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GLOBULIN LEVELS WITH SPECIAL REFERENCE TO THE
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QUANTITATIVE ANTISTREPTOKINASE STUDIES IN PATIENTS INFECTED WITH GROUP A HEMOLYTIC STREPTOCOCCI: A COMPARISON WITH SERUM ANTISTREPTOLYSIN AND GAMMA GLOBULIN LEVELS WITH SPECIAL REFERENCE TO THE OCCURRENCE OF RHEUMATIC FEVER¹

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The phenomenon of liquefaction of human fibrin clots by broth cultures or culture filtrates of *beta* hemolytic streptococci was first described by Tillett and Garner in 1933 (1). The complex mechanism of this reaction, thought at first to be a direct fibrinolytic effect of a streptococcal enzyme, has been elucidated in recent years. Milstone (2) demonstrated that a substance present in plasma, termed "lytic factor," is required for dissolution of the clot; and as a result of the work of Christensen (3, 4), confirmed independently by Kaplan (5), it is now known that the "lytic factor" is a proteolytic enzyme normally present in the plasma as an inactive precursor. The role of the active streptococcal substance is that of an activator of the proteinase precursor, converting it into an active enzyme in a manner analogous to the conversion of trypsinogen to trypsin by enterokinase. The active serum proteinase is responsible for digestion of the fibrin clot. In view of the accumulated evidence regarding the nature of streptococcal fibrinolysis, Christensen and MacLeod (6) have proposed the term *streptokinase* to replace the term fibrinolysin originally applied to the streptococcal component of the system. They have further suggested the name *plasminogen* for the inactive form of the serum proteinase and *plasmin* for the active enzyme. This terminology has been adopted in the present report.

The activity of streptokinase derived from group A hemolytic streptococci in promoting the lysis of human fibrin clots is in sharp contrast to its minimal effect on clots of other animal species (1, 4, 7, 8). Although the basis for the apparent

human specificity remains somewhat uncertain, this property of streptokinase is unique and other products of streptococcal cells, such as streptolysin, erythrogenin, and streptococcal proteinase do not exhibit comparable specificity. Thus, streptokinase deserves special attention in the study of rheumatic fever, which is a sequela of streptococcal infection that appears to be limited to the human species.

Numerous immunological studies on streptokinase (fibrinolysin) have been carried out with human plasma (9, 10, 11, 12, 13) using the anti-fibrinolysin test described by Tillett and Garner (1), but because of the essentially qualitative nature of the test, it has not been possible to obtain definitive information comparable to that derived from the more precise quantitative titrations of other serum antibodies. Kaplan, in collaboration with the Commission on Acute Respiratory Diseases, published in 1946 (14) the first quantitative method for estimation of antibody directed against streptokinase. This method involves a neutralization test in which a constant, standardized amount of streptokinase is incubated with serial dilutions of the serum to be tested, following which an indicator system consisting of fibrinogen, plasminogen and thrombin is added. The end-point of the test is defined as the reciprocal of the highest dilution of serum which completely prevents lysis of the clot during a second period of incubation. The results of individual titrations proved to be sufficiently reproducible on repeated tests to establish the method as a useful quantitative procedure. The most serious objection that can be raised to the method is that it has not been possible up to the present time to standardize the plasminogen-streptokinase system in specific units so that antibody titers obtained in one laboratory can be compared directly with those of other lab-

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oratories. In a given study, however, valuable data can be obtained by using a single lot of streptokinase and of human plasma Cohn Fraction I (containing both fibrinogen and plasminogen) for the entire series of tests.

In the present study, quantitative antistreptokinase determinations have been carried out on serial specimens of serum obtained from patients during an epidemic of scarlet fever. The primary aim of the investigation was to determine whether or not significant differences in the pattern of antistreptokinase response exist between patients who developed rheumatic fever subsequent to the attack of scarlet fever and those who did not. Antistreptolysin O titers and the gamma globulin levels of the same sera are presented for comparison.

MATERIALS AND METHODS

Buffered saline solution: 0.05M veronal buffer, pH 7.5, diluted to 0.01M with 0.85 per cent NaCl solution was used throughout in diluting the various reagents. This solution is hereafter referred to as buffered saline solution.

Fibrinogen and plasminogen: A lyophilized preparation of Fraction I (Cohn) of human plasma² served as the source of both fibrinogen and plasminogen. A 0.6 per cent solution in buffered saline was used both in standardizing the streptokinase preparation and in the quantitative antibody determinations. Solutions of this concentration formed a firm clot promptly upon the addition of thrombin.

Thrombin: Topical thrombin,³ of bovine origin, supplied in ampoules containing 5000 units, was dissolved in 5 cc. of physiological saline solution and further diluted 1:10 in buffered saline solution.

Streptokinase: A single lot of streptokinase was used throughout these experiments. A strain of group A hemolytic streptococcus, H105,⁴ was grown overnight in neopeptone dialysate broth (15). The next morning sterile 50 per cent glucose solution (40 cc. per liter of culture) and phenol red (10 mg. per liter of culture) were added to the culture and incubation continued another five hours during which time the pH of the culture was maintained at or slightly above 7.0 by the frequent addition of 5N NaOH. This procedure resulted in a tenfold increase of streptokinase activity. The culture was centrifuged and the supernate filtered through a Coors No. 3 filter. Approximately 300 cc. of non-dialyzed neopeptone broth had previously been passed through this filter, because it was found that a filter not so treated quantitatively absorbs streptokinase out of

dialyzed neopeptone broth. One-cc. amounts of the sterile filtrate were placed in small tubes and stored in a CO₂ chest at -70° C.

