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M Proteins of Group A Streptococci

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INTRODUCTION

More than a decade has passed since R. C. Lancefield published her comprehensive review on group A streptococcal M antigens (95). Since that publication considerable information on these intriguing proteins has accrued. But in spite of our newer knowledge, group A streptococcal infections and their attending complications are still of world-wide importance, and the M antigens play a pivotal role in the pathogenicity of these microorganisms. It was established, largely through Lancefield's contributions, that the M antigens, among a myriad of cellular and extracellular proteins, are the principal virulence factors elaborated by group A streptococci. The M protein antigens, by virtue of their chemical and physical structure and their peripheral location on the cell wall, permit group A streptococci to colonize a susceptible host. In counteracting this invasion, the host's capacity to eliminate the infection results primarily from the presence of opsonic antibodies against the M proteins. Acquired immunity to group A streptococcal infections in humans is type specific. Only in a few instances is this specificity shared between serotypes. Thus, the task of elucidating the chemical and physical structure of the M proteins (many of which cross-react in various in vitro assays) must account for their structural similarities as well as their differences. Upwards of 60 serotypes of group A streptococci have now been described, and at least 12 of these have been newly identified from epidemic sources in the past 7 or 8 years. This review will attempt to assess contemporary research which has centered around the problems of M protein structure, immunobiology, the imputed role of M-related antigens in the etiology of poststreptococcal sequelae, and the experimental use of M protein vaccines for the prevention of human

streptococcal infection. Lancefield's review presented an historical development of research on M antigens and a discussion of the relevant literature to 1962. The present review is intended to extend this compendium through the first third of 1973.

BIOLOGICAL AND ANTIGENIC PROPERTIES

M Proteins and Cell Structure

The location of the M protein on the streptococcal cell surface was established by Lancefield who observed that the antigen was labile to trypsin and other proteolytic enzymes and could be digested from the streptococcal cell wall without destroying the viability of the streptococci (92). The superficial cellular location of the M proteins has been confirmed by electron microscopy. Cole (24) first noted in ultrathin sections of group A streptococci a dense cell wall surrounding a thin cytoplasmic membrane. On the outside surface of the cell wall was an irregular layer of varying thickness and density which could be removed by trypsin. Added ferritin-labeled anti-M globulin was bound to this outer irregular layer. In all probability, this layer was composed not only of M protein, but a variety of other antigens as well, since it was also seen on other streptococcal groups including C, G, D, and H. More recently, Swanson et al. (155) prepared electron micrographs of ultrathin sections of group A streptococci and demonstrated "hair-like fimbriae" on the outermost surface. This fringe was present on "M-positive" strains and absent from some, but not all, of the "M-negative" strains examined; trypsin-treated cells were denuded of the fimbriae. The observations of Swanson et al. have been confirmed by Ellen and Gibbons (32), who described the M protein layer as "fuzz." Van Boven et al. (162) have carried out ultrathin sectioning of group A streptococci labeled with ferritin-bound anti-M globulin, and their work has also confirmed the observations of Swanson et al., as shown in Fig. 1A–D.

To visualize the structure and topology of group A streptococci one must conceive of a mosaic of antigenic substances on the periphery of the cell. Schematic diagrams (5, 88, 100) depicting concentric layers of antigens no longer obtain in view of our current knowledge of this microorganism. Generally shown schematically is an outer layer of hyaluronic acid surrounding protein antigens on the cell wall. In actuality, this capsule does not mask type-specific opsonization or agglutination by T-specific antisera. Nor do any of these substances interfere with group-specific phage absorption or extracellular lysis by phage-associated enzymes cleaving the glycopeptide cell wall matrix (50). The fimbriated or fuzzy surface of the streptococcal cell must provide a three-dimensional reticulum with interstices exposing most of the cell wall antigens to enzymatic degradation or binding by specific antisera. An artist's conception of this surface configuration is beyond the ken of this reviewer.

In all likelihood, M protein is synthesized on or close to the cytoplasmic membrane and then transported through the cell wall glycopeptide matrix to the superficial location seen in electron micrographs. Several experiments tend to support this functional arrangement. For example, protoplasts and L-forms of group A streptococci synthesize M protein and deposit it into the surrounding medium (57, 83, 139), and membranes from osmotically lysed protoplasts contain small amounts of M protein which has been detected by immunofluorescent techniques (41). Treating whole streptococci with trypsin for short periods digests away the superficial layer containing the M protein, leaving viable cells which, in limiting nutrient media containing penicillin to inhibit cell division, are capable of resynthesizing M protein within 60 min (39, 46).

Nontypable Strains

In the literature on group A streptococci, references to nontypable strains are common. The inability to classify serologically a group A streptococcus on the basis of M specificity may be the result of one or more of at least three possibilities: (i) the total absence of M protein; (ii) the subtle alteration of serological specificity by a few amino acid substitutions within the M protein, giving rise to antigenic determinants only weakly reactive with type-specific antisera; (iii) the emergence of new noncross-reacting serotypes from mutations of typable strains, producing M antigens for which no typing sera are available. These alternatives will be discussed.

The existence of group A streptococci totally without an M antigen has never been convincingly documented. Strains readily phagocytosed in fresh human blood, unreactive with all known typing sera, or avirulent in huge doses for young mice have been described (176). These observations refer to functional definitions of the M antigen and do not necessarily apply on a cellular or biochemical level. Group A streptococci in the throats of patients who have re-

