VARIATION OCCURRING IN GROUP A STREPTOCOCCI DURING HUMAN INFECTION

Progressive Loss of M Substance Correlated with Increasing Susceptibility to Bacteriostasis

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During the course of routine typing of group A streptococci recovered from nasopharyngeal cultures of patients suffering from hemolytic streptococcal infections of the upper respiratory tract, it was noted that, although there was no change in the colony morphology on sheep or rabbit blood agar plates, the streptococci obtained during convalescence often produced less type-specific M substance than the streptococci isolated during the acute phase of infection. It was also observed that the streptococci isolated in the convalescent period were often susceptible to the bacteriostatic action of normal children's blood, whereas the microorganisms isolated in the early period of the infection were invariably resistant.

These findings suggested that following infection in man the antigenic composition of the hemolytic streptococcus may undergo changes similar to those known to occur in the pneumococcus (1) and diphtheria bacillus (2) following natural infections with these bacteria.

Todd (3) and more recently Ward and Lyons (4) have shown that the ability of hemolytic streptococci to multiply in normal human blood is a reliable and sensitive method for distinguishing different strain variants and for determining mouse virulence. Hare (5) has also reported that saprophytic strains of hemolytic streptococci isolated from the human birth canal can be differentiated from pathogenic strains by their susceptibility to the bactericidal action of human blood. Furthermore, various workers (6–9) have shown that the type-specific M antigen is necessary for the exhibition of virulence of group A streptococci; and that the presence of this protein antigen characterizes the matt variant, whereas the glossy variant produces little or no M substance.

Since previous reports of hemolytic streptococcal variation have been confined for the most part to *in vitro* or animal studies, a similar study was undertaken to investigate this phenomenon during the natural course of infection in man. For this purpose the cultures were obtained at weekly intervals from patients during the acute, convalescent, and carrier stages of their streptococcal respiratory infections; and the individual strains were subjected to tests for their ability to resist the bacteriostatic action of normal human blood and to synthesize the type-specific M protein antigen.

Experimental Methods

The source of the hemolytic streptococcal strains, methods, and preparation of material employed was as follows:—

Hemolytic Streptococci.—The streptococcal strains were recovered from nose and throat cultures of patients with acute hemolytic streptococcal infections of the upper respiratory tract at the time of hospital admission and at weekly intervals thereafter during their hospital stay. In some instances streptococci were not found every week during the convalescent period. A number of patients received therapeutic doses of sulfadiazine for 7 to 14 days between the 2nd and 4th weeks of illness in an effort to clear up their carrier state; several were also treated in a similar manner for purulent complications. Since this therapy often failed to clear the carrier state, subsequent strains were usually obtained from these patients.

The culture swabs were streaked immediately in duplicate on fresh 5 per cent rabbit and sheep blood agar plates and incubated at 37°C. for 18 to 24 hours. Several representative colonies of all hemolytic streptococci were picked and streaked on fresh blood agar plates for further identification or transferred directly to broth for serological grouping and typing by the precipitin method (10, 11). Following isolation and classification, the hemolytic streptococci were immediately lyophilized.

To provide uniform handling and to maintain as nearly as possible the original conditions of the culture, each strain was taken from the lyophilized stock and subcultured in 45 cc. of Todd-Hewitt broth. Following incubation at 37°C. for 16 to 18 hours, the streptococci were collected by centrifugation, resuspended in 2 cc. of fresh broth, and distributed in 0.2 cc. portions into a series of small glass tubes which were kept in the dry CO_2 ice box until used.

Bacteriostasis of Group A Streptococci in Normal Human Blood.—The details of the method to determine the capacity of hemolytic streptococci to resist phagocytosis and multiply in normal human blood have been described (12). In this study no added antibody was employed in the bacteriostatic experiments. The day before each experiment the tubes containing the series of strains obtained from a single patient were thawed from the frozen state, a loopful from each was inoculated into 45 cc. of Todd-Hewitt broth, and incubated for 10 to 12 hours in a water bath at 37°C. The streptococcal cells were separated by centrifugation and resuspended in the original volume of fresh broth. Tenfold serial dilutions varying from 10^{-1} through 10^{-6} were prepared from 1 cc. of each culture for use in the bacteriostatic tests; the remainder of the culture was used to prepare the M extracts.

