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# Provisional New Type of Group A Streptococci Associated with Nephritis

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During the summer and fall of 1953 an outbreak of streptococcus infections was observed among children at the Red Lake Indian Reservation in Minnesota. Kleinman (1) has described the clinical manifestations of sore throat, tonsillitis, scarlet fever, and, particularly, acute glomerulonephritis and pyoderma. The outbreak had reached epidemic proportions by September 1953, at which time the Communicable Disease Center was invited to cooperate with the Bureau of Indian Affairs and the Minnesota Department of Health in an epidemiological investigation. This paper (2) deals with the bacteriologic aspects of the resulting study.

Because of limited laboratory facilities at the reservation, the cultures were sent on Loeffler's slants to the Minnesota Department of Health Laboratory in Minneapolis, where the primary isolations were made by one of us (M.S.M.). Isolates of beta hemolytic streptococci were mailed to the Streptococcus Laboratory in Chamblee, Georgia, for serologic identification.

Group A streptococci were isolated from 42 individuals at Red Lake and from six of a "control" group at the Cass Lake Indian Reservation 50 mi away. Each of the 48 cultures was tested with precipitating antiserums for types 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 44, 46, and 47 (3). Since none of the cultures reacted with these antiserums, rabbits were immunized with two of the Red Lake cultures: DS-C300, from a nose and throat culture of a child with albuminuria but with no clinical nephritis, and DS-C274, isolated in almost pure culture from a skin lesion of a child with acute glomerulonephritis. A satisfactory antibody response to C300 was obtained after three immunization series: the first with a vaccine prepared by the standard procedure (4), the second and third with "glow-bead" vaccines (5). The data presented here are based on results obtained with antiserum against C300. Preliminary tests with C274 antiserum were confirmatory.

The C300 antiserum reacted with a majority of the 42 Red Lake cultures, including C274, and with three of the six Cass Lake cultures. It did not react with any of the aforementioned types or with two provisional types that were available for study. Results of the tests with the Red Lake cultures are shown in Table 1. Of particular interest is the fact that the C300 antiserum reacted with 12 (92 percent ) of the 13 cultures from children with clinical nephritis and with 17 (100 percent) of the cultures from those with pyoderma, the two lesions most characteristic of the epidemic.

In view of the experimental results, we believe that the C300 culture is representative of a serologically specific strain of group-A beta hemolytic streptococci and that its antiserum has characterized the etiological agent of the Red Lake epidemic. Therefore, we propose that any culture reacting with C300 antiserum be referred to as a Red Lake strain until

Table 1.	. Serologic ŕ	eactions of	' group-A	streptococci	isolated	from	42 individuals	at Ree	l Lake	Indian	Reservation.
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	Streptococcal			
Diagnosis of individual from which strain isolated	Antiserum to Red Lake strain (DS-C300) only	No available antiserums including DS-C300 (untypable*)	Totals	
Nephritis only	$\left. \begin{array}{c} 3 \\ \end{array} \right\} $ 12	1 1	4 } 13 nephritis	
Nephritis and pyoderma	9∫	0 / ] 0	9 ] 17 pyoderma	
Pyoderma only	8	0	8	
Sore throat, tonsillitis, or scarlet fever	$\frac{1}{2}$	3	5	
No clinical disease	5	11	16	
TOTAL	27 (64%)	15 (36%)	42~(100%)	

\* Tested with antiserums for types listed in text.

† Eleven cultures from skin lesions, six from nose and throat.

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

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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 limeinduced 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.

		Nitrogen ( $\mu g/cm^2$ )					
Description	${ m Dry\ weight}\ ({ m mg/cm^2})$	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	<b>28</b>		
Blueberry, chlorotic (Ni)‡	5.2	161	37	<b>27</b>	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.

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