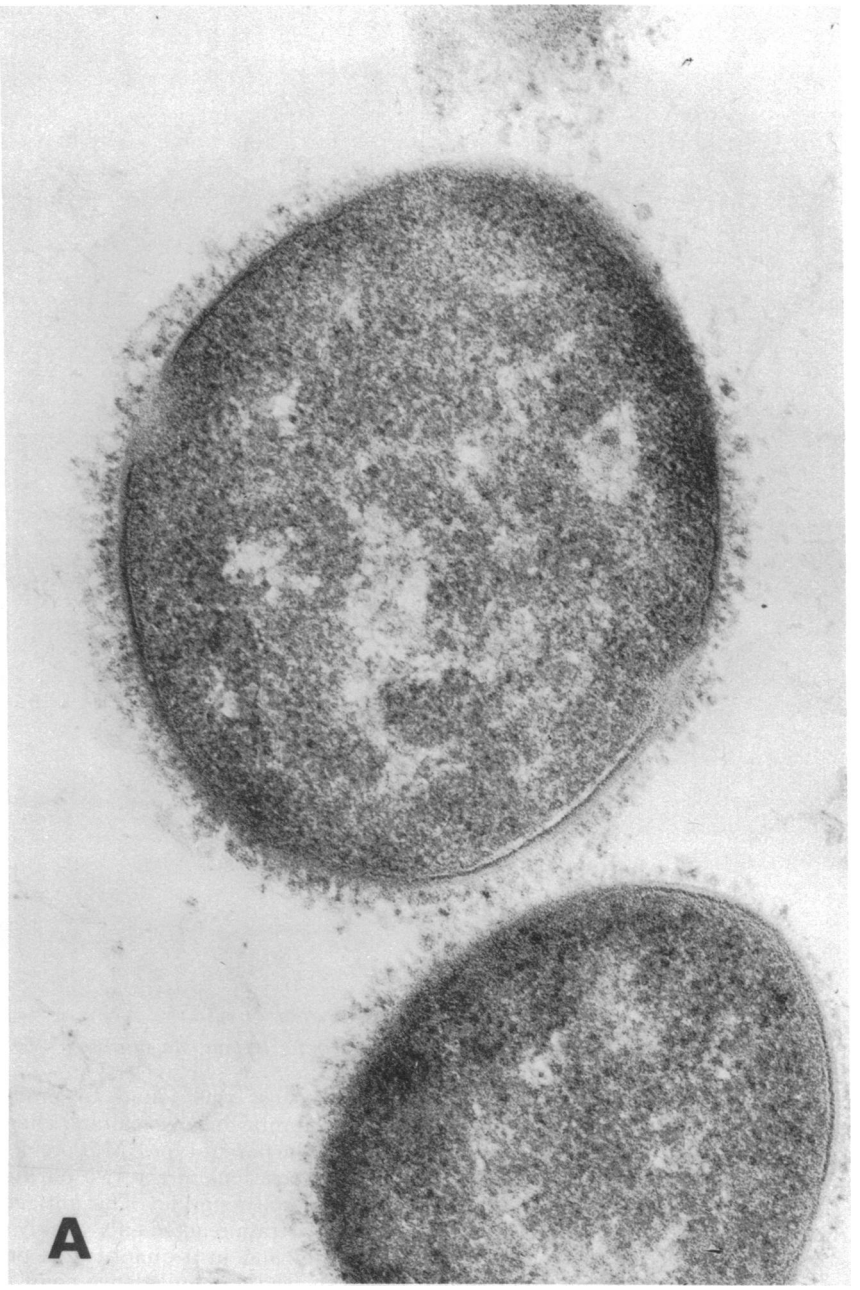

FIG. 1. *Electron micrographs of ultrathin sections of group A streptococci prepared according to the methods of Swanson et al. (155). (These specimens were prepared by C. P. A. Van Boven, Faculty of Medicine, Rotterdam, Netherlands, on leave at the author's laboratory, with the technical assistance of Pei Lee Ho). A, Type 60, 5-h culture, magnification ×50,000.*

covered spontaneously from an upper respiratory infection frequently lose type specificity after a few months of carriage (137). In cases where the serotype of the infecting organism was known, the nontypable strain from the carrier could be passed through mice, and after sufficient successive passages the original serotype could be recovered and identified by capil-

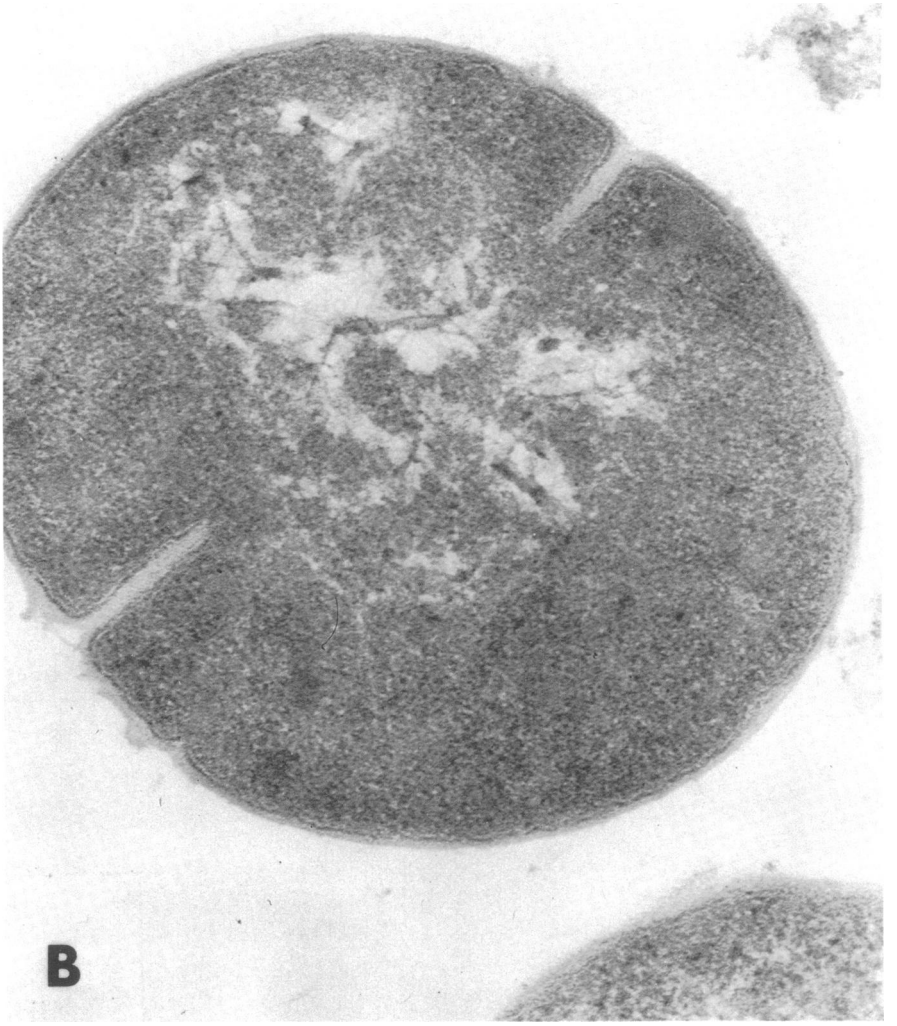

FIG. 1. *B, Type 60, 5-h culture treated with 1% trypsin for 10 min, magnification ×60,000.*

lary precipitin reactions (138). It was demonstrated that various so-called nontypable strains isolated from carriers, in fact, produced an M protein-like antigen, similar in physical properties and amino acid composition to the parent virulent strain (45). Repeated transfer of group A streptococci in laboratory media also leads to a progressive loss of serological typability (95). It was shown that these laboratory strains, as well as those freshly isolated from long-term carriers, possessed an M-like antigen (120), and thus it was proposed that the loss of virulence and typability in these strains resulted from structural modifications, rather than a total loss of the M protein. For example, strain C203S, a well-documented avirulent (so-called M-negative) strain derived from a type 3 streptococcus, was found to produce an M antigen similar in physical and chemical properties to the parent type 3 M protein. The amino acid compositions of the two purified proteins were generally similar. The antigen from the avirulent strain reacted only weakly with type 3 antiserum and in a quantitative precipitation assay showed an equivalence point far into the region of antigen excess when compared with type 3 M protein and homologous antisera (120). The ability of many strains of nontypable group A streptococci to grow in human blood (i.e., to resist phagocytosis) would indicate that these organisms possess an M protein. On the other hand, loss of this growth capacity does not necessarily mean a total loss of the M antigen. Alterations in the protein structure due to

amino acid substitutions and subsequent loss of critical antigenic determinants may be in part responsible for the loss of virulence.