Titration of Type-Specific M Protein Antigen.—Crude extracts made by heating streptococci with hydrochloric acid were prepared as described by Lancefield (10) from each culture studied after removal of a sample for use in the bacteriostatic tests described above. Twofold serial dilutions of these extracts in saline were tested with constant amounts of homologous type-specific absorbed rabbit antiserum in capillary pipettes as described by Swift, Wilson, and Lancefield (11). The titre of the type-specific M protein antigen was taken as the highest dilution of the extract which gave a positive precipitin reaction with the homologous rabbit antiserum. The group-specific C-anti-C reaction was not involved in these titrations, as antibody to the C carbohydrate had been completely removed from the sera by absorption with heterologous-type group A streptococcal cells.

EXPERIMENTAL RESULTS

Resistance of Strains to the Bacteriostatic Action of Normal Human Blood.—Two hundred and fifty-one strains isolated from 54 patients, who suffered 56 different streptococcal infections, were tested for their ability to resist the

bacteriostatic action of normal human blood. The period of observation for each patient ranged from 4 to 55 weeks (average 11 weeks) during which time cultures were made at weekly intervals. From 2 to 15 strains (average 4.5) of the same serological type were obtained on culture from each patient following his infection. Although cultures taken during the 1st week of infection were invariably positive for group A streptococci, those taken later in convalescence were often negative; and in the case of some patients, many weeks separated cultures positive for these microorganisms. In some instances it is not possible, therefore, to state the precise time at which the strains began to lose resistance to the bacteriostatic action of normal blood.

The strains isolated on the initial culture taken within the 1st week of the patients' infections always resisted bacteriostasis and multiplied in all dilutions of the culture from 10^{-1} through 10^{-6}. Such strains are arbitrarily termed "highly resistant." Strains termed "resistant" signify at least a ++ diminution in growth in the 10^{-6} dilution as compared with the corresponding dilution of the initial culture; those strains termed "susceptible" and "highly susceptible" signify similar diminutions in growth in the 10^{-5} and 10^{-4} dilutions respectively. Results with this test have been highly reproducible; and we believe a ++ growth difference in a single dilution is significant. Some of the "highly susceptible" strains showed much greater differences and failed to grow in culture dilutions as low as 10^{-2}, which is equivalent to the destruction of 100,000 streptococcal cells by 0.25 cc. of normal human blood.

Resistant strains of streptococci were found throughout the entire period of observation among 33, or 59 per cent of the 56 infections studied. In the first part of Table I are recorded the serological types of these resistant strains and the last week after infection that each was isolated, which also corresponds to the period of observation. Similar data with regard to the strains which lost resistance to bacteriostasis are tabulated in Table II. From these data it is apparent that in 23, or 41 per cent, of the infections the strains showed a progressive decrease in resistance. Thus, although the streptococci in the initial cultures were always "highly resistant," strains from 13 infections changed during the period that the patient was studied to "highly susceptible" to the bacteriostatic action of normal human blood; an additional 8 became "susceptible;" and 2 others became "resistant." It is also apparent that the time of development of this change was variable despite the fact that the exact time the change occurred could not always be ascertained. The change from "highly resistant" to "resistant" occurred as early as the 2nd week but was frequently noted in the 7th or 8th weeks; the change to "susceptible" took place as early as the 2nd week and as late as the 19th week; and in half the cases studied in which the strains changed to "highly susceptible," this occurred before the 8th week, while in the remainder this degree of change was delayed up to 24 weeks. Those strains which showed decreased resistance were not necessarily carried

by their hosts longer than the strains which remained fully resistant to bacteriostasis.

