Precipitin Reaction Obtainable Between Human Serum and Extracts of Group A, Type 15 Streptococci.* (24808)

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Three different proteins, all antigenic, have been shown to be present on the surface of Group A streptococcal cell(1). Of these antigens designated respectively M, T, and R, only the M antigen has been demonstrated to be associated with virulence. Although it is possible to demonstrate antibody to type specific M protein in post-convalescent human serum by several technics, most satisfactorily by the bactericidal test, it has not been possible by precipitation reaction with human serum to detect such antibody or antibody to any other defined streptococcal cellular component following streptococcal infection. Schmidt has recently reported development of anti-M precipitins following active immunization(2). This report describes a precipitin reaction obtainable between extracts of virulent (M protein containing or so-called matt) strains of Group A, Type 15 streptococci and human serum; it also describes certain properties of the bacterial component. This reaction was first observed while testing a set of streptococcal typing sera (rabbit antisera) for both specificity and potency with homologous and heterologous Group A streptococcal extracts. Ten human sera were initially tested in capillary precipitin tubes with each of the streptococcal extracts used to test the collection of typing sera.

Materials and methods. Streptococcal typing sera were made by the streptococcal laboratory of Communicable Disease Center of U. S. P. H. S., Chamblee, Ga. The large amount of Type 15 rabbit antiserum employed (C.D.C. lot #20) was kindly supplied by Dr. Elaine Updyke. *Normal rabbit sera.* Serum for controls was obtained from 6 normal rabbits. Sera were obtained from 100 patients known to have had rheumatic fever within preceding 5-year period, and from 35 adult patients with a variety of disorders. Solid medium cultures were carried out on sheep blood agar, liquid medium cultures in Todd-Hewitt broth. *Streptococcal strains and extracts.* Four Group A, Type 15 strains were used for making extracts. These strains are designated T15/32/1 (Dr. F. Griffith's T 15), T 15 glossy, D19/20/4, and D19/24 glossy.‡ Three kinds of extracts of washed streptococcal cells were studied: A extracts, M extracts, and extracts of cell wall preparation. A extracts are prepared by Lancefield's method of heating suspensions of Group A streptococcal cells at pH2 in water bath at 100°C for 10 minutes(3). M extracts were prepared by Lancefield's method of repeated alcohol precipitation of A extracts(4). Extracts of cell wall preparation were made by heating an acid (pH2) suspension of cell wall material at 100°C for 10 minutes. Preparation of cell walls of strain D19/20/4 almost completely free of intact cells was made by the method of Salton employing a Mickle disintegrator (5). M proteins of all streptococcal types are destroyed by treatment with trypsin. All strains and extracts employed were treated with crystalline trypsin, to final concentration of 0.01 mg/cc of each preparation. *Capillary precipitin tests.* All tests between streptococcal extracts and serum were carried out in capillary precipitin tubes, by the method of Swift, Wilson and Lancefield(6). Readings were made in terms of pluses, 1+ to 4+ (1+ = approximately 1 mm packed precipitate), at best a very rough quantitation.

Results. All 10 human sera initially tested against A extracts reacted with the extract of D19/20/4 to give 1+ to 4+ precipitin reactions. Following this initial observation, all

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137 sera were set up in capillary precipitin tubes with A extracts of D19/20/4. Again all sera tested reacted to give a precipitate with extract, again giving readings between 1+ and 4+, 115 of them readings of 2+ to 4+.

When several lots of Todd-Hewitt broth, the medium in which the strains were grown, were tested with human sera no precipitation was observed. Likewise, 6 normal rabbit sera when tested with the extract gave no precipitin.

A extracts were prepared from 3 additional Type 15 strains and all 137 sera were tested against them. These 3 strains were T15/32/1, an M containing strain like D19/20/4, and the 2 homologous glossy, avirulent, non-M containing strains, T15 glossy and D19/24 glossy. The results obtained with these strains and with D19/20/4 are shown in Table I. All reactions between human sera and extracts of glossy strains were trace or ± reactions.

M extracts were made of D19/20/4 and of T15/32/1. These extracts which contain no group specific carbohydrate, the streptococcal C substance, and more concentrated and partially purified M reacted to give a precipitate with all human sera tested.

Trypsin treatment of heat killed cultures, of growing cultures, and of reactive extracts of strain D19/20/4 was carried out. Table II shows results obtained. The presence of the reactive streptococcal factor in all extracts correlated remarkably with presence of Type 15 M protein, detected with specific rabbit antiserum to Type 15 M. The tryptic digestion of the unknown factor is suggestive of its protein nature.