An in vitro technique for M protein enrichment of specific group A strains was developed by Becker (10). Avirulent streptococci were rotated in vials containing human blood. Surviving organisms were cultured and the process was repeated in the presence of type-specific sera. The surviving streptococci contained up to eight times as much M protein (based on serological dilutions) as the parent avirulent

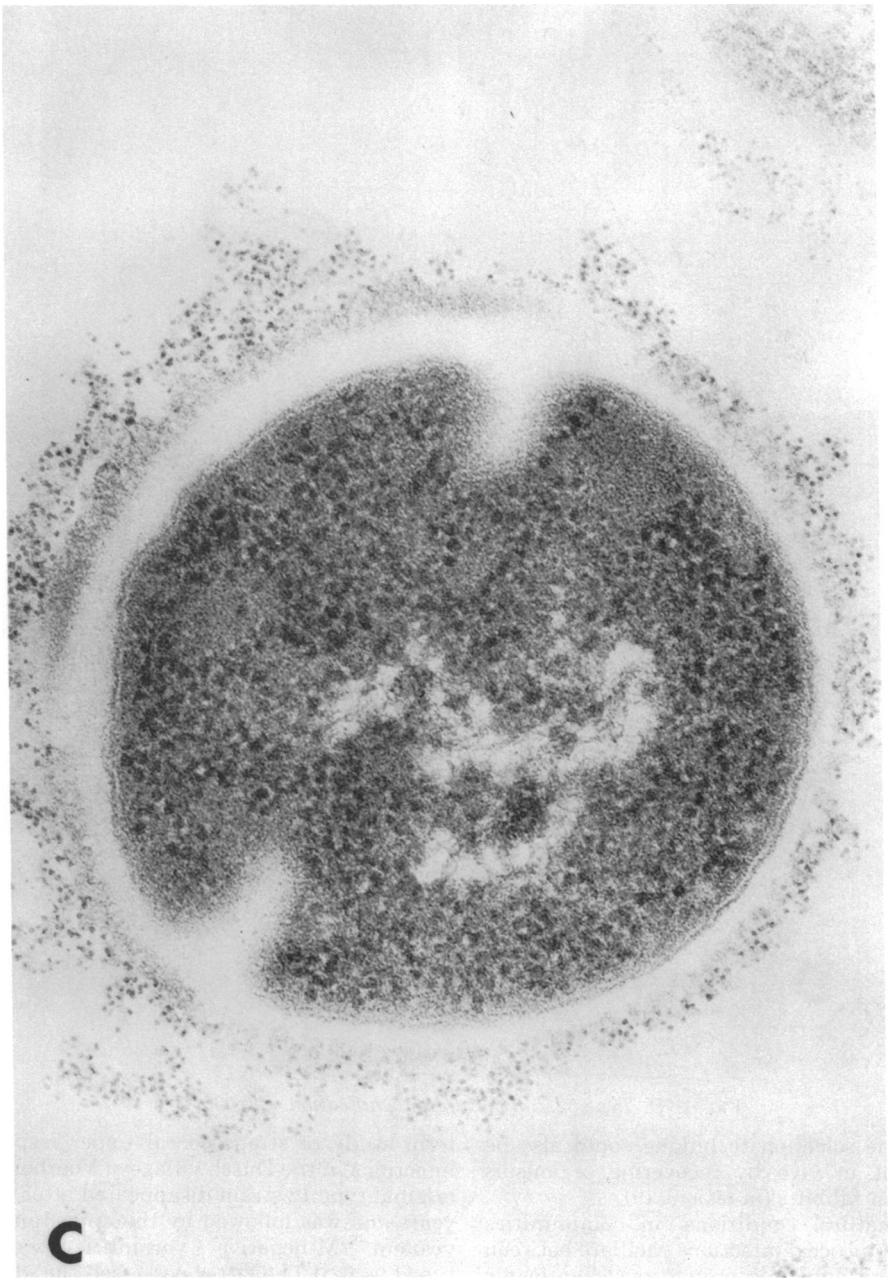

FIG. 1. *C, Type 60, 16-h culture reacted with type-specific rabbit IgG anti-M- and ferritin-labeled goat anti-rabbit IgG, magnification ×60,000.*