A comparison of the length of time that patients with persistently resistant strains were studied with the time at which susceptibility first became apparent in the susceptible strains shows that the former were observed long enough to

TABLE I

*Duration of Resistance to Bacteriostasis of Group A Streptococci Isolated from Patients at Weekly Intervals Following Infection**

Serological type	Strains maintaining resistance‡		Strains losing resistance§	
	No. of infections	Observation period of patients with resistant strains: last wk. in which streptococci were isolated	No. of infections	1st wk. in which susceptible variants appeared
1	1	5	2	2, 3
3	3	5, 11, 13	2	3, 16
5	1	7		
6	2	10, 26	3	3, 5, 6
12	2	4, 4		
14	1	7	1	8
17			1	8
18	1	5		
19	14	5, 5, 5, 6, 6, 6, 7, 7, 8, 11, 12, 12, 19, 21	7	3, 6, 6, 7, 9, 9, 10
26			3	7, 19, 21
29			1	4
30	3	4, 7, 8		
32	1	20		
33			1	2
38	1	6	1	3
NC‖	3	5, 18, 20	1	2

* Streptococci not obtained on every culture from each patient.

‡ From 33 patients (33 infections) 126 strains isolated at weekly intervals maintained resistance to bacteriostasis throughout observation period.

§ From 21 patients (23 infections) 125 strains were isolated at weekly intervals: Susceptible variants first appeared at the times indicated.

‖ NC indicates strains not classified with available sera.

detect such strain variations if they had occurred (Table I). Some of the resistant strains were carried in the patient's throat as long as 20 to 26 weeks following the onset of infection, and most of the patients were under observation for longer periods than it took for variants to appear in the series in which the streptococci became susceptible. This comparison is shown in parallel columns in Table I. The majority of the strains which showed lowered resistance developed these variants comparatively early: 13 within the first 6 weeks, 9 others within 10 weeks, and the remaining 3 within 16, 19, and 21 weeks respectively.

TABLE II

Strains of Group A Streptococci Losing Resistance to Bacteriostatic Action of Normal Children's Blood

Serological type	Bacteriostasis of 125 streptococcal strains isolated at weekly intervals from 21 patients following 23 different infections*			
	Highly resistant‡	Resistant‡	Susceptible‡	Highly susceptible‡
	Wk. following infection streptococci were isolated			
1	1, 2		3, 4	6, 7, 8, 9, 10, 11, 12
1	1	2	3, 4, 5	
3	1			16
3	1, 2	3		4, 5
6	1, 2, 4		6, 10, 16	20
6	1		3, 4, 5, 6, 8, 10, 14	
6	1, 2, 3, 4		5, 6, 7, 9	
14	1, 2, 3, 4, 5, 6, 7	8	9, 10	11
17	1, 2, 3, 4, 5, 6, 7	8, 15	17, 20	24, 28, 51, 55
19	1		9	
19	1		10	
19	1	7		
19	1		9	
19	1			6, 7
19	1	3, 6		
19	1, 5			6
26	1, 2, 3			21
26	1, 2, 6, 15		19, 23	
26	1, 2, 3, 4, 5, 6		7	8, 14, 19
29	1, 2, 3			4
33	1			2, 4, 8
38	1		3, 5, 7	
NC§	1		2, 3	11

* Streptococci not obtained on every culture from each patient.

‡ "Highly resistant" signifies resistance to bacteriostasis in all dilutions of culture from 10^{-1} through 10^{-6}; "resistant" signifies at least a ++ diminution in growth in the 10^{-6} dilution as compared with the corresponding dilution of the initial culture; "susceptible" and "highly susceptible" signify similar diminutions in growth in the 10^{-5} and 10^{-4} dilutions respectively.

§ NC indicates strains not classified with available sera.

Variants were recovered from 9 of 25 patients who received therapeutic doses of sulfadiazine for 7 to 14 days in an effort to clear up the carrier state or purulent complications. From the remaining 16 patients treated with sulfadiazine, only resistant strains were isolated. Not only did sulfadiazine treatment fail to influence the actual number of variants with decreased resistance, but it appeared to have no relation to the serological types in which these variants occurred. Thus 7 of the 9 patients from whom variant strains were obtained were infected with type 19 streptococci, one had a type 3, and another a type 26 infection. Similarly, patients with consistently resistant strains also had infections with a variety of serological types: 11 had type 19 infections, 2 type 30, and one each type 6, 18, or 38 infections.

