anemia being present in all 3 cases. The low iron values in the blood may be due to the secondary anemia present or may be due to the fact that the deposition of iron in the tissues would tend to reduce the quantity of this element available for the production of hemoglobin. According to Ramage and Sheldon[10] there is also increased copper deposition in certain tissues in hemochromatosis. The absence of a high blood copper content in our cases may be the result of retention in the tissues of this element, but it more likely points to the fact that clinical reports indicate the presence of a mild anemia in hemochromatosis. We have found in general that the more severe the anemia the higher is the blood copper content, especially when there is a marked deficiency both in red cells and in hemoglobin.[18]

The blood copper is slightly higher than the normal average by 9.8 to 18.2%, but yet within the range of normal variation. One determination, however gave a blood copper figure corresponding to the average normal. In the small number of cases reported here the increase in the blood copper above the average figure is not high enough to warrant any relationship in hemochromatosis between the copper in the blood and the abnormal iron metabolism in the tissues.

* We wish to thank Dr. Russell M. Wilder of the Mayo Clinic for permitting us to make copper and iron determinations on blood samples from the three patients mentioned in this article.

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Enzyme for Decomposition of Creatinine and its Action on the "Apparent Creatinine" of Blood.

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The nature of the substance in filtrates of whole blood and plasma which gives the color with alkaline picrate (Jaffe's reaction) has been for many years a subject of controversy. One group of investigators believe that this material is true creatinine—others deny that creatinine exists in normal blood. Because of the non-specific methods employed for the identification of creatinine, and the very minute quantities of the chromogenic material available in normal blood, it has been difficult for either group to present convincing evidence.

To obtain a definitive answer regarding the nature of the Jaffe-

reactive material in blood, and also to develop a specific method for the analysis of creatinine in biological fluids, an attempt was made to obtain a specific enzyme for creatinine. By means of a technique similar to that described by Dubos and Avery[1] and Dubos[2] it has been possible to isolate 4 different species of soil bacteria with a high degree of adaptability toward a substrate of creatinine. One strain (NC) has been found to grow with unusual ease in a medium of pure creatinine and inorganic salts. When tested under conditions which do not allow cellular multiplication, the bacterial suspension still decomposes creatinine very readily. The enzyme seems to be intimately associated with cellular structure and so far has not been released into solution without destroying its activity. However, when the cells of another bacterial species (HR) are disrupted, the creatinine decomposing enzyme is readily obtained in aqueous solution. At present, the potency of this soluble preparation is low compared with a cell suspension of the NC organisms.

The present crude enzyme preparation (NC) will decompose its own weight of creatinine in 15 minutes at 37° C. and pH 7.0. Its specificity has been tested with certain creatinine derivatives which give the Jaffe reaction.* 5-Methylcreatinine, 4- (or 5-) benzoylcreatinine, 5-benzylcreatinine and 2-benzylcreatinine are not attacked by the enzyme. Acetyl creatinine undergoes a very slight decomposition which does not progress on further incubation with the enzyme preparation. The enzyme preparation also discriminates creatinine from that fraction of the Jaffe-reactive material in human erythrocytes which has been shown by Hunter and Campbell[3] to be different from true creatinine. The power of this material to form a red product with alkaline picrate is not destroyed by the enzyme.

The "NC" enzyme preparation is active in urine and tungstic acid filtrates of blood. We find that it decomposes approximately 50% of the Jaffe- reactive material in human erythrocytes and a much larger fraction in the plasma. This would appear to offer almost conclusive proof of the existence of true creatinine in human plasma and erythrocytes. Data concerning the ratio of creatinine to other substances in blood capable of giving Jaffe's reaction will be given later, together with details concerning the preparation and mode of action of the enzyme.

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1 Dubos, R., and Avery, O. T., *J. Exp. Med.*, 1931, **54**, 51.

2 Dubos, R., *J. Exp. Med.*, 1932, **55**, 377; *Ibid.*, 1935, **62**, 259.

* We are indebted to Dr. Isidor Greenwald for the derivatives of creatinine. The nomenclature used is that given by Greenwald, I., *J. Am. Chem. Soc.*, 1925, **47**, 1443.

3 Hunter, A., and Campbell, W. R., *J. Biol. Chem.*, 1917, **32**, 195.