Standardization of streptokinase: The procedures used for the standardization of streptokinase and for the quantitative determination of antistreptokinase are similar to, but not identical with, those described by Kaplan (14). In each of a series of tubes were placed the following: 1.0 cc. of varying dilutions of streptokinase, 1.0 cc. of 0.6 per cent Fraction I solution, and 0.2 cc. of the thrombin solution. The tubes were immediately shaken and, when clotting had occurred, were incubated in a water bath at 37° C. for 30 minutes. The highest dilution of streptokinase which just effected complete dissolution of the fibrin clot was used in the determination of antistreptokinase.

Determination of antistreptokinase in serum: Twofold dilutions of serum were used, beginning with a dilution of 1:10. To 0.5 cc. of the serum dilutions was added 0.5 cc. of streptokinase in the dilution determined by the method outlined above. These tubes were mixed by shaking and then incubated in a water bath at 37° C. for 30 minutes to allow for antigen-antibody combination. According to Kaplan (16) this is 95-98 per cent complete in 30 minutes. The tubes were then placed in an ice-water bath to prevent as far as possible any enzymatic activity during the time required for the addition of the indicator system. To each tube was added 1.0 cc. of the 0.6 per cent Fraction I solution (containing plasminogen and fibrinogen). There was then added 0.2 cc. of the thrombin solution and the tubes shaken immediately to insure the formation of a uniform clot. The tubes were re-incubated at 37° C. for 60 minutes. The criteria for reading the rest are those described by Kaplan. The end-point was taken as the reciprocal of the serum dilution which completely prevented lysis of the clot as determined by the failure of the clot to slide when the tube was gently tapped in the inverted position. As a control on the activity of the streptokinase preparation, a tube in which the serum dilution was replaced by buffered saline solution was included in each series of dilutions. Complete lysis was expected in this tube. The largest number of sera which could be conveniently tested at one time was found to be six. An indication of the reproducibility of the test is given by the results of 50 repeat titrations. The end-points were identical in 38, and varied by only one tube in the remaining 12. Therefore, a rise in antibody titer of two or more tubes was considered to be significant.

Antistreptolysin O: The method used for the determination of antistreptolysin O was that described by Todd as modified by Hodge and Swift (17). A two-tube rise in titer was considered significant. It should be noted that the dilution increments in the determination of antistreptolysin O differ from those used in the antistreptokinase test.

Gamma globulin: The gamma globulin levels were determined by the turbidimetric method described by Kunkel (18). The turbidity readings were converted

² We are indebted to Sharp and Dohme, Inc., for a generous supply of Fraction I.

³ Manufactured by Parke, Davis and Co.

⁴ Strain H105 was obtained from Dr. W. S. Tillett and was designated Co in his laboratory (1).

into values expressed in gms. per cent on the basis of standardization of the turbidimetric readings by electrophoretic determination of gamma globulin.

Titration of the streptokinase produced by individual strains of group A hemolytic streptococci: The procedure used is similar to that reported by the Commission on Acute Respiratory Diseases (19). A lyophilized culture of the organism to be tested was inoculated into 5.0 cc. of Todd-Hewitt broth containing 0.1 cc. of defibrinated rabbit blood. After incubation for 22 hours, 0.05 cc. of the culture was transferred to 5.0 cc. of Todd-Hewitt broth and incubated at 37° C. for 18 to 20 hours. After this period of growth, two drops of 0.01 per cent phenol red solution were added to the culture and the pH was adjusted to pH 7.0-7.5 with 1N NaOH. The culture was then centrifuged and the supernate tested undiluted and in dilutions of 1:5, 1:10, 1:20, and 1:30. The

test system consisted of 1.0 cc. of the culture supernate diluted in buffered saline, 1.0 cc. of the 0.6 per cent Fraction I solution, and 0.2 cc. of the thrombin solution. The tubes were incubated in a water bath at 37° C. for 60 minutes, and the end-point was taken as that dilution of culture supernate which produced complete lysis of the fibrin clot. In order to avoid the possible effect of variations in the culture media on streptokinase production, a single lot of broth was used throughout.

Case material: The case material employed in this study was derived from an epidemic of scarlet fever in young adult males at the Great Lakes Naval Training Station, and all patients were admitted between February 26, 1946, and May 2, 1946. With the cooperation of the U. S. Naval Medical Research Unit No. 4, throat cultures and specimens of serum were obtained from these patients on admission to the hospital and at weekly

TABLE I
Detailed bacteriological and antibody data on 90 cases of group A hemolytic streptococcal infection

