

further work has established either (i) the validity of the strain as a new type, to be assigned a number in accordance with the recommendations of the International Bacteriological Nomenclature Subcommittee on Streptococci and Pneumococci, or (ii) its identity with a previously recognized type.

Whatever the outcome of future serologic studies with the Red Lake strain, there is no doubt that, under some circumstances, it is nephritogenic and must therefore be taken into account, together with types 12 and 4, in studies on the relationship of group-A streptococci to acute glomerulonephritis.

References and Notes

1. H. Kleinman, *Minnesota Med.* **37**, 479 (1954).
2. We gratefully acknowledge the technical assistance of Helen Ashworth and the cooperation and assistance with time and materials of the staff of the Red Lake Indian Hospital under the direction of Herman Kleinman; of A. J. Chesley, Minnesota State Health Officer; of the staff of the Minnesota Department of Health Laboratory under the direction of Henry Bauer; of Charles F. Federspiel, statistician, Communicable Disease Center; and of Cecil Reinstein, Epidemic Intelligence Service Officer, Communicable Disease Center, who was in charge of the epidemiological investigation.
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Accumulation of Arginine in Plants Afflicted with Iron-Deficiency Type Chlorosis

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Iron deficiency in plants leads to the development of a characteristic chlorosis of the leaves. The same type of chlorosis develops when certain plants are

grown in calcareous soil, and since analyses of the foliage often show an adequate iron content, this lime-induced chlorosis apparently results from a failure of iron to function in metabolic processes (1, 2). The same type of chlorosis also results from other treatments, such as the presence of nickel or cobalt (3), which apparently interfere with the function of iron.

Plants afflicted with the iron-deficiency type of chlorosis are known to have an increased content of soluble nitrogen in the foliage (4). This suggests that iron may be involved in nitrogen metabolism, a view that is supported by the observation that plants suffering from lime-induced chlorosis frequently recover if nitrogen is supplied as ammonium salts rather than as nitrates (2, 5, 6). Most of the available information on the nitrogen metabolism of chlorotic plants is furnished by the extensive analyses of nitrogenous fractions in chlorotic and green plants that have been reported by Iljin (4). Iljin has concluded that

... residual nitrogen compounds [that is, nitrogen compounds not accounted for as ammonia, amide, protein, or amino nitrogen] are absent in healthy foliage or are present in small amounts only, but may account for 40 to 65 percent of the soluble nitrogen in chlorotic plants.

It seemed to us that the identification of this unknown nitrogenous material would be of interest and might give some clue to the nature of metabolic changes associated with iron deficiency.

Chlorotic and green blueberry, apple, and magnolia leaves were fractionated, and the free amino acids were identified by paper chromatography. The leaves were extracted with 80-percent alcohol and the extract was diluted with one-fifth of its volume of water and mixed with an equal volume of chloroform. The aqueous layer was separated, diluted with water, and the amino acids were adsorbed in a Dowex 50 column. The amino acids were eluted with 10-percent ammonium hydroxide, and the eluate was evaporated to dryness in high vacuum over phosphorus pentoxide

Table 1. Comparison of composition of chlorotic and green leaves.

Description	Dry weight (mg/cm ²)	Nitrogen (μg/cm ²)			
		Total	80-percent alcohol-soluble	Total amino acids	Arginine
Blueberry, chlorotic*	6.1	195	44	25	21
Blueberry, chlorotic (Co)†	3.5	149	55	35	28
Blueberry, chlorotic (Ni)‡	5.2	161	37	27	24
Blueberry, green§	8.1	191	18	3	1
Apple, chlorotic	3.1	115	19	11	6
Apple green#	3.8	122	7	1	0
Magnolia stellata, chlorotic**	3.4	150	31	16	13
Magnolia stellata, green††	5.1	132	11	1	0

* Average of 12 plants, both field samples and sand culture.

† Cobalt-induced chlorotic plant grown in sand culture.

‡ Average of three nickel-induced chlorotic plants grown in sand culture.

§ Average of 14 control plants, both field samples and sand culture.

|| Average of two plants, pot culture, wet.

Average of two plants, pot culture, dry.

** Average of two samples of chlorotic leaves.

†† Average of two samples of green leaves from the same plant.

Table 2. Data showing recovery from nickel-induced chlorosis in blueberry plants following treatment with ferrous ethylenediaminetetraacetate.

Description	Dry weight (mg/cm ²)	Nitrogen (μg/cm ²)			
		Total	80-percent alcohol-soluble	Total amino acids	Arginine
Chlorotic, before treatment	5.1	183	44	32	28
4 days after treatment*	4.7	169	43	31	28
13 days after treatment	6.8	139	14	4	3
26 days after treatment	6.9	107	11	1	0.2

* Approximately 30 ml of ferrous ethylenediaminetetraacetate solution containing 20 ppm of iron was injected into a branch bearing four actively growing shoots. Almost complete regreening had occurred after 26 days.

The dried samples were stored at -18°C . Aliquots were chromatographed quantitatively on Whatman No. 1 paper using procedures similar to those of Levy and Chung (7) and Thompson and Steward (8). (The data have not been corrected for any losses that may have occurred during the isolation procedure. Controls indicated that the recoveries of amino acids were at least 80 percent.) The content of arginine in the samples was also determined colorimetrically (9), using the difference between determinations before and after treatment with arginase. Results are expressed in terms of leaf area. Total nitrogen and 80-percent-alcohol-soluble nitrogen were determined by the micro-Kjeldahl method.

The results (Tables 1 and 2) indicate that an accumulation of free arginine is characteristic of the nitrogen metabolism of plants afflicted with iron-deficiency chlorosis. The disappearance of free arginine during recovery from chlorosis is shown in Table 2. Amino acids, other than arginine, that commonly occur in the leaves studied are (identification by position on the chromatogram) alanine, aspartic acid, asparagine, glutamic acid, and glutamine, with occasional traces of γ -aminobutyric acid, glycine, lysine, proline, serine, threonine, and valine.

Three of the four nitrogen atoms present in arginine (the three nitrogens in the guanidino group) would have been missed by Iljin's analyses and would have been included in his "residual nitrogen" fraction. Therefore, a high free-arginine content, which seems to be characteristic of chlorotic leaves, would account for the "residual nitrogen" described by Iljin.

It is of interest in connection with the findings reported here (10) that von Euler and Burström (11) found a high free-arginine content in the white borders of leaves of a variegated *Pelargonium* plant. (Leaves from two other variegated plants showed normal arginine content.) It should also be mentioned that Hewitt *et al.* (12) have reported that free arginine increases more than other amino acids during manganese deficiency in cauliflower, and these authors suggest that manganese is involved in amino acid metabolism.

References and Notes

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Differential Sensitivity of the Eye to Intermittent White Light

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The eye has often, in the past, been scorned as a temporal analyzer, the ear always seeming to take precedence. Strangely, few data exist that allow a direct, valid comparison between the two modalities. This paper (1) reports the results of an experiment that measured the speed of response of the human eye in a new way. The procedure was to measure the difference-limens for intermittent white light at 16 frequencies in the range of 1 to 45 cy/sec.

The intermittent stimulus light was produced by a Sylvania R1131C glow-modulator tube operated independently from two variable-frequency square-wave generators. Glow-modulator tubes follow precisely an electric input, and the square-wave generator was determined to have an accuracy of 0.5 percent in the range of the frequencies studied.

The stimulus spot provided by the tube subtended 1° of visual angle and had a homogeneous luminance of 98 millilamberts. It appeared in the center of a white surround subtending 71° held at a luminance of 44 millilamberts.