

definite prolongation of survival time was caused by II and VI, however, neither of these appeared worthy of extended study because II failed to afford complete protection during therapy and VI exhibited a relatively high acute oral toxicity in mice.

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### 13428

#### Graying of Hair Produced by Ingestion of Phenylthiocarbamide.

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The results of previous experiments demonstrated the high degree of toxicity of the bitter tasting phenylthiocarbamide for rats (Richter and Clisby<sup>1</sup>), 1 to 2 mg usually being lethal. With these and slightly larger doses the rats died within 3 to 18 hours with one outstanding symptom, pleural edema and effusion. In some rats from 10 to 14 cc of a clear serous fluid accumulated in the thoracic cavity within only a few hours' time. By administering the drug in sublethal doses for a few days and then gradually increasing the doses over a period of several months, we succeeded in increasing the tolerance to 18 mg per day in some animals. At autopsy rats treated for several months did not show any thoracic changes, but all of them demonstrated marked hyperplasia and hyperactivity of the thyroid gland. Histologically such a gland had all the characteristics of an exophthalmic goiter. Two of these rats which originally had been solid black became gray over a saddle blanket area on the back. The observations on this effect of the drug were limited to these 2 rats since the other rats used for the experiments were either white or tan hooded.

Further observations have now been undertaken to determine (1) the consistency with which feeding phenylthiocarbamide causes the hair to turn gray, (2) the constancy of the gray hair patterns which it produces, and (3) whether after the phenylthiocarbamide is no longer given the hair returns to normal.

Including the 2 rats from the previous experiments we have a group of 23 black rats which received phenylthiocarbamide for

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<sup>1</sup> Richter, C. P., and Clisby, K. H., *Arch. Pathol.*, in press.

periods which ranged from 41 to 125 days. Each rat was kept in a separate cage containing a food cup with the stock diet and a graduated inverted 100 cc bottle with the phenylthiocarbamide solution. The concentration of the solution was started at 0.001% and increased at intervals of approximately 12 days as follows: 0.01%, 0.02%, 0.04%, 0.06%, 0.08%, 0.1%. During the experiment the actual amount of phenylthiocarbamide received by the rats per day changed from 1.3 to 14.1 mg. Thus, in order to satisfy their thirst, the rats were forced to take a certain amount of the drug at frequent intervals throughout the day and night.

With one exception the 23 rats showed definite graying after they were started on the phenylthiocarbamide. In the one exception the hair showed patterns of slightly different degrees of blackness but no definite graying. Graying of hair was first noticed as soon as 27 days in some of the rats, though it may have been present earlier and escaped notice. In most instances it was very distinct after 58 days on the phenylthiocarbamide solution.

All except 3 of the rats showed essentially the same pattern of graying, which developed progressively during the period of treatment. The hair first turned gray in back of a line which paralleled that of the ears at a distance of about 1 cm. (See Fig. 1-A.) In front of this line the hair remained black. Progressively with the increased dosage and length of time of treatment, the graying process moved along the back until it reached the lumbar region where a clear-cut pattern in the shape of a saddle blanket manifested itself. (See Fig. 1-B.) Later the graying involved all of the hair down to the tail. (See Fig. 1C.) At the same time that the graying process descended along the back, it proceeded forward under the ear and eyes and included most of the snout. In all instances, however, the top of the head remained black. (See Fig. 2-A.) It is probably significant that in human beings also the hair on the top of the head

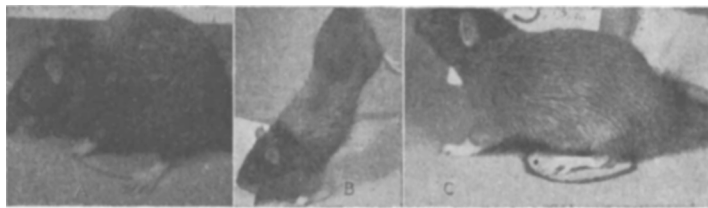


FIG. 1.