Although an insufficient number of strains of the various serological types were available to determine whether this variation has any relationship to the type or is characteristic of particular strains, some of the data may be significant in this respect. Of the 21 series of type 19 strains studied, 20 were isolated from individual patients who developed scarlet fever in the same epidemic (13). In 7 of these 20 patients, type 19 variants which proved susceptible to bacteriostasis appeared during convalescence; while from the remaining 13 patients, only resistant strains were isolated during the period of observation. Although in several other types all the strains were on the contrary alike in resistance or susceptibility, the numbers in each type were small: the strains recovered from the 3 patients with type 26 infections all developed susceptible variants; and only resistant strains were recovered from the 3 patients with type 30 infections and the 2 patients infected with type 12 streptococci. In the remaining types some strains remained resistant throughout the study of the patient and some in each serological type encountered became susceptible. Consequently, no correlation could be established between these strain variations and the serological types involved.

Todd and Lancefield (6) have demonstrated that mouse-virulent matt variants of group A streptococci can be converted to mouse-avirulent glossy variants by repeated subculture in media containing homologous type-specific rabbit antisera. It appeared of interest, therefore, to determine whether any relation existed between the occurrence of variation among these strains and the appearance of type-specific antibodies in the sera of patients from whom the streptococci were obtained. In a previous study (14) reported elsewhere, type-specific bacteriostatic antibody determinations had been made on the sera of 37 patients whose series of strains were studied in this present investigation. Both resistant and susceptible strains were found among patients who developed bacteriostatic antibodies: 16 patients had resistant strains and 15 had susceptible strains. Among the remaining 6 patients with no type-specific antibody response, susceptible variants appeared in every case. It is, therefore, apparent that there was no correlation between the appearance of susceptible

variants and type-specific antibody formation. Moreover, no correlation was found between the time of appearance of the variant strains and the formation of antibodies, nor was there any relation between the height of the antibody response and the degree of variation. Apparently, the variation of these streptococcal strains cannot be explained by the concomitant appearance of type-specific bacteriostatic antibodies.

Since 23 of the 56 streptococcal infections were followed by complications, an attempt was made to ascertain whether there was any correlation between the appearance of this variation phenomenon and the development of these complications, but no such relationship was found. Of the 23 complicated infections, strains which showed some degree of variation were recovered from 11 (3 purulent and 8 rheumatic fever), and only stable strains were isolated from the remaining 12 (3 purulent and 9 rheumatic fever). As far as could be determined, the complications appeared in all instances before the isolation of the variant strains of streptococci. From the 33 remaining infections which were uncomplicated, 12 yielded strains which showed variation and 21 yielded strains which showed no change.

Relation of the Type-Specific M Protein Antigen to Bacteriostasis.—In a previous study (12) it has been shown that bacteriostatic susceptible variants produce less M protein than do the parent resistant strains. To determine whether this relationship held in the present study, the 251 strains were also tested for their capacity to produce the M protein antigen. From 4 patients (4 infections), 17 strains were not included because they did not fall into known serological types. From the remaining 50 patients with 52 infections, 234 strains of known serological types were tested. In 42 per cent of the 52 infections, strains isolated in the convalescent and carrier stages showed an increasing susceptibility to bacteriostasis correlated with a progressive loss of M substance; whereas in the remaining 58 per cent, resistance to bacteriostasis and the capacity to produce M protein were maintained throughout the observation period.

In Table III are recorded the results of titration for the M protein antigen prepared from the highly resistant strain recovered on the initial culture and the less resistant strain isolated on the last culture. The streptococcal extracts containing M protein antigen were diluted in a twofold serial manner, and tested with type-specific absorbed rabbit antisera. The data in Table III reveal that the susceptible variant strain in each series produced less M substance by at least a 2-tube dilution difference than did the highly resistant parent strain; 4 of the strains showed a 4-tube dilution difference; 5, a 5-tube difference; 2, a 6-tube and 1, a 7-tube dilution difference. Similar titrations were also done on each of the series of stable strains which showed no evident variation, but these results are not tabulated because no difference was found in the amount of M substance produced by the strains in each respective series.