Group	Case	Streptococcus type*					Antistreptokinase† titer					Antistreptolysin O† titer					Gamma globulin grams per cent				
		0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.	0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.	0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.	0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.
I	49	30	30	30	30		40	40	40	80		250	300	300	300	150	0.72	0.86	0.86	0.89	
	123	30	30	30	30	30	40	40	80	160	150	150	200	200	200	150	0.83	0.86	0.89	1.05	0.98
	192	30	30	30	30		40	80	320	320	75	100	150	150		0.77	0.72	0.81	0.92		
	198	30	30	30	30		10	10	320	80	300	250	200	600		1.17	1.22	1.14	1.50		
	279	30	30	30	30	NC	80	80	160	320	300	250	250	250	250	250	1.14	0.98	1.08	1.22	1.14
	290	30	30	30	30		40	80	160	160	150	400	300	250	250	100	0.81	1.17	1.17	1.14	
	304	30	30	30	—	—	40	80	80	80	80	150	150	100	100	100	0.72	0.83	0.89	0.81	0.81
	11	30	NC	—	—	—	40	80	80	80	50	100	150	150	150	100	0.72	0.75	0.81	0.81	
	324	30	30	30	NC	NC	160	160	640	640	150	200	250	250	250	200	0.77	0.77	0.81	0.89	
	63	19	19	19	19	19	10	20	40	40	150	150	200	250	200	100	0.83	0.92	0.92	0.98	0.89
	108	19	19	NC	19	19	0	40	40	160	160	0	100	200	200	100	0.89	0.89	0.89	0.92	0.98
	377	19	19	19	19	19	40	40	160	320	75	100	150	100	100	100	0.70	0.72	0.83	0.89	
	295	19	19	19	NC	NC	20	40	40	40	50	100	150	100	100	100	0.64	1.17	0.95	0.75	0.86
	316	19	19	19	19	19	160	320	640	640	75	100	150	250	250	300	0.77	0.95	0.75	0.86	
	131	17	17	17	17	17	20	320	320	320	25	250	200	250	250	100	0.62	0.72	0.77	0.98	
	288	3	3	3	—	—	40	40	40	40	80	150	250	400	500	100	0.83	0.98	1.00	1.14	0.89
	310	1	1	1	1	1	160	160	160	160	150	150	200	250	250	100	0.81	0.95	1.00	0.95	
	Average					55	99	216	214	140	129	184	213	241	150	0.81	0.91	0.93	0.99	0.95	
II	55	17	30	30	19	NC	40	160	640	640	25	25	50	50	200	0.72	0.83	0.89	1.05		
	97	30	30	17	—	NC	160	160	640	1280	100	100	150	250	200	0.95	1.08	1.38	1.55	1.38	
	99	30	30	1	1		0	40	80	80	0	0	25	25	25	0.72	0.81	0.95	0.95		
	58	30	30	17	17	30	160	320	320	320	150	200	150	150	800	0.92	1.00	1.08	1.14	1.50	
	125	17	17	3	3	NC	20	80	320	320	320	75	250	500	250	800	0.77	0.89	1.00	1.08	1.50
	158	30	30	—	1	1	40	40	80	80	80	200	250	300	250	250	0.70	0.81	0.77	0.77	0.81
	222	30	19	—	1	1	80	80	320	320	150	200	400	250	250	300	0.83	0.83	1.08	1.22	
	235	3	3	30	30	30	40	80	80	80	160	100	150	200	250	300	0.81	0.89	1.05	1.05	1.14
	265	19	17	17	17	17	160	160	320	320	300	500	500	500	300	300	0.77	0.92	1.08	1.08	
	266	17	19	—	—	—	40	160	160	160	160	300	300	300	300	300	1.00	1.14	1.38	1.22	1.22
	208	17	17	—	1	1	80	320	640	320	250	250	300	250	250	100	1.00	1.22	1.44	1.41	
	14	17	NC	17	19	1	0	20	160	80	80	50	75	500	600	600	0.72	0.92	1.31	1.28	1.08
		Average					68	135	313	334	240	141	191	281	261	408	0.83	0.95	1.12	1.17	1.20
	III	41	30	—	—	—	80	80	80	80	100	200	200	250	150	1.05	1.38	1.17	1.33		
280		30	—	—	—	80	80	80	80	400	400	400	400	150	1.08	1.28	1.17	1.22			
281		30	—	—	—	20	20	20	20	20	100	200	150	150	150	0.72	0.89	1.08	1.00		
94		19	—	—	—	20	20	20	20	20	25	25	25	25	25	0.72	0.77	0.72	0.86	0.77	
191		19	—	—	—	40	80	80	80	200	300	300	300	300	300	0.89	0.98	1.14	1.00		
299		19	—	—	—	80	160	160	160	100	150	150	150	150	150	0.77	1.17	1.14	1.28		
323		19	—	—	—	40	40	40	40	250	300	250	200	200	200	0.83	1.05	1.00	1.05		
151		19	—	—	—	20	20	20	20	50	75	75	150	150	150	0.77	0.89	0.92	1.17		
171		19	—	—	—	20	20	20	10	100	100	150	200	200	200	0.89	0.89	1.00	1.17		
201		17	—	—	—	80	80	160	160	50	50	50	50	50	50	0.77	1.00	0.95	1.00		
202		17	—	—	—	40	40	40	40	150	150	150	150	150	150	0.83	1.05	1.11	1.11		
286		17	—	—	—	40	40	40	40	40	150	150	200	200	200	0.72	0.77	0.77	0.86	0.89	
261		17	—	—	—	20	20	40	20	20	75	200	150	150	150	150	0.77	1.22	1.08	1.05	1.05
275		17	—	—	—	80	160	160	160	100	250	150	150	150	150	100	0.70	0.89	0.81	0.81	
292		3	—	—	—	80	80	80	80	80	100	100	150	150	150	150	0.89	1.00	1.11	1.05	1.17
258		3	—	—	—	40	20	20	20	40	50	100	100	100	100	100	0.77	0.67	0.77	0.77	0.83
184		1	—	—	—	40	40	40	40	80	150	100	100	100	100	100	0.67	0.77	0.83	0.77	0.77
		Average					48	59	66	63	43	126	168	162	169	125	0.81	0.99	0.99	1.02	0.92