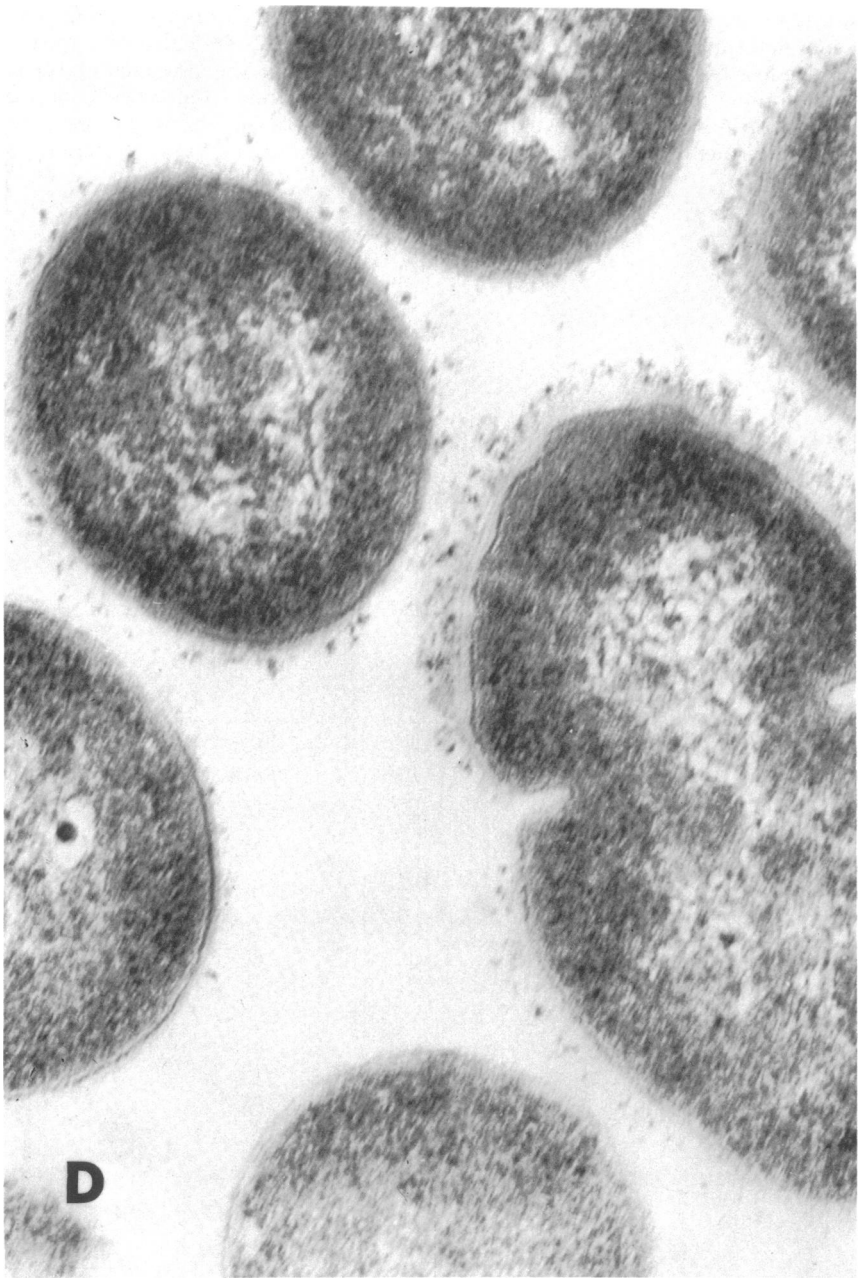

FIG. 1. *D, Type 52, 5-h culture, magnification ×40,000.*

strains. The selection technique could also be carried out in vivo by recovering organisms surviving in rabbit skin lesions (9).

Under natural conditions, in communities where streptococcal infections vacillate between endemic and epidemic proportions, "antigenic drift" may occur with respect to M serotypes. Maxted and Valkenburg (109) described a long-term study of streptococcal upper respiratory infections in the Dutch village of Voorhout. The original type 12 strain disappeared after several years and was followed by the appearance of a virulent "M-negative" variant (subsequently typed as 62). The latter possessed the identical T antigenic pattern of the original, and strains isolated at various times gave both a positive

and negative serum opacity reaction. The authors concluded that over a period of time this variant had arisen in the indigenous streptococcal population and survived, whereas the parent type 12 strain did not.

M Antigens and Opsonization

Most evidence points to opsonization as the primary defense against streptococcal infection in man and experimental animals (95). Antibodies in adequate titer, specific for the M antigens, are singularly responsible for the stimulation of opsonization by leucocytes. The mechanism by which the M antigen permits streptococcal cells to resist phagocytosis has not been explained. Moreover, in addition to the M protein, the hyaluronic acid capsule has been shown to contribute a measurable degree of nonspecific resistance to phagocytosis by group A streptococci (37, 38, 84). Cinephotomicrography studies by the late Armine Wilson (178) demonstrated what appeared to be surface phenomena enabling virulent group A streptococcal chains to slither off the surfaces of leucocytes vainly attempting to engulf them. Addition of type-specific sera to the system or removal of the M antigen by tryptic digestion altered the surface chemically or physically, permitting the phagocytes to engulf and kill the streptococci. The presence of bound immunoglobulin on the surface of streptococci cannot be the entire explanation of these surface phenomena inasmuch as antibodies specific for the C carbohydrate or T antigens are not opsonic.

Mechanisms of streptococcal opsonization have been studied in several laboratories and conclusions are somewhat conflicting, particularly concerning the roles of complement and "accessory plasma factors." Normally, virulent group A streptococci will grow in fresh heparinized human blood where type-specific antibody is absent. In the indirect bactericidal test (93, 106), dilutions of streptococci are mixed with whole heparinized blood from a nonimmune donor as a source of leucocytes and immune antiserum to be tested for opsonic activity is added to the system. The bactericidal action of the mixture is assayed after 2 or 3 h of incubation at 37 C in tubes slowly rotated end-over-end to ensure mixing. The surviving organisms are then plated on blood agar, and the numbers are compared with the original inocula. Stollerman et al. (153) demonstrated a factor in normal blood, possibly hyaluronidase, that stimulated immune phagocytosis, particularly with highly encapsulated streptococci. Although the factor was thermolabile and other anticomplementary manipulations of sera or plasma also removed this "co-opsonin," its relationship to complement was not established. In a later study (151) they observed a patient with hereditary angioneurotic edema and an accompanying C2 deficiency. This patient's plasma exhibited normal co-opsonin activity in a streptococcal opsonic system. The investigators concluded that opsonization of virulent streptococci can proceed in the absence of an intact complement sequence. Another experiment negating the participation of a complete sequence of complement components during in vitro opsonization was carried out by Perkins and Hahn (129), who prepared purified F $(ab')_2$ and Fab' fragments from immunoglobulin (IgG) possessing anti-M activity and demonstrated that these immunoglobulin fragments, like the parent antibody, were type-specifically opsonic. They assumed that removal of the Fc portion of the molecule prevented complement fixation, although they did not assay for complement or depletion of its components in their tests. Saito (142) also reported opsonic activity with type-specific anti-M antibody digested with papain. He claimed that univalent IgG fragments exhibited type-specific bactericidal activity in vitro and via mouse protection tests. The problem is by no means solved. Kahlich and Procházka (75) studied the participation of nonspecific serum fractions in type 12 streptococcal phagocytosis. By using only the criteria of zymosan absorption and heat inactivation to demonstrate significant reduction in opsonic activity in a reconstituted "indirect" system, they claimed that the first four components of the complement sequence were stimulatory and in some cases necessary for type-specific opsonic activity in vitro.

Michael and Massell (116) carried out active and passive mouse protection tests to assess the relative contributions of cellular and humoral factors in resistance to streptococcal infection. They observed in passive transfer that sera from mice in the early phases of immunization with streptococcal cell walls contributed only minimal protection to recipient mice, although the donor mice were comparatively resistant to both homologous and heterologous challenge. They examined the peritoneal leukocytes from these immunized donor mice and observed that in vitro phagocytosis was more vigorous in these cells when compared with cells from normal mice. They concluded that nonspecific cellular factors, or possibly cytophilic antibody, participated in the destruction of lethal doses of streptococci during the early phases of infection

in their immunized mice.