Presence of the reactive factor on the surface of the streptococcal cell is indicated by its removal on trypsinization of the growing

TABLE I. Precipitin Reactions Obtained between 137 Human Sera and Extracts of 4 Group A, Type 15 Streptococcal Strains.

Extract	No. of sera giving precipitin with extract	No. of sera not giving precipitin with extract
D19/20/4	137	0
D19/24 glossy	12	115
T15/32/1	137	0
T15 glossy	7	130

TABLE II. Effect of Trypsinization on Group A, Type 15 Streptococci and Extracts (Strain D19/20/4).

Extracts of	Precipitin tests: With rabbit antiserum to Type 15 M	Precipitin tests: With reactive human serum
Heat killed culture D19/20/4	+++	++++
Trypsin treated heat killed culture D19/20/4	—	—
18 hr culture of D19/20/4	+++	++++
Trypsin treated 18 hr growing culture of D19/20/4	—	—
D19/20/4	+++	++++
Trypsin treated extract of D19/20/4	--	--

18-hour culture. Further evidence for this was obtained from a cell wall preparation of D19/20/4. Acid extraction of washed, dried cell wall preparation yielded an extract which reacted strongly with human serum. Trypsin treatment of a saline suspension of this cell wall preparation prior to extraction eliminated the reaction between the extract and human serum as well as the reaction with Type 15 rabbit antiserum.

Discussion. A substance, protein in nature and extractable from Group A, Type 15 matt streptococcal cells, reacted to form a precipitate with human serum. This substance, a cellular component, correlates with the presence of Type 15 M protein, but is probably distinct from it. It is not a T antigen, as T antigens are destroyed by heating suspensions of streptococcal cells at pH2; moreover, all established T antigens, unlike the type specific M antigen, are resistant to tryptic digestion. The reactive material derived from Type 15 streptococcal cells is destroyed by treatment of A extracts with trypsin, by treatment of living 18 hour cultures by trypsin and by treatment of Type 15 streptococcal cell walls by trypsin. These findings indicated some similarity between the M protein of Group A streptococcal cells and the undefined cellular factor. Indeed, M extracts prepared by repeated alcohol precipitation of A extracts contained the new reactive factor as well as Type

15 M protein. In addition to these similarities obtained by extraction methods and by trypsin treatment, extracts of glossy variants of the 2 Type 15 strains available for study did not contain the factor which formed a precipitate with human sera. This finding served to differentiate the unknown reactive factor from an R antigen, as R antigens, almost by definition, are extractable by usual methods from glossy strains, and react to form a precipitate with rabbit antisera prepared against R containing matt strains of the homologous type.

It was thus evident that the unknown protein factor extractable from Group A Type 15 matt strains of streptococci was not a T antigen and not an R antigen. Certain of its properties were similar to those of M antigens, but precipitin reactions between M containing extracts of Group A streptococci and sera of patients who have had infections even with homologous strains have never been obtained. Even were precipitin reactions between sera of patients convalescent from streptococcal infections and extracts of the infecting strains obtainable, it would be most improbable that a random population of 137 individuals would all have had infections with Type 15 streptococcus. Human infection with Group A, Type 15 streptococci is extremely uncommon.

Summary. A precipitation reaction between extracts of Group A, Type 15 streptococci and human serum has been described. The streptococcal factor bears some similarity to the M protein, but is almost certainly a separate substance, probably protein in nature. Like the M, T and R antigens it is present on the surface of the streptococcal cell. The reactive factor has been differentiated from T and R antigens. It is not yet known whether or not the bacterial factor is antigenic, nor is it known yet whether the reaction between this factor and human serum is of other than a happenstance nature.

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Effect of Histamine on F_{cells} Value in Splenectomized Dog.* (24809)

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The overall cell percentage venous cell percentage (F_{cells}Factor) is less than one in the splenectomized dog(1,2) as well as in man (3). The generally accepted explanation is that the blood in the small vessels and capillaries has a lower cell percentage than that in the large veins and arteries(1,4,5,6). Hence, the F_{cells} value is presumably influenced by the fraction of blood present in the minute vessels. Superficially at least, circulation through the small vessels and capillaries appears to be quite variable and subject to many influences (temperature, bleeding, infusions, psychic states, functional demands of the tissues and a variety of other neural and humoral stimuli). And yet, in the splenectomized dog, drastic changes in volume and composition of the blood appeared to have little effect upon the F_{cells} value(2). It was reported from this laboratory by Deyrup that in unanesthetized dogs subcutaneous injection of histamine causes prolonged reduction in blood pressure(7) and cardiac output(8), but

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