TABLE I—Continued

Group	Case	Streptococcus type*					Antistreptokinase† titer					Antistreptolysin O† titer					Gamma globulin grams per cent					
		0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.	0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.	0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.	0 wk.	1 wk.	2 wks.	3 wks.	4-6 wks.	
IV	283	19	—	30	30	—	160	320	320	640	640	50	25	50	50	50	0.67	0.67	0.75	0.75	0.77	
	289	17	17	—	17	17	20	80	640	640	640	50	200	1200	1200	1000	0.89	0.89	1.38	1.28	1.17	
	300	17	—	17	—	—	20	40	40	80	100	100	150	150	100	0.67	0.67	0.77	0.67	—		
	180	30	—	19	19	19	80	80	80	80	640	25	25	50	100	100	0.67	0.62	0.72	0.62	0.98	
	309	30	—	—	19	19	160	320	320	320	320	100	100	150	150	200	0.77	0.83	0.77	0.75	0.75	
	196	30	—	—	30	19	40	80	80	1280	2560	50	100	100	150	200	0.67	0.72	0.67	0.98	1.05	
	334	19	—	30	—	—	80	80	160	160	640	150	150	250	250	250	0.89	1.17	1.11	1.17	1.17	
	206	30	—	—	—	—	20	40	40	40	80	200	150	300	200	300	0.95	0.98	1.11	1.22	1.17	
	267	30	—	30	—	—	80	80	80	80	80	250	250	250	250	150	0.75	0.89	0.95	0.98	—	
	269	17	—	—	—	17	160	320	160	160	160	100	200	100	100	100	0.72	0.81	0.89	0.81	0.83	
	249	30	—	3	—	—	320	640	640	640	640	250	250	300	300	400	0.62	0.67	0.67	0.72	0.77	
	379	17	17	17	17	—	20	40	80	80	80	100	200	400	400	1.25	0.95	1.00	1.00	—		
	54	17	17	17	17	—	40	80	160	160	160	100	150	150	200	0.62	0.59	0.72	0.75	—		
	56	17	17	17	17	—	40	40	40	80	80	100	100	100	150	250	0.62	0.75	0.75	0.83	0.81	
	68	30	30	30	17	NC	17	160	40	160	640	640	50	100	100	100	150	0.77	1.00	1.22	1.28	
	71	19	19	19	1	—	0	0	320	320	20	25	25	150	200	0.81	0.95	1.11	1.11	—		
	73	30	17	1	1	—	40	40	80	160	160	100	100	150	250	300	0.95	1.05	1.22	1.25	1.11	
	126	19	17	17	—	—	320	320	320	20	640	200	250	300	300	300	0.75	0.75	0.67	0.83	0.95	
	133	30	17	17	17	—	10	10	40	40	40	40	25	50	100	150	150	0.81	0.81	0.89	1.17	1.05
	305	30	—	19	—	—	160	320	160	160	160	200	300	300	300	400	0.75	0.81	0.81	0.83	0.83	
209	19	—	—	17	—	80	80	160	2560	160	100	100	100	500	0.77	0.81	0.83	1.44	—			
	Average					96	145	194	416	541	111	139	226	257	286	0.77	0.82	0.89	0.98	0.98		
V	13	30	—	—	—	1	80	80	320	1280	2560	150	250	300	300	300	1.02	1.28	1.44	2.48	2.64	
	16	19	19	—	1	3	20	20	160	320	320	100	100	100	200	200	0.89	1.05	1.22	1.28	1.33	
	23	17	—	17	17	17	40	160	640	640	640	150	250	300	700	300	0.95	1.02	1.38	1.33	1.28	
	30	3	—	—	19	19	40	320	80	80	80	150	400	2000	3000	2000	0.95	1.11	1.67	1.38	1.55	
	51	19	19	17	17	17	40	40	80	320	640	300	300	500	600	600	1.05	0.95	1.38	1.72	1.55	
	52	17	17	17	3	3	160	160	320	640	640	100	200	250	400	1600	0.81	0.89	1.05	1.38	1.72	
	65	19	19	19	—	—	20	20	160	640	640	250	250	300	700	500	0.89	1.05	1.38	1.83	1.67	
	88	17	17	—	17	17	20	10	80	80	80	25	25	300	400	400	0.92	0.98	1.67	1.96	1.55	
	92	1	1	30	17	—	40	80	160	160	160	80	100	150	300	500	1000	0.72	0.95	1.17	1.44	1.97
	120	30	30	30	—	—	80	80	320	640	1280	150	200	200	200	250	0.98	1.00	1.17	1.20	1.88	
	234	19	19	—	—	—	80	160	320	640	640	1200	1600	1400	1200	1400	1.17	1.28	1.22	1.55	1.44	
	247	—	—	17	17	17	640	640	640	1280	1280	150	200	200	200	200	0.92	1.08	0.95	1.08	1.08	
	251	—	30	30	—	—	0	0	80	640	1280	200	200	200	500	700	0.81	0.81	0.95	1.83	1.88	
	61	30	30	30	—	3	320	320	2560	2560	2560	100	150	250	300	400	0.67	0.75	1.00	1.00	1.05	
	42	NT	NT	NT	NT	NT	40	80	80	80	80	100	150	200	150	150	150	0.77	0.77	0.95	1.00	1.17
	45	17	17	—	—	30	40	80	160	20	160	100	100	150	150	100	250	0.77	0.83	1.11	0.98	1.00
	181	30	—	—	17	17	40	160	320	320	320	200	600	1400	2000	2000	0.70	0.75	1.00	1.17	1.14	
	175	19	—	17	17	—	0	0	10	40	40	80	250	250	250	250	1.05	1.22	1.14	1.14	1.22	
	164	17	17	17	—	—	40	320	320	320	320	300	700	800	600	700	1.33	1.99	2.21	2.21	2.54	
	173	30	3	30	17	—	160	160	320	320	640	100	150	150	250	250	1.17	1.33	1.44	1.28	1.17	
217	17	—	17	—	—	17	40	160	320	640	1280	250	300	500	800	0.89	1.05	1.00	1.17	1.55		
337	30	—	—	—	19	20	20	40	80	160	200	200	200	200	250	0.81	0.95	0.95	1.11	1.11		
44	30	17	17	—	30	160	160	160	320	640	250	200	500	600	700	1.05	1.44	1.38	1.33	1.50		
	Average					92	140	332	524	737	212	301	459	602	661	0.92	1.06	1.25	1.43	1.52		