According to Domingue and Pierce (30) extracted M antigens had the capacity to be absorbed by glossy avirulent strains of group A streptococci. The absorbed antigen retarded the rate of in vitro phagocytosis of the live glossy streptococci. As yet unexplained was the additional observation that these phagocytosed glossy strains with M antigens absorbed were more susceptible to intracellular killing than were the normal glossy variants without the passively absorbed M antigen.

Gill and Cole (59) described an immunofluorescent technique for studying the fate of bacterial surface antigens, including M protein, after macrophage ingestion. They coated type 1 streptococci with fluorescein-labeled anti-M globulin and mixed them with washed mouse peritoneal macrophages. After various periods of incubation, the live phagocytes were examined by phase microscopy. The intracellular fluorescence patterns suggested that the M protein, or large fragments thereof, with intact bound fluorescent anti-globulin was removed from the cell wall and pooled within intracellular macrophage vacuoles. From a description of their methods for observing phagocytosis of the globulin-labeled streptococci, one must assume that opsonization and intracellular destruction occurred without the participation of complement in the medium.

Multiple Types and Shared Antigenic Determinants

The concept of employing individual serotypes of group A streptococci for inducing specific rabbit antisera to identify these types is not always tenable. Even in Lancefield's original description of the procedures for preparing absorbed type-specific sera we were advised that unless selected heterologous strains are used for absorption, all or most of the type-specific antibody is removed in the procedure (91). Owing to the discovery that various serotypes of M proteins share antigenic determinants (53), that certain strains of streptococci contain multiple serotypes (64, 121, 174, 175), and that other nontypable strains ("no-types") do, in fact, contain what may be defined functionally or chemically as M protein (98, 129, 171, 173), her observations have a sound basis.

Wiley and Wilson (177) carried out a study demonstrating the existence of two M antigens within individual strains. They found that clones from a number of strains of type 14 contained a second distinct M antigen which they newly characterized as type 51. Many of the type 14 strains they examined contained the type 51 antigen; four strains of the new type 51 were without type 14 activity, and only one of the eight isolates contained the type 14 antigen without the type 51. When the multiple strains were used to immunize rabbits, antibodies to the type 14 antigen predominated. The authors discussed the possibility that the multiple determinants could be on the same M protein molecule, although the two antigens appeared to be able to exist independently.

Shared antigenic determinants between M proteins of different serotypes were described by Fox and Wittner (53). Spurred precipitin lines in immunodiffusion tests with rabbit antisera specific for purified M-3 and M-12 proteins, when reacted with their respective M antigens, confirmed that shared antigenic determinants were responsible for cross-reactions between these serotypes. Although these cross-reactions were observed by precipitin and passive hemagglutination tests, the bactericidal assay did not show this reciprocity. Similar cross-reactions were found by Wiley and Bruno (175, 176) with types 33, 41, 43, and 52; in certain cases, rabbit antisera to these strains were reciprocally bactericidal.

Years earlier, Hirst and Lancefield (68) alluded to shared antigenic determinants between serotypes when they observed some degree of immunity against heterologous serotypes in mouse protection tests, and in recent years a number of laboratories have observed both in human infection and in laboratory tests individual serotypes inducing protective and bactericidal antibodies against heterologous serotypes. Harrell et al. (64) found reciprocal cross-protection in the indirect bactericidal test between types 3 and 31 and "one-way" cross-protection between types 46 and 51. In the latter, strains of type 46 contained a type 51 antigen as well, although strains of type 51 did not induce antibodies bactericidal for type 46. Similar results with one-way cross-protection between nephritogenic serotypes 55 and 60 were found by Bergner-Rabinowitz et al. (14) in sera of convalescent patients during an epidemic of type 55 strains involving both pyoderma and pharyngitis.

Cultural Conditions for M Protein Synthesis and Inhibition

Group A streptococci are capable of growing in a synthetic medium consisting of approximately 20 amino acids, purine and pyrimidine bases, B vitamins, trace elements, glucose, and buffering salts (114, 117). The synthesis of M

proteins, proteinase and streptolysin O by resting cells was shown by Fox (39) to depend upon the presence of glucose for an energy source and free amino acids supplied by hydrolyzed casein; the system was strongly stimulated by peptides from enzymatically digested proteins. The role of peptides has never been fully explained, although Kihara and Snell (85) published evidence that bacterial growth stimulated by peptides was a result of permeability effects in which the required amino acid in peptide linkage was better assimilated than the free amino acid and was preferentially utilized in semisynthetic media.

Mickelson and Slade (118) devised an entirely synthetic medium capable of supporting the growth of group A streptococci. However, virulent "M-positive" strains soon lost their typability after repeated transfers in this medium. When peptides from enzymatically hydrolyzed protein or sulfhydryl-reduced albumin were added to the medium, the streptococci regained the M protein. Davies et al. (27) cultivated group A streptococci in synthetic media under steady-state conditions in a chemostat and demonstrated that strains of types 6 and 14 group A streptococci could produce M protein for at least 480 generations. No peptides were required in the medium which included 17 amino acids, B vitamins, purines, pyrimidines, trace elements, glucose, thioglycolate, and buffering salts to maintain a pH of 7.3. They claimed that the amount of M protein produced was comparable to that obtained from stationary cultures in Todd-Hewitt broth.

Cohen (22) was able to obtain streptococcal growth and M protein formation in a semisynthetic medium with acid-hydrolyzed casein as a source of amino acids, provided the pH was held above 7; no peptides were required. Cohen's study on the composition and pH of media optimal for M protein or proteinase production demonstrated a valuable relationship concerning these two antigens. It was shown previously by Elliott (33) that under reduced conditions and at a pH below about 7.5, many strains of group A streptococci secreted extracellular proteinase which rapidly destroyed the M protein. It has been conjectured that small amounts of proteinase production by various nontypable strains could be responsible for the inability to detect the M antigen. Cohen's study demonstrated that many of the nontypable strains examined were unreactive with typing antisera for reasons unrelated to proteinase production. He showed that under controlled conditions, where traces of proteinase could be detected in strains known to contain the enzyme, no proteinase could be found in most of the nontypable streptococci. Pine and Reeves (132) also studied M protein production under various cultural conditions. They observed a number of factors affecting the amount of antigen per dry weight of cells. In a semisynthetic medium with limited glucose, more M protein was produced aerobically than anaerobically, although in Todd-Hewitt broth the effect was not observed.