Photograph showing pattern and location of gray hair at 3 typical stages of graying in the phenylthiocarbamide-treated rats.

A. Gray hair starting on transverse line about 1 cm back of ears.

B. Saddle blanket shaped area on back which appeared later.

C. Final stage of graying. Note that hair on top of head still is black.



FIG. 2.

A. Facial pattern of graying in a black hooded rat. The gray pattern included the area between the eyes and below a longitudinal line which ran just below the eyes and ears.

B. Graying with irregular spots of white hair on different parts of the body found in three rats.

C. Pattern of brownish red area of hair found between the ears in a rat which had been well grayed but which for over a month had not received any phenylthiocarbamide.

is the last to turn gray. As is well known, the graying process usually starts at the temples and gradually works up to the top of the head.

The 3 rats which formed exceptions to the more usual pattern had irregularly shaped areas of white hair on different parts of the body. (See Fig. 2-B.)

In answer to the third question—does the hair return to normal after cessation of the drug?—five of the rats were given tap water in place of the phenylthiocarbamide solution after 41-91 days on the drug. In each of these animals the hair turned black again over the entire surface of the body, the return of color requiring approximately 83 days and taking place in reverse order to the graying process; that is, the hair along the line back of the ears was the last to return to normal. There was usually an intermediate stage during which the hair had a distinctly brownish cast, and it was noticed that in 3 rats there were sharply defined but irregular patches of a brownish red color between the ears—these too turned black again within the 90-day period. Fig. 2-C shows a photograph of such an area.

The graying areas found in most of the rats did not follow, so far as we were able to determine, any known anatomical or developmental pattern of hair coloring, except that the line over the shoulders at which the graying started coincided fairly closely with the lower edge of the hood in hooded rats. These patterns differed quite markedly from those produced by Morgan and Simms<sup>2</sup> in rats with diets which lacked one or two of the filtrate factors. None of our

<sup>2</sup> Morgan, A. F., and Simms, H. D., *J. Nutrition*, 1940, **19**, 233.

rats showed the dermatitis. The general appearance of the rats grayed by the two methods is quite different.

The results of our experiments indicate that graying of the hair may be produced by a positive factor in the diet as well as by the lack of some factor, such as pantothenic acid. It is possible that the phenylthiocarbamide may combine with the pantothenic acid or some other factor in the body and thus produce an actual deficiency of the latter.

These experiments bring more evidence for the close relationship between graying of the hair and the activity of the thyroid gland. However, the fact that graying was found in several animals in which supposedly all of the thyroid gland had been removed indicates that the phenylthiocarbamide probably has a more direct effect on the hair.

*Summary.* (1) After being placed on a stock diet and given phenylthiocarbamide solution as the sole source of fluid, with one exception 23 black or black hooded rats showed definite graying, some as early as 27 days and all within 58 days. (2) Approximately 83 days after the phenylthiocarbamide solution was replaced by tap water, five rats that had shown marked graying turned completely black again. (3) The experiments showed that graying may result from a positive factor in the diet, a poison, as well as from a deficiency of some factor.

## 13429

### A Phosphorylase in Calcifying Cartilage.\*

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It has been shown by histochemical<sup>1, 2, 3</sup> and chemical<sup>3, 4</sup> methods that cartilage cells preparatory to calcification accumulate large stores

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<sup>1</sup> Creighton, C., *Microscopic Researches on the Formative Property of Glycogen*, Adam and Charles Black, London, 1896.

<sup>2</sup> Harris, H. A., *Nature*, 1932, **130**, 996.

<sup>3</sup> Glock, G. E., *J. Physiol.*, 1940, **98**, 1.

<sup>4</sup> Hoffman, A., Lehmann, G., and Wertheimer, E., *Arch. f. d. ges. Physiol.*, 1928, **220**, 183.