* NC No culture obtained.
 — No hemolytic streptococci.

NT Group A, no type.

† Antistreptokinase titers of less than 10, and antistreptolysin O titers of less than 25 are listed as 0.

intervals thereafter. The strains of streptococci isolated from the throat cultures were classified serologically in our laboratory by the precipitin technique of Swift, Wilson and Lancefield (20), and preserved for subsequent study by freezing and drying. The sera were stored at 4° C. Patients developing rheumatic fever were transferred to the Naval Hospital at Dublin, Georgia, and late specimens of serum were obtained from these individuals. Summaries of the clinical records of these patients were provided by the medical officers of Naval Research Unit No. 4.⁵

⁵ The authors are indebted to Lt. Comdr. John R. Seal, MC, USN, and the personnel of the U. S. Naval Medical Research Unit No. 4 for collection of sera and cultures and for supplying the clinical data on the patients. Dr. Robert F. Watson and Dr. Rebecca C. Lancefield represented the Hospital of the Rockefeller Institute for Medical Research in setting up this cooperative project.

As indicated by serological studies of the streptococcal strains isolated, three types of group A hemolytic streptococci (Types 17, 19, and 30) accounted for the great majority of the infections, but at least three other types (Types 1, 3, and one or more non-typeable strains) were involved in the epidemic. Individual isolation of the patients was not feasible, and the presence in the same ward of individuals infected with different types of streptococci resulted in a high incidence of cross infection. This occurrence of cross infection, together with other variables such as treatment and response to treatment, have been taken into consideration in the grouping of the case material chosen for serological studies. Cases were excluded from the study if clinical and bacteriological data were incomplete. Out of a total of 380 cases studied in the epidemic, detailed antibody studies were carried out on 90, grouped according to the following scheme:

Untreated, uncomplicated scarlet fever:

Group I: 17 patients from whom only a single type of group A hemolytic streptococcus was isolated. Although the failure to isolate more than one type of streptococcus from the serial throat cultures does not entirely eliminate the possibility of cross infections in these patients, it reduces the likelihood that clinically significant cross infections occurred.

Group II: 12 patients from whom more than one type of group A hemolytic streptococcus was isolated during the first two weeks of illness.

Penicillin-treated scarlet fever:

Group III: 17 patients from whom group A hemolytic streptococci could not be isolated after the initiation of treatment. Thus, from the bacteriological point of view, this group comprises those patients successfully treated with penicillin.

Group IV: 21 patients who showed persistence of, or recurrence of, a positive culture for group A hemolytic streptococcus of the same or different type.

Scarlet fever followed by rheumatic fever:

Group V: 23 cases. This group is not large enough to justify subdivision for purposes of analysis into sub-groups according to occurrence of cross infection and presence or absence of penicillin therapy. A majority of the patients were treated with penicillin, but none fell into the group of "successfully" treated cases comparable to Group III in which organisms disappeared permanently from the throat after the initiation of therapy. The selection of these rheumatic fever patients was of necessity based on rather rigid criteria, similar to those set down by other investigators. Unequivocal evidence of at least three of the following clinical or laboratory manifestations of rheumatic fever was required: arthritis or arthralgia; fever (subsequent to the initial streptococcal infection); elevated erythrocyte sedimentation rate; prolongation of the P-R interval (over 0.20 seconds); other electrocardiographic abnormalities; pericarditis; occurrence of valvular lesions as evidenced by characteristic murmurs. As a result of the use of these criteria, borderline and doubtful cases are not included. The objection that only the more severe cases of rheumatic fever have been accepted is perhaps valid, although in general these patients had relatively mild, monocyclic attacks, and few of them would be considered severe according to the usual clinical standards.

EXPERIMENTAL RESULTS

The results of antistreptokinase, antistreptolysin O, and gamma globulin determinations on the sera of the 90 patients are recorded in detail in Table I. Although certain facts are apparent from inspection of the table, the significant conclusions to be

drawn from the large mass of data are better illustrated by graphic treatment. The complete data are given for reference in conjunction with the figures which summarize the various aspects to be discussed.

In Figures 1-3, the arithmetic means of the three determinations are plotted against the week of disease. It will be seen at once from an inspection of these figures that, in general, the pattern of response within each group is similar in all three determinations. For example, the group of rheumatic fever patients (Group V) has consistently higher antibody titers and gamma globulin levels throughout the period studied than any of the other groups. The possible significance of this finding will be considered in detail below. In addition, there is a consistent difference between the degree of response of those untreated scarlet fever patients with single type infections (Group I) and those with multiple type or cross infections (Group II). The higher average response of the latter group suggests that the occurrence during infection of more than one type of streptococcus may augment the antibody response.