From 3 patients (one with a type 14 infection and 2 with type 26 infections),

TABLE III

Production of Type-Specific M Substance of Group A Streptococci Highly Resistant to Bacteriostasis as Compared to Their Susceptible Variants

Serological type	Bacteriostatic variant			
	Highly resistant		Susceptible*	
	Wk. isolated	M titre‡	Wk. isolated	M titre‡
1	1	1:32	12	1:2
1	1	1:8	5	1:1
3	1	1:16	16	1:4
3	1	1:32	5	1:1
6	1	1:32	20	1:4
6	1	1:16	14	1:2
6	1	1:16	9	1:4
14	1	1:32	11	0
17	1	1:16	55	1:4
19	1	1:32	9	1:4
19	1	1:16	10	1:4
19	1	1:32	9	1:8
19	1	1:64	7	1:2
19	1	1:32	6	1:2
19	1	1:32	7	1:2
19	1	1:64	6	1:2
26	1	1:64	21	0
26	1	1:64	23	1:2
26	1	1:32	19	0
29	1	1:32	4	1:1
33	1	1:32	8	1:2
38	1	1:8	7	1:1

* Indicates variants which show any degree of diminution of resistance from original highly resistant strains.

‡ Titre signifies highest dilution of type-specific M protein extract which gave a positive reaction with homologous rabbit antiserum.

strains were recovered which were so degraded that no M protein could be demonstrated in acid extracts of these variants by the precipitin technique. It is possible that others would have become degraded to a similar degree had the patients been followed longer. In Tables IV *a*, IV *b*, and IV *c* are recorded the

degrees of resistance to bacteriostasis and the M titre for every strain isolated in series from each of these 3 patients. From Table IV *a* it is evident that at about the 8th week after the onset of a type 14 infection a beginning loss of resistance to bacteriostasis occurred but with little apparent variation in M protein synthesis; however, during the 9th week, a further decrease in resistance to bacteriostasis occurred and for the first time a definite decrease in the titre of M was noted. By the 11th week there was little resistance to bacteriostasis and no M substance was demonstrable on precipitin test. After 25 passages through mice, the capacity of this degraded variant to resist the bacteriostatic action of normal human blood and to produce the M protein substance was restored to the levels noted on primary isolation.

From Tables IV *b* and IV *c* it can be seen that strains degraded to a similar degree were recovered from the other 2 patients. Unfortunately the cultures taken on one of these patients between the 3rd and 21st week revealed no streptococci so that the gradual change is not shown in Table IV *c*. The most degraded variants in these 2 series also regained after mouse passage their capacities to elaborate the M substance and to resist the bacteriostatic action of human blood.

The series of strains shown in Table IV *c* is of particular interest because special means were available for identification of the variant strain, 24 RS50, and the original strain, 11 RS50. This latter strain was unusual in two respects: It lacked the T antigen found in most type 26 strains; and it had a peculiar and characteristic T antigen which has so far not been observed in any other strain studied in this laboratory. Because after an interval of 18 weeks, during which time no group A streptococci were obtainable on serial weekly cultures, the group A streptococci recovered from this patient no longer synthesized type 26 M substance, a special study was undertaken to determine whether the peculiar T antigen of the original strain was still present in the later or variant strain. These 2 strains were used to immunize respective groups of rabbits, and antibodies specific for the peculiar T antigen of the original strain 11 RS50 were demonstrated in the sera of both groups of rabbits by direct agglutination reactions and specific cross-absorption of the 2 kinds of antisera with the 2 strains studied. Control absorption tests with type 26 strains, which contained the usual T antigen of type 26, failed to remove the T antibodies peculiar to the RS50 series of strains. Finding this unusual T antigen in both of these strains is additional evidence that the variant strain 24 RS50 was actually a derivative of the original strain 11 RS50 isolated from this patient.

Moreover, by means of agglutination tests with rabbit antisera directed toward the type 14 and type 26 T antigens respectively, it was also possible to show that the variants listed in Tables IV *a* and IV *b* had the same T antigens as those in the original strains isolated from each of these patients.

