditions is further evidence in favor of the view expressed by Andrews[2] that the formation of sulfate in the oxidation of cystine is through a side oxidation of some intermediate substance preceding cysteic acid. In studies on the oxidation of cystine to cysteic acid in 5.9 N HCl by atmospheric oxygen Andrews found that the oxidation could be greatly accelerated by the addition of Cu^{++} salts whereas Fe^{+++} salts were far less effective. It was noted in this instance that small amounts of inorganic sulfate were formed but that cysteic acid did not yield any. Andrews,[2] furthermore, found that treatment of cysteic acid with pure bromine yielded no inorganic sulfate.

In support of the views expressed by Andrews, Toennies and Lavine[3] found that upon iodine treatment of the partially oxidized cystine obtained by the action of perchloric acid small amounts of sulfate appeared, whereas cysteic acid was entirely stable in the presence of iodine.

In a detailed study of the determination of cysteine and cystine Lugg[4] noted that when either of these compounds was heated with $FeCl_3$ in acid solution that some of the substance disappeared. Lugg states that apparently cysteic acid was formed although it seems no test for cysteic acid was made. From our results it would seem possible that the disappearance of cystine or cysteine as noted by Lugg was at least partly due to the formation of sulfate from the sulfur of these 2 amino acids.

---

[2] Andrews, J. C., *J. Biol. Chem.*, 1933, **102**, 263.

[3] Toennies, G., and Lavine, T. F., *J. Biol. Chem.*, 1934, **105**, 107.

[4] Lugg, J. W. H., *Biochem. J.*, 1933, **27**, 1022.