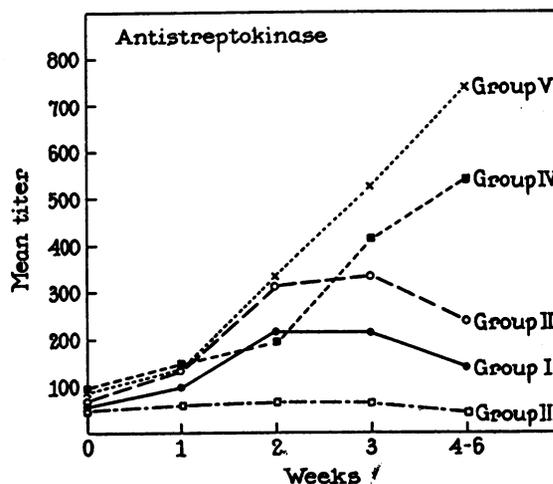


FIG. 1. MEAN ANTISTREPTOKINASE TITERS AT WEEKLY INTERVALS FROM TIME OF ADMISSION TO HOSPITAL

Group I: Uncomplicated scarlet fever; single type of streptococcus.

Group II: Uncomplicated scarlet fever; multiple types of streptococcus.

Group III: Penicillin-treated scarlet fever with disappearance of organism from throat.

Group IV: Penicillin-treated scarlet fever with persistence or reappearance of streptococci in throat culture.

Group V: Scarlet fever followed by rheumatic fever.

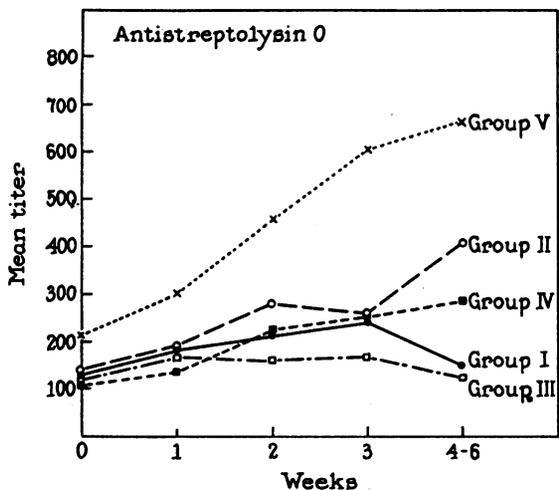


FIG. 2. MEAN ANTISTREPTOLYSIN TITERS AT WEEKLY INTERVALS FROM TIME OF ADMISSION TO HOSPITAL Groups as in Figure 1.

The failure of production of antibodies to streptokinase and streptolysin O in those patients "successfully" treated with penicillin (Group III) is apparent from inspection of Tables I and II, and of Figures 1 and 2. In no case was there a significant rise in antistreptokinase, while only eight of the 17 patients showed a significant, though minimal, response of antistreptolysin O. It should be remembered, however, that because of the differences in dilution increments used in the measurement of the two antibodies, a significant (or two-tube) rise in antistreptolysin O implies a smaller

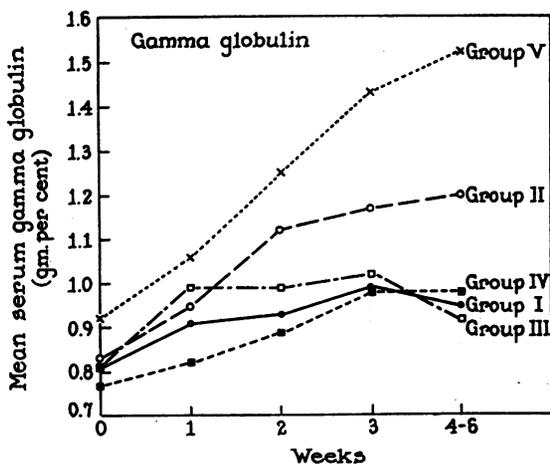


FIG. 3. MEAN SERUM GAMMA GLOBULIN LEVELS AT WEEKLY INTERVALS FROM TIME OF ADMISSION TO HOSPITAL

Groups as in Figure 1.

actual increase in antibody than in the case of antistreptokinase. Similar observations on the decreased antistreptolysin O response in patients with hemolytic streptococcal infections treated with large doses of penicillin have been reported by Rantz, Boisvert, and Spink (21), and by Weinstein and Tsao (22). In contrast to the flattened curves seen in Figures 1 and 2 for this group (Group III), the average gamma globulin levels (Figure 3) show a rise comparable to those of Groups I and IV. This suggests that the antibody response as a whole may not be greatly suppressed by the administration of penicillin. The two specific antibodies measured are directed against extracellular products elaborated by growing streptococcal cells, and may represent special cases with respect to the effect of penicillin on antibody production.