The degraded variants in these 3 series of strains appear to be relatively stable

TABLE IV *a*

Resistance to Bacteriostasis Correlated with Production of Type-Specific M Protein Antigen by Group A Type 14 Streptococci Isolated from a Patient with Acute Pharyngitis

Streptococcal strain No.	Serological type	Wk. of infection strain isolated	Bacteriostasis of streptococcal strains — Dilution of culture						Titration of type-specific M extract — Dilution of extract						
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	1:1	1:2	1:4	1:8	1:16	1:32	1:64
23 RS84	14	1	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0
24 RS84	14	2	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0
25 RS84	14	3	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0
26 RS84	14	4	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0
27 RS84	14	5	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0
28 RS84	14	6	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0
29 RS84	14	7	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0
32 RS84	14	8	++++	++++	++++	+++	++	0	+++	+++	++	++	++	+	0
33 RS84	14	9	++++	++++	++++	++±	9	0	+++	+++	++	+	±	0	0
34 RS84	14	10	++++	++++	+++	++	0	0	+++	+++	++	+	±	0	0
35 RS84	14	11	++	2	0	0	0	0	0	0	0	0	0	0	0
35 RS84/25/O*	14	—	++++	++++	++++	++++	+++	++	+++	+++	++	++	++	+	0

In bacteriostasis of strains, degree of growth is indicated on a ++++ to + scale; fewer than 10 colonies are represented in arabic numerals; 0 indicates no growth.

In titration of M antigen, the degree of precipitation is indicated on a ++++ to ± scale; 0 represents no precipitation.

* This strain was passed through mice 25 times and regained its capacity to produce the M substance.

TABLE IV *b*

Resistance to Bacteriostasis Correlated with Production of Type-Specific M Protein Antigen by Group A Type 26 Streptococci Isolated from a Patient with Acute Pharyngitis

Streptococcal strain No.	Serological type	Wk. of infection strain isolated	Bacteriostasis of streptococcal strains — Dilution of culture						Titration of type-specific M extract — Dilution of extract						
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	1:1	1:2	1:4	1:8	1:16	1:32	1:64
14 RS15	26	1	++++	++++	++++	++++	+++	++	++±	++	++	++	++	+	0
15 RS15	26	2	++++	++++	++++	++++	+++	++	++±	++±	++	++	++	+	0
16 RS15	26	3	++++	++++	++++	++++	+++	++	++	++	++	++	++	+	0
17 RS15	26	4	++++	++++	++++	++++	+++	++	++	++	++	++	++	+	0
18 RS15	26	5	++++	++++	++++	++++	+++	++	++	++	++	++	++	±	±
19 RS15	26	6	++++	++++	++++	++++	++	+	++	++	++	++	+	±	0
20 RS15	26	7	++++	++++	++++	++++	+	8	++	++	++	++	+	±	0
21 RS15	26	8	++++	+++	++±	++	0	0	++	++	++	+	0	0	0
22 RS15	26	14	+	7	0	0	0	0	±	0	0	0	0	0	0
23 RS15	26	19	+	0	0	0	0	0	0	0	0	0	0	0	0
23 RS15/25/O*	26	—	++++	++++	++++	++++	+++	++	+++	+++	+++	++	++	+	0

Same symbols for bacteriostasis of strains and titration of M antigen as used in Table IV *a*.

* This strain was passed through mice 25 times and regained its capacity to produce the M substance.

TABLE IV *c*

Resistance to Bacteriostasis Correlated with Production of Type-Specific M Protein Antigen by Group A Type 26 Streptococci Isolated from a Patient with Acute Pharyngitis

Streptococcal strain No.	Serological type	Wk. of infection strain isolated	Bacteriostasis of streptococcal strains — Dilution of culture						Titration of type-specific M extract — Dilution of extract						
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	1:1	1:2	1:4	1:8	1:16	1:32	1:64
11 RS50	26	1	++++	++++	++++	++++	++++	+++	+++	++±	++±	++	+	+	±
12 RS50	26	2	++++	++++	++++	++++	+++	+++	+++	+++	++	++	+	±	0
13 RS50	26	3	++++	++++	++++	++++	+++	++	+++	+++	++	++	+	+	0
24 RS50	26	21	++++	++	7	0	0	0	0	0	0	0	0	0	0
24 RS50/21/O*	26	—	++++	++++	++++	++++	+++	++	+++	+++	++	++	+	±	0

Same symbols for bacteriostasis of strains and titration of M antigen as used in Table IV *a*.