The effects of antibiotics and metabolic inhibitors on M protein synthesis have been studied. A handy procedure for measuring M protein formation has made use of Lancefield's original observation that trypsin added to suspensions of group A streptococci will strip the antigen from the cell without affecting the viability (92). Fox and Krampitz (46) used this system in resting cell suspensions and demonstrated that M protein could be resynthesized in an incomplete growth medium consisting only of amino acids, peptides, and glucose. Metabolic inhibitors of glycolysis such as iodoacetic acid prevented the M protein from being resynthesized. A concentration of penicillin approximately 1,000 times that required to inhibit cell division did not interfere with regeneration of M protein. Brock (20) pursued these regeneration experiments with a large number of inhibitory substances and classified them into three general categories. A number of inhibitors including tyrocidin and diisopropyl fluorophosphate supposedly acted upon the mechanisms of fermentation and energy utilization and therefore prevented M protein synthesis. Another group of substances, mostly antibiotics such as penicillin, bacitracin, and gramicidin, interfered with cell wall mucopeptide synthesis, but did not prevent the regeneration of M protein. The third category of compounds which prevented cell growth as well as M protein synthesis were antibiotics which Brock classified essentially as "growth inhibitors," such as chloramphenicol, erythromycin, and tetracycline.

Reaction with Fibrinogen

It was demonstrated by Kantor (76) that M proteins will combine stoichiometrically with fibrinogen to form insoluble complexes. He estimated that fibrinogen (molecular weight about 400,000) combined with M protein (average molecular weight 40,000) in a one-to-one molar ratio. The M protein-fibrinogen complexes, prepared from partially purified M proteins precipitated with purified human fibrinogen, were immunogenic in mice and protected them against type-specific infection. Rabbit

antisera to the complexes also induced type-specific bactericidal antibodies. Hryniewicz et al. (70) found that fibrin monomers as well as intact fibrinogen also combined with M protein to form precipitates. Kantor was able to induce renal lesions in mice and rats by the intravenous inoculation of relatively large amounts of partially purified M protein (77). In renal biopsies from these animals it was revealed by immunofluorescence with antisera specific for M protein or fibrinogen that glomerular lesions contained complexes of M protein and fibrinogen as well as fibrin. The induction of the syndrome in mice required up to 18 mg of M antigen per animal, and thus one could question the relevance of these experiments to the pathology of human poststreptococcal glomerulonephritis in which fibrin or fibrinogen deposits in the glomerulus are not generally pathognomic (157). Further data on M proteins and poststreptococcal sequelae will be discussed in the section on immunologic injury.

Association of M Protein with Teichoic Acid

A teichoic acid polymer of D-alanyl polyglycerophosphate (PGP) was first shown by McCarty to be a constituent of group A streptococcal cell walls (111, 112). The teichoic acid polymer could be removed from cell walls by phenol extraction, and small amounts could also be obtained by washing whole cells with water. Jackson and Moskowitz (71) characterized D-alanyl PGP as one of the so-called "cell-sensitizing factors" found in streptococcal culture supernatants or extractable from cells by phenol. This factor had the capacity to bind to erythrocytes and other mammalian cells (29), and antibody to the cell-sensitizing factor could agglutinate sensitized red cells or, in the presence of complement, exert cytotoxic or lytic effects on a variety of sensitized mammalian cells. Wittner and Fox (179) found traces of PGP associated with the purified M protein of four serotypes. The PGP could only be released by enzymatic digestion of the purified M protein. However, anti-PGP did not agglutinate tanned sheep red blood cells sensitized with the M proteins nor would rabbit hyperimmune anti-M sera react with isolated PGP. The addition of PGP did not diminish the titer of anti-M sera in a passive hemagglutination inhibition test. Approximately 1 mole each of phosphorus and glycerol was detected in 1 "mole" of M protein with a minimum molecular weight of 20,000. However, a covalent attachment of PGP to M protein was not established. The authors concluded that this trace amount of PGP in purified M proteins was not immunogenic in vaccines administered to rabbits, even in large doses.

Serum Opacity Reaction

Culture supernatants and cellular extracts of certain group A streptococcal strains have the capacity to produce opalescence in horse serum as well as sera from a number of other mammalian species (89, 169). The opacity reaction was shown to be most likely the result of a streptococcal lipoproteinase acting upon the α-1-lipoprotein of the serum plus an activation or augmentation of normal serum enzymes esterifying cholesterol esters (141). Gooder (61) was the first to observe an inverse relationship between the capacity of group A streptococci to produce the opacity factor (OF) and the formation of the M antigen. This was confirmed by Köhler (86) and Top and Wannamaker (159). With few exceptions, strains that produced M antigen poorly or in amounts undetected by available antisera were shown to be good OF producers. Köhler examined over one thousand strains, and only about 10% of those producing M antigen were also positive for OF. Widdowson et al. (170) selected matt ("M-positive") and glossy ("M-negative") variants from eight individual serotypes and compared the opacity reactions; only the "M-negative" strains which no longer grew in blood produced the OF in culture supernatants. Rabbit antisera were prepared against "cell wall-membrane" fractions of each strain and tested for the capacity to inhibit the opacity reaction. They observed that inhibition of the serum opacity reaction by antisera for the various strains was generally type specific. This was also the case in the experiments of Top and Wannamaker (158) who examined a large number of streptococci typable by the M or T systems or both. Antisera to cell wall-membrane fractions capable of inhibiting the OF were generally strain specific and, particularly among the strains typable only by T-specific sera, they found a specificity for inhibition that closely followed the T antigen patterns of the streptococci. A possible antigenic, biochemical, and structural relationship of the OF to M proteins was claimed by Widdowson et al. (171). They separated the OF from cell walls by extraction and purification techniques used to obtain M proteins. The OF was associated with a high-molecular-weight fraction of the M antigen after partial purification. However, anti-M sera did not inhibit the opacity reaction and anti-OF sera did not precipitate M proteins in homologous serotype systems. Their work was in part at variance with that of Hill and Wannamaker (66) who

showed that OF was destroyed by hot acid extraction of streptococcal cell walls and was resistant to the action of streptococcal proteinase. Maxted and Widdowson (110) suggested the interesting possibility that the OF and M protein are part of the same molecule, although the OF activity is usually lost when M protein is fragmented during acid extraction; they suggested further that the immunodominant portions of OF and M protein are for the most part not shared. Thus, the physical or chemical relationships of OF and M protein have not been resolved.

M-Associated Protein

Maxted, Widdowson, and Pinney (110, 172) detected an antigen ("MAP") associated with purified M proteins from a variety of serotypes. This MAP antigen was "co-purified" with their preparations of M proteins and serologically cross-reacted with all M preparations tested. The antigen was assayed by a macro complement fixation test in which crude acid extracts of streptococci served as the MAP antigen reacting with various anti-MAP sera. The MAP of certain strains was reported to be resistant to proteolytic digestion when whole cells were subjected to streptococcal proteinase (110), although acid-extracted MAP was susceptible to proteolysis. Sera from rheumatic fever patients generally had higher titer anti-MAP activity than sera from patients with uncomplicated streptococcal infections. The MAP antigen has not been shown to be related serologically to the heart-specific cross-reacting antigens of streptococcal cell walls, nor have the chemical or physical properties of the MAP been elucidated. Wittner and Fox (179) were unable to detect the presence of extraneous cross-reacting antigens in their preparations of M proteins assayed by a sensitive micro complement fixation assay, indicating that the presence of MAP may be related to the methods used to obtain purified M proteins.

