

FEATHER DEPIGMENTATION AND PANTOTHENIC ACID DEFICIENCY IN CHICKS

DEPIGMENTATION of the hair due to deficiency in the filtrate fraction of the vitamin B₂ complex has been demonstrated in rats, dogs and foxes.¹ Similar graying has been reported² in rats on a diet low in pantothenic acid. In order to determine whether these deficiencies produce depigmentation in the feathers of birds, fifty-nine Black Minorca chicks, one week of age, were placed on a purified, vitamin-free diet similar to that used by Almquist.³ In addition, the following crystalline vitamin supplements were given daily by pipette to each chick: 100 micrograms each of riboflavin and thiamin chloride and 50 micrograms each of pyridoxine and nicotinic acid.⁴ Vitamins A and D as liver oil were added directly to the diet. Forty-seven chicks received daily in addition to the above supplements 5 micrograms of calcium pantothenate or an amount of yeast extract containing this amount of pantothenic acid as determined by microbiological assay. The yeast extract was made from brewers' yeast extracted with acetone and treated with fuller's earth. Twenty-one chicks as positive controls received 280 micrograms of calcium pantothenate or its equivalent in assayed yeast extract. The average weight of the birds at the beginning of the experimental period was 60 grams. The average weight in-

acid supplements showed little or none of this depigmentation or distortion.

On the thirtieth day, depigmentation was most evident in the newly developed feathers of the ventral and femoral tracts in the low pantothenic acid group. In four of the birds on high pantothenic acid supplements, depigmentation of a lesser degree was noted. The entire group on low pantothenic acid appeared much lighter in color than the positive controls. It was clear that a high level of either calcium pantothenate or yeast extract protected against this distortion and depigmentation of the feathers, whereas the low levels of either, while allowing the birds to grow slightly, did not prevent feather changes.

The depigmentation was manifested microscopically as both a reduction in size of pigment granules and in numbers of these granules. The loss of barbules produced a barring effect particularly evident in the feathers of the femoral tract.

Eight chicks (average weight, 104 gm) receiving the low pantothenic acid supplements and nine (average weight, 212 gm) receiving the high pantothenic acid supplements were killed at the conclusion of the experimental period and tissue samples of liver, kidney, leg muscle and brain were assayed⁵ for pantothenic acid by the microbiological method of Pennington, Snell and Williams.⁶ The results of this assay are given in Table I.

TABLE 1

	P. A. intake daily	Liver	Kidney	Muscle	Brain
Aver. and range of P. A. content per mg. tissue	Adequate Low	53.6[51.7-55.7] 40.9[37.5-44.4]	34.5[33.3-36.3] 31.1[23.2-39.1]	11.6[10.7-13.8] 3.3[2.7- 3.9]	22.7[19.8-27.9] 8.9[7.5-10.3]

crease over the 66-day experimental period was 46 grams for the chicks on low pantothenic acid and 151 grams for the birds on high pantothenic acid supplements.

Feather samples taken from the chicks on the low pantothenic acid supplements on the sixteenth day of the experiment showed definite areas of partial depigmentation in both shafts and barbs with some distortion of the shafts and loss of barbules. Such areas were particularly striking in the primary feathers of the wing. Feathers of the group on high pantothenic

acid supplements showed little or none of this depigmentation or distortion. It is evident that the pantothenic acid content of the kidneys and to a lesser degree the livers of the deficient chicks was only a little less than that of the controls and that the greatest differences were present in the brain and muscle. This is not completely in agreement with Snell, Pennington and Williams,⁷ who found that all the tissues of their deficient chicks were considerably lower than those of their positive controls.

The depigmentation phenomenon may be due wholly to pantothenic acid deficiency or some other factor may also be involved. In another experiment on a new group of fourteen chicks, the attempt was made to discover whether the feather depigmentation and distortion were solely a vitamin deficiency effect or

¹ A. F. Morgan, H. G. Davison and B. B. Cook, *Jour. Nutrition*, 15: 27, 1938; A. F. Morgan and H. D. Simms, *Jour. Nutrition*, 19: 133, 1940.

² K. Unna, G. V. Richards and W. L. Sampson, *Jour. Nutrition*, 22: 553, 1941.

³ H. J. Almquist and E. Meechi, *Proc. Soc. Exper. Biol. and Med.*, 48: 526, 1940.

⁴ We are grateful for gifts of crystalline pyridoxine and of calcium pantothenate from Merck and Company, Rahway, New Jersey.

⁵ We are indebted to Relda Cailleau for these microbiological assays.

⁶ D. Pennington, E. E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 135: 213, 1940.