TABLE II
Percentage incidence of significant rises in antistreptokinase and antistreptolysin O titers

Group	No. of cases	Per cent showing significant rises in—			
		ASK	ASO	ASK and ASO	Either ASK or ASO
I	17	65	76	53	88
II	12	75	58	42	92
III	17	0	47	0	47
IV	21	71	76	62	90
V	23	87	83	74	96

The proportion of patients in each group showing significant rises in antistreptokinase and antistreptolysin O titers may be seen in Table II. With the exception of Group III, the incidence of significant responses of the two antibodies is similar within each group. The percentage difference in Group III is probably more apparent than real for the reason mentioned before. As demonstrated by the data given in the last two columns of Table II, patients with a significant antibody response to one antigen did not necessarily have a corresponding response to the other. The percentage of patients showing significant simultaneous rises in both antibodies is much smaller than the percentage showing a rise in either antistreptokinase or antistreptolysin O. No criterion for significance of an increase in gamma globulin has yet been rigidly defined, although with this

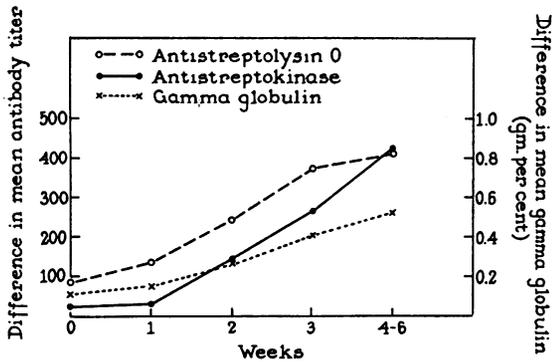


FIG. 4. DIFFERENCE IN ANTISTREPTOKINASE, ANTISTREPTOLYSIN AND GAMMA GLOBULIN LEVELS BETWEEN THE RHEUMATIC FEVER GROUP (GROUP V) AND THE NON-RHEUMATIC GROUPS (GROUPS I-IV)

method the normal range has been found to be about 0.67-1.0 gm. per cent (18).

The occurrence of consistently higher mean values for the two antibodies as well as for the gamma globulin levels in that group of patients with rheumatic fever (Group V) as compared with the other groups is worthy of special reference. Although the number of uncontrollable variables inherent in a study of this kind may cast some doubt on the validity of applying statistical methods, the results of statistical analysis of these data suggest that the observed differences are significant. For example, in the case of the mean value for the serum gamma globulin at three weeks after the onset of scarlet fever, the observed difference between the rheumatic fever patients and the entire non-rheumatic group is four times its standard error. According to statistical theory, the probability of this difference occurring by chance is 1 in 15,000.

There are additional considerations which appear to support the interpretation that the observed difference is significant. To provide graphic representation of the observations, the mean weekly value of each of the three determinations for the 67 scarlet fever patients in Groups I-IV have been subtracted from the corresponding mean values for the rheumatic fever patients (Group V) and the differences plotted in Figure 4. It will be seen that in the case of antistreptokinase and antistreptolysin O the absolute differences, as well as the rate of increase of the differences, are comparable. Although not directly comparable because they are expressed in different

units, the difference in the gamma globulin levels shows a rise similar to that of the antibodies. The mechanism and technical procedures involved in the three determinations vary widely. It is all the more striking, therefore, that the differences observed are of the same order in the case of each test.

The possibility must be considered that the arithmetic means for the various determinations may be unduly influenced by the occurrence of both very high and very low values with resultant exaggeration of minor differences. Consequently,

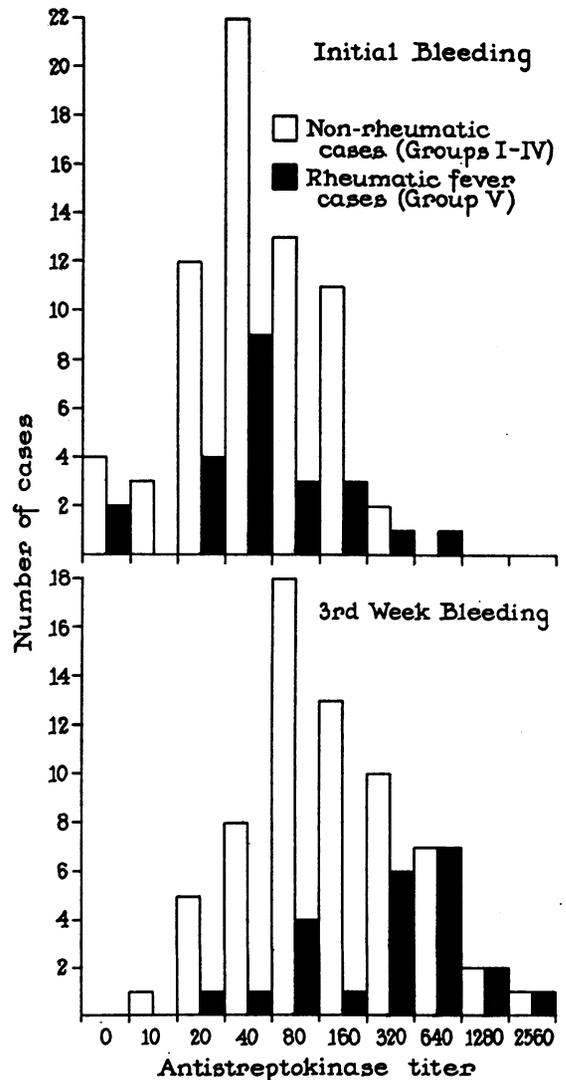


FIG. 5. COMPARISON OF DISTRIBUTION OF ANTISTREPTOKINASE TITERS OF RHEUMATIC (GROUP V) AND NON-RHEUMATIC (GROUPS I-IV) GROUPS AT TIME OF ADMISSION WITH SCARLET FEVER AND AFTER THREE WEEKS

frequency distributions were analysed for the three determinations, and are illustrated by the chart for antistreptokinase reproduced in Figure 5. At the time of the initial bleedings, the frequency distribution of the titers is remarkably similar for the two groups. By the end of 21 days, however, the two distributions have lost their similarity, and the one for the rheumatic fever group has shifted more to the right than has the one for the non-rheumatic fever group. Similar analyses of the antistreptolysin O and gamma globulin data yield comparable results. Thus this treatment of the data appears to support the significance of the differences between the rheumatic and non-rheumatic groups that was first suggested by the mean values of the various determinations.