* This strain was passed through mice 21 times and regained its capacity to produce the M substance.

antigenically since no evidence of spontaneous reversion to the original state has been noted after several years. It may also be noteworthy that only 2 of the 3 patients from whom these degraded variants were isolated developed type-specific bacteriostatic antibodies following infection. This indicates, as previously noted, that the production of type-specific bacteriostatic antibodies does not explain the variation.

The colony morphology was carefully investigated on fresh sheep and rabbit blood agar plates to see whether any difference could be observed by this means between the bacteriostatic-resistant M-producing parent strain and the degraded bacteriostatic-susceptible non-M-producing variant. No significant difference was detected, nor was capsule formation of any help in differentiating the 2 variants of the various strains.

In an attempt to explain the loss of M antigen, the variants which completely lost their ability to produce M were repeatedly tested for the production of streptococcal proteinase, an extracellular proteolytic enzyme which digests the M protein (15). This proteolytic enzyme could not be demonstrated in cultures of any of the parent strains or their variants.

These data show that group A streptococci may lose their capacity to elaborate the type-specific M protein antigen and become susceptible to the bacteriostatic action of human blood while the bacteria are residing in the host's tissues; but the factor or factors which are responsible for the occurrence of these phenomena are still unknown.

DISCUSSION

From the evidence presented in this report, it appears that in the course of group A streptococcal infections in man, variant strains often occur which are characterized by a decreased capacity to produce the type-specific M protein antigen, and a concomitant decrease in resistance to the bacteriostatic action of normal human blood. This variation occurs gradually over a period of weeks during convalescence and the carrier period while the streptococci persist in the nasopharyngeal tissues of the host. In the 3 series of strains each characterized by the development of variants which lost their capacity to produce the M antigen, the same T antigen was retained in each instance as that present in the strain originally isolated from the patient. These degraded variants are relatively stable since reversion after numerous subcultures on bacteriological media has not occurred; the original capacities, however, to produce the M substance and to resist bacteriostasis can be restored by serial mouse passage. Since the T antigen was found to be similar in both the variant and original strains, and since the M-producing capacity was restored to the variant on mouse passage, it appears that these degraded strains were derived from the original strains which caused the patient's infection.

In seeking an explanation for the variation, it was found that its occurrence

was not related to the production of type-specific antibody by the host, nor was it dependent on the production by the variants of a proteolytic enzyme (15) which is known to destroy the M protein.

The possibility that the degraded variants were present at all times during the infection and that in convalescence they were selected on culture by chance seems very unlikely; nevertheless this must be considered. The fact that multiple colonies picked from a single culture failed to reveal differences in variation seems to be against this possibility. Moreover, the gradual and progressive changes only in the direction of further degradation also suggest that no chance selection occurred.

The decrease in resistance of streptococcal strains to bacteriostasis by normal blood has uniformly been associated with a diminished capacity of the streptococcal cells to produce the M protein antigen. The M substance, which is probably a surface antigen, has been shown to play an important rôle in the exhibition of virulence, and in these respects is analogous to the type-specific polysaccharide of the pneumococcus. From the evidence at hand it would appear that the M protein plays an important part in protecting the streptococcal cell from phagocytosis by the leukocytes of normal blood. In some instances the decreased resistance to bacteriostasis was evident before a definite decrease in the capacity of the streptococcal cells to produce the M protein could be demonstrated. This is probably explained by a difference in the sensitiveness of the two tests: the bacteriostatic test being more sensitive for demonstrating a slight loss of the M substance than the precipitin test.