⁷ E. E. Snell, D. Pennington and R. J. Williams, *Jour. Biol. Chem.*, 133: 559, 1940.

resulted partly from inanition. All the chicks in both high and low pantothenic acid-supplemented groups were forced-fed the same amounts of food each day. This was unsuccessful as growth ceased entirely in the chicks on the low pantothenic acid level, and nearly all of them died after two weeks.

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IDENTIFICATION OF SELENIUM INDICATOR SPECIES OF *ASTRAGALUS* BY GERMINATION TESTS

INDICATOR plants belonging to the genus *Astragalus* are of great aid in locating seleniferous areas that may be capable of producing toxic forage and grain.¹ From chemical analyses of plants collected in the field, 28 species of *Astragalus* are known to be selenium accumulators and indicators, whereas 54 other species of *Astragalus* absorb only small amounts of selenium and are not limited to seleniferous soils.² It is important that many of the remaining 218 species in North America and 1,200 species in the rest of the world be examined for their ability to absorb selenium.

The present paper describes a simple test with germinating seedlings that enables one to determine whether or not a given species of *Astragalus* is capable of accumulating selenium. The test is based on an earlier observation that a selenium indicator species tolerates and is even stimulated by a high concentration of sodium selenite, whereas a non-indicator species is severely poisoned by a low concentration of this salt.³ No other case has been reported in which closely related species differ so markedly in their response to a mineral poison.

It has been found that the addition of 20 parts per million of selenium (as sodium selenite) to the culture solution has no observable effect upon the early seedling growth of an indicator species of *Astragalus*, whereas it completely inhibits root development of a non-indicator species.

The culture technique is an adaptation of a method that has been used for many years.⁴ Seeds of most of the species included in these tests were collected during the summer of 1941. To soften their coats, the seeds were treated for from 30 to 60 minutes with concentrated sulphuric acid. They were then soaked

in tap water for three hours and subsequently allowed to germinate on wet filter paper in a glass moist chamber. After about 24 hours, when the seedlings were approximately 10 mm in length, they were transferred to culture vessels. A piece of paraffined bobbinet (1/16-inch mesh) was stretched over the top of a 300-cc beaker and fastened by a ligature of paraffined linen thread. This beaker was placed in a 600-cc beaker, and both the inner beaker and the space around it were filled to the level of the netting with culture solution. Twenty-five seedlings were placed upon the netting, with their roots dipping into the solution. Two cultures of each species of *Astragalus* were prepared, one supplied with the usual mineral salts⁵ and the other receiving in addition 20 parts per million of selenium (as sodium selenite). The cultures were kept in darkness at 28°–29° C., and a moist chamber was provided for each during the first day by covering the larger beaker with an inverted watch glass. At the end of three days, the seedlings were put in chrom-acetic fixing solution, and the lengths of roots and hypocotyls were later recorded. I am indebted to Dr. Sydney S. Greenfield for assistance in making these tests.

The results are summarized in the following tabulation, in which a *plus* sign signifies a selenium indicator species and a *minus* sign denotes a non-indicator species. The roots of the indicator species made the same amount of growth (25–30 mm) in the absence of selenium and in the presence of 20 parts per million. Root growth of the non-indicator species was completely inhibited in the solution containing 20 parts per million of selenium but amounted to 25–30 mm in the selenium-free solution. The hypocotyls of the non-indicator species in the selenium solution grew only about 5 mm, while those of all the other cultures grew 20–30 mm.

BISULCATI

- + *Astragalus bisulcatus* (Hook.) Gray (Wyoming)
- + *A. haydenianus* Gray (Colorado)
- + *A. oocalycis* Jones (Colorado)

GALEGIFORMES

- + *A. racemosus* Pursh (South Dakota)
- *A. drummondii* Hooker (Wyoming)

LONCHOCARPI

- + *A. osterhoutii* Jones (Colorado)
- *A. lonchocarpus* Torr. (New Mexico)

OCREATI

- + *A. confertiflorus* Gray (Utah)
- + *A. flavus* Nutt. (Wyoming)

PODO-SCLEROCARPI

- + *A. grayi* Parry (Wyoming)
- + *A. pectinatus* Dougl. (Wyoming)
- + *A. rafaelsensis* Jones (Utah)
- + *A. toanus* Jones (Nevada)
- *A. canonis* Jones (California)

⁵ See footnote 3.

¹ For a general review of the selenium problem, see: S. F. Trelease, *The Scientific Monthly*, 54: 12, January, 1942.

² O. A. Beath, C. S. Gilbert and H. F. Eppson, *Am. Jour. Bot.*, 28: 887, 1941.

³ S. F. Trelease and H. M. Trelease, *Am. Jour. Bot.*, 26: 530, 1939.

⁴ S. F. Trelease and H. M. Trelease, *Bot. Gaz.*, 80: 74, 1925.