In vitro production of streptokinase: Because previously published work (19) suggested that the antistreptokinase response of the patient was somewhat proportional to the quantity of streptokinase produced *in vitro* by the infecting organism, a number of group A hemolytic streptococci isolated from patients in this investigation were studied for their ability to produce streptokinase. Ninety-four assays were performed, using 62 different strains. Supernates of ten Type 1 and ten Type 3 streptococcal cultures were tested, and, without exception, all failed to lyse a standard clot even when tested undiluted. These two types were far less common during the epidemic than were the other types, so that untreated patients from whom only a Type 1 or a Type 3 streptococcus was isolated were few in number, and only two are represented in Table I. Neither has a significant antistreptokinase response. Forty-two group A hemolytic streptococci of Types 17, 19, and 30 were tested, and all produced measurable amounts of streptokinase. The supernates of two strains could be titered only to a dilution of 1:5; all the rest titered to dilutions of 1:10 or 1:20. There was no difference in ability to produce streptokinase between those organisms isolated from patients with rheumatic fever, and those from patients who did not develop rheumatic fever.

DISCUSSION

The results of the present studies provide no evidence that those patients who develop rheumatic fever following group A hemolytic strepto-

coccal infections have a pattern of antibody response to streptokinase that differs from the pattern of their response to other antigenic stimuli. On the contrary, the quantitative differences in antistreptokinase production which are observed in a comparison of the rheumatic and non-rheumatic patients are paralleled by comparable differences in the production of a second antibody, antistreptolysin O, and in the increase in serum gamma globulin. The data on gamma globulin suggest that the greater production of the two antibodies in those patients who developed rheumatic fever is a reflection of a general augmentation of antibody formation.

It is important to point out that in the present study the increased antibody production is made apparent only by the collective analysis of the two groups of cases, and no basis is provided for differentiating between individual patients. Thus, some of the patients with uncomplicated scarlet fever showed a greater rise in antibody titer than certain of the rheumatic fever patients, and a few of the latter have relatively insignificant increases.

Quantitative differences in production of antibody have not been emphasized in previous investigations of group A hemolytic streptococcal infections in which rheumatic and non-rheumatic patients have been compared (23, 24, 25, 26, 27). In a recent study, Rothbard, Watson, Swift, and Wilson (28) found that the average antistreptolysin O response, as indicated by the ratio between the maximal titer and initial titer, was greater in patients who developed rheumatic fever following a streptococcal infection than in those who had uncomplicated infections. However, they found in addition that a group of patients with purulent complications showed a much greater average response than either of the other two groups. The fact that the differences described in the present study are so readily apparent may be in some degree due to the homogeneity of the case material. These patients were selected from a single epidemic of scarlet fever occurring within a period of two months, and a relatively small number of streptococcal strains was involved. Furthermore, the patients represented an unusually uniform sample of the population, since all were males between the ages of 17 and 27; 82 (91 per cent) of the men were included in the age group of 17 to 20 years.

Accordingly, some of the uncontrollable variables commonly present in this type of study were eliminated, and it is reasonable to suppose that small differences of the sort described might become apparent under these conditions.

The possible significance of the apparent enhancement of antibody formation in the rheumatic subjects is difficult to assess. Conceivably it might mean that on the average these patients received a greater antigenic stimulus in the form of a more serious or extensive streptococcal infection, although there is nothing in the clinical histories to support this point of view. A second possible interpretation is that persons susceptible to rheumatic fever may in general respond to a given stimulus with greater production of antibody. It is well known, for example, that individual differences in degree of antibody formation occur among experimental animals injected with identical amounts of antigen. It is also worthy of note that even at the onset of the streptococcal infection, those patients who later developed rheumatic fever had, on the average, higher antibody titers and higher gamma globulin levels than did those who did not develop rheumatic fever. Regardless of the interpretation one wishes to put on the results, it seems premature and fruitless to attempt to reconcile them with any of the current theories concerning the pathogenesis of rheumatic fever.

SUMMARY AND CONCLUSIONS

1. A procedure for the quantitative determination of antistreptokinase has been employed to follow the antibody response of patients with scarlet fever, including those who developed rheumatic fever. Antistreptolysin O titers and gamma globulin levels on the same sera are presented for comparison.

2. There is suggestive evidence that the presence of two or more types of streptococci during an infection calls forth a greater antibody response than does the presence of only a single type.

3. Early and effective penicillin therapy which removed the infecting organism promptly from the nasopharynx either prevented entirely or greatly decreased the expected antibody response to streptokinase and streptolysin O. No effect was apparent on the total antibody response as measured by the serum gamma globulin.

4. Development of the rheumatic state is not accompanied by a pattern of antistreptokinase response that differs significantly from the pattern of the general immune response in the same state.

5. Of the patients included in this study, it would seem that on the average those who developed rheumatic fever as a sequela to a streptococcal infection exhibited a greater antibody response than those who did not develop rheumatic fever.

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