While yeast generally produced a greater regularity and ease of evacuations, the moisture of the feces did not seem to be noticeably or consistently increased. The yeast feces were more bulky and easier to mix and sample. This change in the character of the feces was probably the result of yeast fermentation and greater porosity.

There were no consistent changes which could be correlated with the ingestion of yeast for the blood and urine glucose, creatinine, acidity, urea, etc.

In our study, the changes following the ingestion of one cake of yeast per meal permit the following conclusions: 1. The yeast had little effect on nitrogen metabolism. Most of the added yeast nitrogen was excreted in the feces. 2. The yeast had little effect on phosphorus metabolism. Most of the added yeast phosphorus was excreted in the feces. 3. The yeast protein does not seem to be well utilized. 4. There is no retention of uric acid following yeast ingestion as is evidenced by no change in the blood uric acid. The ingestion of yeast does not cause an increased excretion of uric acid unless the level of uric acid excretion is already high, then the ingestion of yeast causes an increased excretion of uric acid which promptly falls off when the yeast is discontinued. 5. The ingestion of yeast caused a change of intestinal flora as evidenced by the reduction of urinary phenols. 6. The ingestion of yeast caused no consistent changes in the moisture content of the feces; however, the greater bulk and porosity due to fermentation caused evacuations to be easier.

3893

Studies on Hemoglobin Formation in the Rat.

GEORGE F. CARTLAND AND F. C. KOCH.

From the Physiological Chemistry Laboratories, University of Chicago.

The purpose of these experiments was to study the relationship between the protein and vitamin composition of the diet and the hemoglobin forming process in the rat. In a study of this type, hemoglobin estimations and red corpuscle counts are of diminished significance unless the blood volume factor is controlled. For control of this factor we have developed a micro-modification of the Kieth-Rowntree plasma-dye method which makes possible repeated blood volume determinations in the rat. The method tested upon measured samples of blood *in vitro* shows an error not exceeding

4%. Applied to the rat the method yields consistent and reproducible results. With proper manipulation the mortality does not exceed 5%.

In this work 2 experimental methods have been applied. The first is of the type commonly used for anemia studies in rats with the additional precaution of determining blood volumes. Young, growing rats were fed upon various synthetic diets and the changes in hemoglobin, red corpuscle count, and blood volume recorded over periods of 8 to 12 weeks. The changes in the number of grams of circulating hemoglobin per 100 gm. body weight are taken as an index of the rat's ability to form hemoglobin upon the diet under consideration.

In later work we resorted to a severe anemia type of experiment similar to that used by G. H. Whipple[1] and his co-workers upon the dog. The hemoglobin level is reduced to approximately half its normal value and maintained at this level by repeated bleedings. The rat is bled by cardiac puncture under ether anesthesia. If, over any period of time, the blood volume and hemoglobin concentration are the same at the beginning and end of the period, the amount of hemoglobin removed by bleeding during this time represents the amount of hemoglobin produced by the rat over and above the maintenance factor. Thus, this second method enables us to measure the rate of hemoglobin formation in the rat upon various diets and under conditions of maximum stress.

Using the first type of experimental method described above, the possible relation of tryptophane to hemoglobin formation was studied. As a control for tryptophane feeding a synthetic diet was used containing 10% of wheat gluten. The mineral content of all the diets used in this work was complete and adequate since we wished to study only organic factors. A tryptophane analysis on the gluten showed that the rats upon the control diet received less than 5 mg. of tryptophane daily. The rats upon this diet did not become anemic and an increase of the tryptophane consumption to 65 mg. per rat daily failed to increase their ability to form hemoglobin.

In another series of rats, casein was compared to gluten and was found to be no better utilized by the rat for hemoglobin formation than was gluten. The effect of feeding red blood corpuscles was also studied using the 10% gluten diet as a control. The results were negative.

Using the severe anemia type of experiment described above, the rate of blood regeneration upon the 10% gluten diet was determined. It was found that under these conditions the rats regenerated the total hemoglobin content of their blood every 10 to 13 days,

and maintained this high rate of blood formation for weeks. Upon a casein diet the rate of blood regeneration was found to be no higher than upon the gluten diet. Feeding red corpuscles to the extent of 15% of the diet failed to increase the rate of blood formation over that of a control period.

The relations of vitamin A, B, and E to hemoglobin regeneration were studied, using the severe anemia type of experiment. It was found that rats in a marked state of avitaminosis due to the long continued absence of vitamins A, B, or E in the diets could regenerate their blood at the normal rate. Furthermore, the addition of vitamins A, B and E to diets deficient in these substances failed to produce any effect upon the rate of blood regeneration.

Conclusions: 1. Wheat gluten is an adequate dietary protein for promoting hemoglobin synthesis in the rat. Casein is not superior to wheat gluten for this purpose. 2. Hemoglobin and tryptophane in the diet are no better utilized than gluten for hemoglobin production in the rat. 3. The blood forming process in the rat is not dependent upon the presence of vitamins A, B, or E in the diet.

[1] Whipple, G. H., *Am. J. Physiol.*, 1925, lxxii, 395.

3894

Rate of Liberation of Tryptophane from Proteins by Enzymes.

IDA KRAUS-RAGINS. (Introduced by F. C. Koch.)

From the Physiological Chemistry Laboratories, University of Chicago.

Casein, edestin, Witte peptone and squash seed globulin were subjected to trypsin hydrolysis. At different intervals of time a portion of the respective hydrolysates was taken and tryptophane determined by the indirect Vanillin-HCl reaction.[1] At the end of one hour three-fourths of the total available tryptophane in casein was liberated, a little less than one-half was liberated from edestin and two-fifths from squash seed globulin. Witte peptone had one-third of the total tryptophane available before incubation with trypsin and at the end of the first hour two-thirds was available. Equilibrium was established in the case of Witte peptone in 24 hours, casein in 72-96 hours, edestin and squash seed globulin in 120 hours. The latter 3 proteins were subjected to the action of pepsin, trypsin and erepsin in the order given and aliquot portions were taken and analyzed for amino nitrogen and for tryptophane.