are due to Dr. V. E. Nelson and Mrs. Gladys Timson Stevenson of Iowa State College for valuable information relative to the preparation of the diets.

8422 P

Preservation of Fertility in Male and Female Rats on a Supplemented Milk Diet.

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This work is the outgrowth of the studies made by the authors on the relation of iron and copper to nutritional anemia and hemoglobin synthesis. It is well known that cow's milk is inadequate for the normal maintenance of the rat. Additions of copper and iron salts to an exclusive milk diet protect animals from nutritional anemia and produce an apparent well being in the animals of the first generation from the standpoint of hemoglobin formation, growth, and reproduction. Underhill, Orten, Mugrage, and Lewis[1] have observed no abnormalities in the organs of rats fed milk, copper, and iron for periods of more than a year. Waddell[2] reported sterility in males of the first generation receiving a milk, copper, and iron diet. The sterility was marked by a progressive testicular degeneration together with an absence of motile sperm in the seminal fluid. Ferric chloride, when added to the ration, was found to produce symptoms of sterility as early as 10 weeks after administration. Keil, Keil, and Nelson[3] found the animals on a milk, copper, and iron ration grow more slowly than those on a good stock diet. Reproduction occurred in animals of the first generation, but the second generation did not reproduce. Our results on second generation rats, together with the findings of Waddell, led us to investigate both males and females of the first and second generations in order to ascertain whether or not the sterility on this diet could be ascribed to degeneration of sex organs.

All of the animals (rats) were fed whole milk obtained from Holstein cows in the dairy herd at Iowa State College. The milk was collected directly into glass jugs, in order to avoid contamina-

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[1] Underhill, F. A., Orten, J. M., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **99**, 469.

[2] Waddell, J., *J. Nutr.*, 1931, **4**, 67.

[3] Keil, H. L., Keil, H. H., and Nelson, V. E., *Am. J. Physiol.*, 1934, **108**, 215.

tion with inorganic substances. Purified copper as $CuSO_4.5H_2O$ and iron as $FeCl_3.6H_2O$ were added to the fresh milk daily. Copper and iron salts were examined spectrographically, in order to insure a high degree of purity. Hemoglobin was determined by the Newcomer method. The animals were bled by the tail.

The trimmed testes from the males were weighed and placed in Bouin's fixing solution prior to histological sectioning. The epididymis was cut and semen squeezed into a drop of Ringer's solution on a microscopic slide, which was immediately examined for motile sperm. The males were also subjected to mating tests with unbred females from the stock colony. Many females of the second generation on a diet of milk, copper, and iron were tested for sterility at the end of an experiment by placing them with mature males from the stock diet. These animals were given the milk, copper, and iron diet. Other females were tested in a similar way, except that all of the rats were given stock ration during the period of breeding. The ovaries of the female rats were trimmed and weighed; and they were then fixed in Bouin's solution and examined histologically.

Galvanized iron wire cages placed on glass funnels were employed for metabolism cages in the studies on the composition of the urine. Analyses of urine were made on 3 groups of animals. One group had been fed milk, copper, and iron from the time of weaning; a second lot was depleted of its copper and iron stores by an exclusive milk diet until a state of anemia existed; and a third group was given the stock colony ration. The following determinations were performed on the urine: volume, specific gravity, total nitrogen, urea, ammonia, uric acid, creatine, and creatinine. The results of the experiments may be summarized as follows:

No sterility or degeneration of the testes was observed in male albino rats maintained on milk, supplemented with various amounts of purified iron as $FeCl_3$ together with copper as $CuSO_4.5H_2O$. The testicular weights from both first and second generation males on the above ration compared favorably with those obtained on the stock diet; motile sperm were found in all cases, and mating tests were positive.

The average weights of paired ovaries from female rats reared on a diet consisting of milk supplemented with pure iron as $FeCl_3$ along with copper as $CuSO_4.5H_2O$ is slightly lower than that of controls on a stock diet. This apparent abnormality did not denote sterility, since normal litters have been obtained in the second generation after the rats were given a stock ration in lieu of the milk, copper, and iron diet.

A nitrogen partition in the urine of 3 groups of female rats fed stock ration, milk, and milk plus copper and iron, respectively, indicates that the addition of copper and iron to the milk diet affects the excretion of nitrogen bodies, particularly ammonia, creatine, and creatinine.

8423 P

Effect of Methods of Preparation upon Fermentative Activity of Yeast Zymin.

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Stavely, Christensen and Fulmer[1, 2, 3, 4] presented data on the effect of several electrolytes and of ethanol upon the fermentative activity and phosphate content of yeast zymin. The zymin was prepared from brewers' yeast (furnished by Anheuser-Busch of St. Louis) by the procedure outlined by Harden.[5] Five hundred grams of yeast and 3 liters of acetone were stirred together for 10 minutes and filtered on a Buchner funnel. The mass was then mixed with one liter of acetone for 2 minutes and again filtered. The residue was coarsely powdered, stirred with 250 cc. of ether for 3 minutes, filtered, and spread out on paper in a thin layer. After standing for one hour at room temperature it was dried for 24 hours at 40-45°.

The present communication deals with data on several factors as they affect the activity of the zymin preparation. The apparatus of Harden, Thompson and Young,[6] with some modification, was employed in the determination of the evolution of carbon dioxide. The

[1] Stavely, H. E., Christensen, L. M., and Fulmer, E. I., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 877.

[2] Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 771.

[3] Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 785.

[4] Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 791.

[5] Harden, A., Alcoholic Fermentation, p. 39. Longmans, Green and Co.

[6] Harden, A., Thompson, J., and Young, W. J., *Biol. Chem. J.*, 1910, **5**, 230.