The thyroid epithelial response in this cold-blooded animal was very uniform to any given dosage level. The average control epithelial cell measured 1μ in height, with extreme variations of 0.5μ from this dimension. In strongly stimulated thyroid tissue, epithelia as high as 12μ were seen. It was found convenient to recognize different grades of stimulation. A 50% increase in average follicle cell height (cells 2μ high) was considered the minimal reaction. Increases of 175% (3.5μ), 300% (5μ), and 500% (9 to 10μ), respectively, were adjudged the arbitrary limits for delineation of the reactions referred to as mild, strong, and very strong.

11830

Further Experiments on Nutritional Achromotrichia in Rats and Mice.

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From the results of previous experiments it has been concluded that "concentrates of pantothenic acid, with a purification up to 40 to 50%, appear to contain one factor but not the only factor concerned in the cure of nutritional achromotrichia in rats."[1]

Later, it became evident that this factor, which proved to be heat labile in alkaline solution, is identical with pantothenic acid.[2] In a group of rats kept on a diet free from pantothenic acid, administration of daily doses of from 75 to 100 μg of synthetic pantothenic acid† brought about cure of the nutritional achromotrichia in from 5 to 7 weeks.[2] In some rats the cure was slower and in a few it was never quite complete.

These experiments were repeated with black mice‡ kept under

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1 György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, 1940, **132**, 789.

2 György, P., and Poling, C. E., *Science*, 1940, **92**, 202.

† Synthetic pantothenic acid was generously put at our disposal by Merck & Co. Inc., Rahway, N.J.

‡ Grateful acknowledgment is made to Dr. Agnes Fay Morgan, University of California, Berkeley, for the strain of black rats used in the experiments on nutritional achromotrichia and to Dr. William C. Barrett, Department of Anatomy, School of Medicine of Western Reserve University, Cleveland, for the C-57 strain of black mice.

similar nutritional conditions. The diet used was particularly conducive[3] to the production of acrodynia in rats. It consisted of 18 parts of purified casein,§ 10 of dried heated egg white, 58 of sucrose, 8 of melted butter fat, 2 of cod liver oil and 4 of salt mixture. Raw egg white was heated over a steam bath for 3 hours in order to destroy the injurious effect ("egg white injury"[4]) it has on animals. The diet fed both to the rats and the mice was supplemented with 20 μg each of thiamin chloride, pyridoxine and riboflavin.

In from 3 to 5 weeks, apart from the skin lesions, the fur of the mice became gray or brownish, similar in color to the fur of wild house mice or more silvery. Daily doses of from 50 to 100 μg of calcium pantothenate (synthetic, Merck) produced quick and definite effect on the depigmentation of the fur, with practical cure in from 3 to 5 weeks. The therapeutic effect on the cutaneous manifestations was even more rapid.[5] These lesions were similar to those described in rats as Type II[6] and to those recently observed in mice under similar conditions by Norris and Hauschildt.[7]

A group of 24 black rats and 8 black mice were kept under prolonged observation without the institution of any further change in the experimental conditions. They received the supplement of pantothenic acid for as long as 6 months after the initial achromotrichia had been cured.

It became evident that the diet fed these animals could not be regarded as complete. Changes in the pigment metabolism of the fur again became apparent in both the rats and the mice. In addition there were (1) cutaneous lesions in some of the rats similar to those reported by Morgan and Simms,[8] (2) a predominantly scaly dermatosis and dandruff in some of the rats and mice, and (3) a thinning of the pelt in a few rats and mice. The pathological changes in the content of pigment in the fur never reached the stage of depigmentation seen in rats and mice kept on a diet deficient in pantothenic acid, and they were characterized, as a rule, either by brownish discoloration of the black fur or by single gray hairs interspersed in the fur without generalized graying, or by both. The brownish discoloration was more conspicuous than the moderate, diffuse gray-

[3] György, P., and Eckardt, R. E., *Biochem. J.*, 1940, **34**, 1143.

§ Generously furnished by the S.M.A. Corporation, Chagrin Falls, Ohio.

[4] György, P., *J. Biol. Chem.*, 1939, **131**, 733; György, P., Kuhn, R., and Lederer, E., *J. Biol. Chem.*, 1930, **131**, 745; Birch, T. W., and György, P., *J. Biol. Chem.*, 1939, **131**, 761.

[5] György, P., and Poling, C. E., data to be published.

[6] György, P., and Eckardt, R. E., *Nature* (London), 1939, **144**, 512.

[7] Norris, E. R., and Hauschildt, J., *Science*, 1940, **92**, 316.