M Proteins from Other than Group A Streptococci

Maxted and Potter (108) discovered a type 12 M antigen in three strains of group G streptococci isolated from the throat and skin lesions of children during a glomerulonephritis epidemic in Trinidad. Rabbit antisera prepared against these group G strains reacted in immunodiffusion with M antigens from group A, type 12 strains. These antisera also exhibited bactericidal activity against group A, type 12 streptococci as well as the homologous group G, "type 12" strains and passively protected mice against lethal doses of group A, type M-12 streptococci. Maxted (107) subsequently tested 146 other group G strains and 10 group C strains for precipitin reactivity with anti-M-12 sera, but was unable to find type-specific activity in any of them. To this reviewer's knowledge no other streptococcal groups have been shown to contain M antigens. In comparing group A and group C streptococci, the structural and antigenic similarities of these two serological groups far outweigh their differences. The immunodominant carbohydrate determinant of group A is *N*-acetylglucosamine and group C is *N*-acetylgalactosamine, each on a rhamnosyl-rhamnose backbone (88). Consequently, epimerase- and transglycosidase-mediated reactions are responsible for the main structural differences between the two organisms, indicating only a minor genetic divergence between groups A and C. It would thus seem reasonable to find M proteins (and possibly T antigens) in group C streptococci; further screening for these antigens may reveal their presence in group C streptococci, particularly in those strains freshly isolated from human upper respiratory infections.

M-like antigens were described in certain strains of *Diplococcus pneumoniae* by Austrian and MacLeod (4). The crude antigens were obtained by the hot acid extraction procedure of Swift et al. (156) used routinely for streptococcal typing. These protein antigens conferred no antiphagocytic property to the pneumococci and did not appear to be related to the pathogenicity of the organisms. Antisera to the putative M antigens were not opsonic or bactericidal. The M antigens were found in both smooth and rough variants of pneumococci, but antipneumococcal M sera (free of anti-carbohydrate activity) agglutinated only the rough variants. Antisera specific for streptococcal M antigens (16 were tested) did not cross-react with pneumococcal M proteins nor did antisera to the latter cross-react with streptococcal M antigen extracts from any of the 27 serotypes tested. From these data one must conclude that the structural relationship of the so-called pneumococcal M antigens to those of group A streptococci has not been established.

M ANTIGENS, STREPTOCOCCAL INFECTION, AND EPIDEMIOLOGY

Type-Specific Immunity

Epidemiological studies have demonstrated that an untreated upper respiratory group A streptococcal infection probably affords lifelong type-specific immunity (95). Lancefield

(94) examined the antibodies of individuals who had been infected with known serological types of streptococci for as long as 10 to 32 years previously. Type-specific bactericidal antibodies to these types were still present in many of the subjects. It is difficult to explain the persistence of these antibodies in persons who do not carry group A streptococci in the upper respiratory tract (31, 148). In noncarriers one may presume that in the absence of subsequent infections, casual contact or exposure to these serotypes affords sufficient antigenic stimulus to maintain a measurable secondary antibody level.

In contrast to upper respiratory group A streptococcal infections, little if any data have been published demonstrating immunity in untreated self-limiting pyodermic infections caused by skin-associated strains. In recent years, reports of epidemics of acute glomerulonephritis (AGN) have shown that the group A streptococci responsible are frequently associated with skin infections rather than upper respiratory infections (73, 150, 168). The serological typing of these pyodermic nephritogenic streptococci has presented problems owing to difficulties encountered in preparing absorbed typing sera specific for these organisms. The latter have often been better characterized serologically by their T agglutination patterns (119). The data of Table 1, assembled by M. D. Moody and R. R. Facklam (personal communication from the Center for Disease Control, Atlanta, Ga.) from a number of studies throughout the world list the newer serotypes of group A streptococci associated with skin infections and accompanying AGN. Serotypes 52 through 63 are relatively new designations for types isolated predominantly from impetigo infections leading to AGN. Many of the pyodermic strains among these serotypes grow in normal human blood, although convalescent sera from AGN patients are occasionally bactericidal (13, 72, 160), and therefore these streptococci are presumed to contain an M antigen. Titers of type-specific antibodies in patients recovered from AGN of pyodermic origin are rarely high; antistreptolysin O titers are frequently low and serological evidence of a recent streptococcal infection is found primarily in the elevated anti-deoxyribonuclease and anti-hyaluronidase titers (168).

Several laboratories have described experimental animal models presumed to simulate the impetigenous lesion of human streptococcal pyoderma. Cushing and Mortimer (25) found that the shaved skin of hamsters inoculated intradermally with live streptococci isolated from human impetigo lesions produced a pustular lesion grossly and histopathologically resembling the human infection. These infections were generally self-limiting and without systemic complications (including the absence of renal pathology). Dajani and Wannamaker (26) repeatedly infected hamsters cutaneously with types 12, 49, and 57, but (as in human infections) were unable to induce type-specific immunity or demonstrate an altered course of infection in animals previously infected.

Carriage of group A streptococci in the upper respiratory tract after a clinical infection or via asymptomatic acquisition has been shown in a number of studies to maintain type-specific antibody at elevated levels. See, for instance, the reviews of Rammelkamp (137) and Wannamaker (168). Paradoxically, the presence of these antibodies does not always lead to the elimination of the streptococci, which may persist in carriers for many months and occasionally for several years. Physicians recognizing this situation are faced with the dilemma of either treating the asymptomatic patient to eliminate the streptococci or not treating the patient and thus allowing him to develop type-specific immunity at the risk of acquiring post-streptococcal sequelae. From an epidemiological standpoint, selective pressures by host antibody may lead to the emergence in carriers of mutant streptococci possessing M antigenic determinants recognized neither by laboratory typing sera nor the immunological defenses of a susceptible host to whom the organism may be transmitted.

