ing urine was acidified, hydrolized, and extracted with benzene, following the method of Smith and Smith.[4] The residue was dissolved in glycocol propionate and injected into immature white mice following the suggestion of Lauson, Heller, Golden and Sevringhaus.[5] We have repeatedly convinced ourselves that the tannic acid precipitates were free of any estrogenic substance.

Results. The curves depicting the cyclic excretion of estrogen and gonadotropin are pictured in Fig. 1. It appears that 1 to 3 peaks of prolan excretion may occur in a single cycle. The excretion of estrin either parallels or in some instances precedes the prolan excretion. Two distinct peaks have been observed in all our cases. In Patient D they were so close to each other that a separation may seem arbitrary. If we wish to deduce from the appearance of the hormone levels in the urine the time and incidence of ovulation, we must agree with Gustavson's statement that "the time of ovulation may vary considerably in different individuals, and that the corpus luteum may require varying periods of time to reach its full development and activity."

11458 P

Non-Identity of Gray Hair Produced by Mineral Deficiency and Vitamin Deficiency.

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The recent recognition of a specific organic food factor[1,2] necessary for the maintenance of the black fur of black or piebald rats has raised the question as to whether the phenomenon of graying noted by older workers[3,4,5,6] particularly in rats on an exclusive milk diet is due to this cause or is due to a specific mineral deficiency. Jukes and

[4] Smith, G. V., and Smith, O. W., *Am. J. Physiol.*, 1935, **112**, 340.

[5] Lauson, H. D., Heller, C. G., Golden, J. B., and Sevringhaus, E. L., *Endocrinology*, 1939, **24**, 35.

[1] Morgan, A. F., Cook, B. B., and Davison, H. G., *J. Nutrition*, 1938, **15**, 27.

[2] Lunde, G., and Kringstad, *Z. physiol. Chem.*, 1938, **257**, 201.

[3] Hartwell, G. A., *Biochem. J.*, 1923, **17**, 547.

[4] Kiel, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1931, **93**, 49.

[5] György, P., *Biochem. J.*, 1935, **29**, 741.

[6] Gorter, F. J., *Z. Vitaminforsch.*, 1935, **4**, 277.

Richardson[7] have pointed out that milk is not a good source of filtrate fraction vitamins.

The black fur of young piebald rats fed an exclusive diet of either powdered whole milk or of fresh certified milk rapidly and uniformly became gray. Addition of a supplement of 0.25 mg of iron, 0.05 mg of copper and 0.05 mg of manganese cured the grayness if it had developed or prevented any change in the color of the fur of rats which were fed milk with the above mineral supplements from the time of weaning. Attempts to ascertain which of the 3 metals is responsible have not at present yielded entirely conclusive results.

Rats developing a nutritional anemia show a marked diminution of appetite and the possibility exists that the graying of rats may be due to a diminished intake of vitamin accompanying the reduced intake of the vitamin-poor milk. This has been disproven since in 2 groups of rats fed isocaloric amounts of milk, those supplemented with iron, copper and manganese did not develop grayness whereas those fed milk alone became quite gray. It has also been found that rats that have developed grayness will become black when supplemented with the above minerals whereas the isocaloric controls without minerals remain gray. Rats, following the production of gray hair by a milk diet, when supplemented with a rice bran extract* rich in the factors of the B complex very slowly regained their black color. However, control rats fed equal quantities of the ashed rice bran extract likewise slowly became black. Hemoglobin regeneration slowly occurred in both the rats receiving rice bran and in the rats receiving rice bran ash. This indicates that the effect with milk-fed gray rats is entirely due to inorganic substances in the preparation.

Rats fed a synthetic diet consisting of purified casein, cane sugar, butter, salt mixture, cod liver oil, and supplements of synthetic vitamins B_1, B_6, and riboflavin† developed gray hair. The hemoglobin level in these rats was normal. Iron, copper, and manganese supplements in no way altered the response of these animals. However, rice bran extract in the same amount used above was effective in the cure of these animals. With the vitamin-deficient animals the ash of the rice bran was without effect. Rats given a daily ration of 50 cc of milk plus 1 g of the synthetic diet showed no graying or were cured when placed on such a diet after production of the gray hair either by mineral or vitamin deficiency.

All of the present evidence based on experiments with approxi-

[7] Jukes, T. H., and Richardson, G. A., *J. Agr. Res.*, 1938, **57**, 603.

* Obtained through the courtesy of Dr. Paul György.

† Kindly supplied by Merck and Co., Inc., Rahway, New Jersey.

mately 50 rats seems to indicate that graying of black hair in rats may result from a deficiency of a factor or factors present in the vitamin B complex and may also result from a deficiency of iron, copper, and manganese.

11459

Effects of Testosterone Propionate on Female Roller Canaries under Complete Song Isolation.

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It is commonly assumed that secondary sexual characteristics are conditioned by the secretions of the respective gonads of each sex. Singing in canaries is normally limited to the male of the species and so may be considered as a male secondary sexual characteristic.

Baldwin and Goldin[1] indicated that when testosterone propionate was administered to the female viviparous teleost, *Xiphophorus helleri* Heckel, the male secondary sexual characteristics were induced in all cases. Noble and Wurm[2] treated adult females and immatures of both sexes of the black-crowned night heron, and produced male sexual behavior. They concluded that the differences between the sexual behavior of adults of this form seem to be regulated only by proportionate differences in the amounts of male hormone normally found in these birds. Allee and Collias[3] reported crowing in hens treated with testosterone propionate, and cessation of this crowing soon after the treatment was stopped, indicating the dependence of this behavior on the male hormone.

Leonard[4] treated female roller canaries with testosterone propionate and reported that they produced song that differed from normal male song only in the greater sound volume produced by the males. He also stated that his best results were obtained when the females were "isolated" by putting them in individual cages but keeping them in

[1] Baldwin, F. M., and Goldin, H. S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 813.

[2] Noble, G. K., and Wurm, M., *Anat. Rec.*, 1938, **72**, Sup. 1, 60.

[3] Allee, W. C., and Collias, N., *Anat. Rec.*, 1938, **72**, Sup. 1, 60.

[4] Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 229.