[8] Morgan, A. F., and Simms, H. D., *J. Nutrition*, 1940, **19**, 233.

ing, the fur in the latter case being only slightly different from normal fur. These secondary changes appeared usually from 4 to 12 weeks after the initial nutritional achromotrichia had been cured by the administration of pantothenic acid.

When 100 μg of a highly purified concentrate of biotin[9] were added to the daily diet fed this group of rats and mice, or 0.25 μg of crystalline biotin methyl ester[‖] to the diet of 2 rats, definite and quick improvement in the pigment metabolism of the fur was noted.

These observations are in agreement with those made in rats kept on a diet that produced typical egg white injury,[10] the pelts of which showed distinct signs of depigmentation, such as mild graying, brownish discoloration and rusting. The present group of rats and mice was fed a diet which contained only a small amount of egg white (10%) and even this amount was detoxified to a large extent by heating. However, the possibility that it may have affected the pigment metabolism of the pelt cannot be denied. Be that as it may, the experiments here reported prove that there is a possible interrelationship between biotin and pigment metabolism of the fur in rats and mice.

The order in which pantothenic acid and biotin were administered was found to be of decisive importance in the present series of experiments. When supplements of biotin were given before supplements of pantothenic acid, achromotrichia was somewhat aggravated, and the curative effect of biotin became evident only when it was given during the period of secondary changes in the fur, after the initial achromotrichia had been relieved by pantothenic acid.

In black rats and mice treated with pantothenic acid and biotin, pigmentation of the fur still did not necessarily become entirely normal. It should be borne in mind, however, that the fur of black rats kept on the stock diet[11] shows, as a rule, with progressing age, a slight brownish discoloration and is often interspersed with single gray hairs. The question arises whether this condition should be considered a function of age rather than one of incomplete diet.

Conclusion. Pantothenic acid is only one although probably the most important factor in the prevention of nutritional achromotrichia as it is seen in rats and mice used in experiments on the

[9] du Vigneaud, V., Melville, D. B., György, P., and Rose, C. S., *Science*, 1940, **92**, 62.

[‖] Kindly furnished by Professor F. Kögl, Utrecht, Netherlands.

[10] György, P., in Pfaundler, A., and Schlossmann, M. v., *Handbuch der Kinderheilkunde*, 1935, **10**, 55.

[11] Sherman, H. C., and Muhlfeld, M., *J. Biol. Chem.*, 1922, **53**, 41; Smith, A. H., and Bing, F. C., *J. Nutrition*, 1928, **1**, 179.

vitamin B_2 complex. Biotin is an additional factor that is connected with the maintenance of the normal pigment metabolism of the fur in rats and mice under special dietary conditions.

11831

Effects of Epinephrine and Amphetamine on Respiration and Blood Pressure in Different Postures.

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The cause of the overventilation of the erect posture, as indicated by lowering of the alveolar CO_2 tension, is unknown. Main[1] suggested that it might be due to the fact that the pressure in the carotid sinus is lowered about 20 mm Hg on standing,[2] which should therefore stimulate respiration. Turner[3] believed it might be due to cerebral ischemia. I decided to test the former hypothesis by raising the blood pressure of a standing subject, to see if this would remove the excess respiratory stimulation by increasing the pressure in the carotid sinus back to the normal level.

I decided to use epinephrine subcutaneously to raise the blood pressure. Two reports in the literature on the effect of epinephrine on alveolar CO_2 are somewhat at variance; Arnoldi[4] reporting a rise, and Peters,[5] a slight fall. However, the side effects of this drug, such as marked tremor, cold perspiration, feeling of apprehension, and the possible production of a lactacidemia,[6] made it advisable to control the results with some other vasopressor drug which would not produce such deleterious side effects. Amphetamine sulfate subcutaneously was selected because of its entirely different side effects: euphoria, and the complete lack of tremor and sweating.

It was soon found that the subjects, males between the ages of 20 and 30, varied greatly in their response to epinephrine; some being severely affected by 0.5 cc, and others showing very little ill effects from 0.75 cc of a 1 to 1000 solution in ampules (Parke-Davis). No ill effects were noticed in any case from amphetamine

1 Main, R., *Va. Med. Monthly*, 1937, **64**, 330.

2 Loman, J., and Myerson, A., *Am. J. Psychiat.*, 1936, **92**, 791.

3 Turner, A., *Am. J. Physiol.*, 1927, **80**, 601.

4 Arnoldi, W., *Deut. Med. Woch.*, 1924, **50**, 1397.

5 Peters, J., *Am. J. Physiol.*, 1917, **44**, 84.

6 Cori, C., *J. Biol. Chem.*, 1925, **63**, 253.