color appears in the toluene preservative. Further investigation of this question and also of the question of relationship of vitamin deficiency to the appearance of these substances in the urine, is in progress. It seems noteworthy that both urorosein and indirubin have been encountered in a variety of diseases such as cancer, tuberculosis, and diabetes,[6, 7, 8] all of which, however, are frequently associated with various deficiency states. Nencki and Sieber[6] first encountered urorosein in the urine of a diabetic patient; it is therefore of interest that Spies and his associates[5] have recently suggested correlation between cozymase deficiency in diabetic patients, and the presence of a positive B.E.S. test.

Conclusions. 1. The color noted in positive Beckh-Ellinger-Spies tests is not due to porphyrin. While a marked increase in urinary porphyrin, such as occurs in porphyria, would be productive of color, the color reaction as observed in pellagra and other diseases, is due to urorosein, first described by Nencki and Sieber. There is no evidence that the color reaction is due to any bile pigment derivative. 2. The urines of pellagra patients may contain either the chromogen of urorosein, or a red pigment extracted by the toluene preservative. It appears highly probable that this pigment is indirubin, although exact identification has not yet been made. 3. Both of the red substances may be noted in the urines of patients not having clinical pellagra. Further investigation is necessary to decide whether their appearance is related to deficiency of nicotinic acid or other essential substances.

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Metabolism of Two Di-deuterobutyric Acids as Indicated by Deuterium Content of Excreted Beta-Hydroxybutyric Acid.

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In order to obtain direct information as to what portions of the fatty acid molecule are convertible to the acetone bodies, a study has been made of the metabolism of the di-deuterobutyric acids. In the first series of tests alpha-beta and beta-gamma deuterobutyric

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acids were fed in doses of 50 mg (as acetone) per 100 sq cm to male rats having an endogenous ketonuria as a result of a previous high-fat diet. The betahydroxybutyric acid separated by extraction of the urine was analyzed for deuterium content by the pressure float method of Rittenberg and Schoenheimer.[1] In 3 tests on alpha-beta deuterobutyric acid only approximately 4% of the extra betahydroxybutyric acid contained deuterium, while in a similar number of tests with the beta-gamma acid 17 to 25% of the betahydroxybutyric acid retained the deuterium. Control rats which received an equivalent concentration of deuterium oxide, excreted no deuterobetahydroxybutyric acid.

In the second series of tests beta-gamma deuterobutyric acid was fed to fasting female rats, which did not have an endogenous ketonuria, in amounts of 150 mg (as acetone) per 100 sq cm per day. In order to prove that the betahydroxybutyrate separated from the urine was not contaminated with sufficient deuterobutyrate (unmetabolized) to account for the deuterium found in the samples of the first series, the hydroxy acid was further purified by precipitation as the silver salt. In 9 different tests an average of 23% of the betahydroxybutyrate was found to contain deuterium. The purity of the silver salt was sufficiently high so that the deuterium could not have been present as a component of unmetabolized deuterobutyrate.

It is concluded that deuterium is retained to a considerable extent in betahydroxybutyrate when present on the gamma carbon (as occurs after feeding beta-gamma di-deuterobutyric acid) although it is almost completely lost when present on the alpha carbon (after administering alpha-beta deuterobutyrate). This procedure may be used for identification of the source of the acetone bodies. It also proves that ingested butyric acid is the source of urinary betahydroxybutyrate.

[1] Rittenberg, D., and Schoenheimer, R., *J. Biol. Chem.*, 1935, **111**, 169.