Although knowledge of the factor or factors which are responsible for the occurrence of variation during the natural course of group A streptococcal infections remains obscure, there is ample evidence to show that this phenomenon does occur frequently enough possibly to be of considerable importance from the standpoint of preventive medicine. There is some evidence to indicate that certain persons harboring hemolytic streptococci are more likely to disseminate infection than others (16, 17). It is possible that among other factors which make one host less dangerous than another is the fact that in some the strains may undergo variation with an associated decrease in virulence and invasiveness. What perhaps is more important is that the majority of strains do not lose their capacity to synthesize the M protein for relatively long periods after the onset of infection and are therefore potentially dangerous pathogens.

SUMMARY

A study was made of the variation occurring in group A streptococci during the natural course of infection in man. From 54 patients with 56 different group A streptococcal infections of the upper respiratory tract, 251 strains of streptococci, isolated at weekly intervals following infection, were tested for their capacity to resist the bacteriostatic action of normal human blood. In 52 of the infections the streptococci were of recognized serological types and were

also tested for variation in their ability to produce the type-specific M protein antigen. Strains isolated in the 1st week of infection were uniformly highly resistant to bacteriostasis and elaborated large amounts of M substance. In 42 per cent of the 52 infections, strains isolated in the convalescent and carrier stages showed an increasing susceptibility to bacteriostasis correlated with a progressive loss of M substance; whereas in the remaining 58 per cent resistance to bacteriostasis and the capacity to produce M protein were maintained throughout the observation period.

In 3 different infections, the streptococci became so degraded that no M protein could be demonstrated in acid extracts of these variants. Concomitantly these strains became highly susceptible to bacteriostasis. Spontaneous reversion did not occur, but serial mouse passage reestablished these functions. These degraded variants had the same T antigen as their respective original strains.

No evidence was obtained that variation of group A streptococci in resistance to bacteriostasis or in the ability to produce the type-specific M antigen was associated (*a*) with the appearance of type-specific bacteriostatic antibodies; (*b*) with any particular serological type of streptococcus; (*c*) with the production of streptococcal proteinase which digests the M protein; (*d*) with the therapeutic administration of sulfadiazine; or (*e*) with the development of complications.

The possible relationship of these observations to the problem of the "dangerous carrier" of group A hemolytic streptococci is discussed.

BIBLIOGRAPHY

1. Shibley, G. S., and Rogers, E. S., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30,** 6.
2. Yü, H., *J. Bact.*, 1930, **20,** 107.
3. Todd, E. W., *Brit. J. Exp. Path.*, 1927, **8,** 289.
4. Ward, H. K., and Lyons, C., *J. Exp. Med.*, 1935, **61,** 515.
5. Hare, R., *J. Path. and Bact.*, 1934, **38,** 129.
6. Todd, E. W., and Lancefield, R. C., *J. Exp. Med.*, 1928, **48,** 751.
7. Lancefield, R. C., and Todd, E. W., *J. Exp. Med.*, 1928, **48,** 769.
8. Hirst, G. K., and Lancefield, R. C., *J. Exp. Med.*, 1939, **69,** 425.
9. Lancefield, R. C., *J. Exp. Med.*, 1940, **71,** 521.
10. Lancefield, R. C., *J. Exp. Med.*, 1928, **47,** 91.
11. Swift, H. F., Wilson, A. T., and Lancefield, R. C., *J. Exp. Med.*, 1943, **78,** 127.
12. Rothbard, S., *J. Exp. Med.*, 1945, **82,** 93.
13. Watson, R. F., Schwentker, F. F., Fetherston, J. E., and Rothbard, S., *J. Am. Med. Assn.*, 1943, **122,** 730.
14. Rothbard, S., Watson, R. F., Swift, H. F., and Wilson, A. T., *Arch. Int. Med.*, in press.
15. Elliott, S. D., *J. Exp. Med.*, 1945, **81,** 573.
16. Coburn, A. F., *U. S. Nav. Med. Bull.*, 1944, **42,** 325.
17. Hamburger, M., Jr., Green, M. J., and Hamburger, V. G., *J. Infect. Dis.*, 1945, **77,** 68.