Frequency and Distribution of Serotypes

Although M antigen specificities have been described for about 63 serotypes of group A streptococci, some are exceedingly rare and others possibly have disappeared from the natural environment. The most recent strains isolated from endemic infections and epidemics of AGN associated with pyoderma have been characterized as new M serotypes, and in all likelihood additional new serotypes will continue to arise. For the experienced epidemiologist, M typing as a means of identifying and classifying group A streptococci is reliable and convenient when sera are available. However, many of the typing sera are difficult to prepare, and only a few laboratories have a complete inventory of typing sera. These laboratories include the World Health Organization Headquarters of the International Streptococcal Reference Center in

TABLE 1. *Group A Streptococcus provisional new M types 52 through 63*

M type	T agglutination pattern	Reference strain or source	Other designations of sample strains	Ref.
52	3/13/B3264	CV686 (Top and Wannamaker) Red Lake and Cass Lake, Minnesota	CDC SS686 Colindale 100, 186 Lancefield A871/14/2	160
53	3/13/B3264	CV832 (Same as 52)	CDC SS733 Colindale 100, 187 Colindale R66/3441	160
54	15/17/19/23/47	CV581 (Same as 52)	CDC SS725 Colindale R66/3443	160
55	(8)/25/Imp 19	Trinidad A (Potter)	CDC SS735 Colindale R65/4127 Rabinowitz (Israel) 2004	
56	(8)/25/Imp 19	"Baker" (Stollerman, Memphis, Tenn.)	CDC SS743 Lancefield A963 Colindale R67/3281	72
57	(8)/25/Imp 19	(V. Ramkissoon)	CDC SS790 Lancefield A995	
58	25/Imp 19	Trinidad (Bassett)	CDC SS872 Lancefield D315	
59	(14)/25/Imp 19	2853 Birmingham, Ala. (Dillon)	CDC SS914 Lancefield D307, D304 Colindale R67/2991	
60	4	2797S Birmingham, Ala. (Dillon)	CDC SS876 Colindale R69/2709	
61	(9), 11	2998T Birmingham, Ala. (Dillon)	CDC SS875 Colindale R66/2714	
62	12	Colindale R68/485 (Maxted, Parker)	"Voorhout"	109
63	4	Colindale, Trinidad (Parker, Maxted)	CDC SS985	

Prague, the Center for Disease Control, United States Public Health Service, Atlanta, Ga., and the Central Public Health Laboratory (Colindale), London. Unofficially, many of the streptococcal reference sera are also exchanged between these laboratories and R. C. Lancefield (Rockefeller University, N. Y.), whose collection of group A streptococcal cultures and type-specific sera is extensive.

In 1964 and 1965 an international survey was carried out to determine the distribution of serotypes of group A streptococci infecting man. The data were compiled by M. T. Parker (128) from information submitted by 12 laboratories throughout the world. Nearly 6,000 cultures from representative samples of streptococcal disease in each of the participating countries were typed by T agglutination and M precipitation tests. Over 80% of all cultures were typable by the T agglutination pattern, but only about 30 to 50% of the strains were typable by M precipitation (depending on the individual laboratories and their supply of anti-M sera). By both techniques, over 90% of all cultures were

typable. The typing results from the various laboratories were compared for the same cultures and 86% of all cultures typed similarly in each laboratory. The M serotypes seen most frequently in that 2-year period were 1, 3, 4, 5, and 12. The most common strains identified by their T agglutination patterns included [3, 13, B3264], [5, 11, 12, 27, 44], and [8, 25, Imp. 19]. Although certain types (as listed above) were common in most countries, there were differences in national distribution as well as differences in types responsible for scarlet fever, sore throat, and extrarespiratory infections.

Neonatal Antibodies

The transplacental acquisition of type-specific antibodies may account in part for the infrequency of group A streptococcal infections in infants during the first 6 months of life. Several studies have compared anti-M titers of maternal and neonatal (cord) blood. Quinn and Lowry (136) used a passive hemagglutination assay with seven serotypes to show that antibody titers of infants were approximately equal to those of the serum of the respective mothers. Zimmerman and Hill (183) also carried out the same type of study by using both passive hemagglutination and bactericidal tests to measure type-specific antibody in maternal and neonatal serum. They assayed over 100 pairs of sera and found each transplacental antibody titer, except in a few instances, to be equivalent to that of the mother. Follow-up studies showed that the anti-M titers of infants declined markedly by 3 months and virtually disappeared after 6 months.

No definitive studies have been carried out to assess the role, if any, of colostral antibody in human neonatal protection against group A streptococcal infection. Experimentally, Fox and Wittner (55) examined the type-specific antibodies in the colostrum of rabbits immunized by intramammary injection of M protein vaccines administered at the time of conception and 10 days before parturition. The crude colostral whey was type-specifically bactericidal; after fractionation of the secretory immunoglobulins, all the bactericidal acitivity was shown to reside in the IgG. The purified colostral IgA, although representing about 90% of the total globulins, was not bactericidal and could be shown only by passive hemagglutination to possess type-specific anti-M activity.

Adherence Phenomenon

A possible clue to the mechanism of colonization of upper respiratory mucosal membrane by group A streptococci in a susceptible host has been found by Ellen and Gibbons (32). They observed that "M-positive" strains had the capacity to stick to mucosal epithelial cells in vitro, whereas "M-negative" streptococci did not. This adherence of virulent streptococci to mucosal surfaces was inhibited with type-specific antibody (including secretory IgA) or by pretreating the microorganisms with trypsin. The authors observed the phenomenon in vitro with human buccal mucosal cells and hamster cheek pouch membranes.

CHEMICAL AND PHYSICAL PROPERTIES OF M PROTEINS

Extraction Techniques

Lancefield's discovery 45 years ago of the extractability of M proteins from streptococcal cells with hot dilute acid has proved to be both an advantage and a hinderance in the elucidation of the structure of these antigens (90). In a single step of heating cells at 95 C in saline acidified to pH 2.0, the M antigens are dissociated from the cells, and the majority of cell wall and cytoplasmic debris may be discarded after separation by centrifugation. Although the crude, solubilized antigen is contaminated with extraneous protein, carbohydrate, and nucleic acid, the bulk of cellular material is removed. On the other hand, relatively drastic hot acid extraction of a washed culture (or cell walls) results in the production of an M protein preparation comprised of a multiplicity of polypeptides ranging in molecular weight from 20,000 to 40,000 (51). The structure of M proteins will be covered later in this section.

Several enzymatic methods for removing M proteins from cells have been described. The lytic enzyme liberated during bacteriophage infection of group C streptococci has been used as a muralytic agent to solubilize the M antigen from group A cells (5, 17, 78, 87). In another procedure extracellular muralytic enzymes produced by *Streptomyces albus* have been used to degrade streptococcal cell walls. These latter enzymes were characterized by McCarty (111) primarily for the purpose of obtaining the liberated group-specific carbohydrate of streptococci, and the presence of proteolytic activity in the crude *S. albus* extracts destroyed the M protein. However, Schmidt (144) was able to inhibit proteolysis by treating a partially purified *S. albus* enzyme preparation with diisopropyl fluorophosphate, and lysis of group A streptococci by this system liberated M protein into solution. Although enzymatically liberated M

proteins are immunogenic and may represent a more "native" configuration of the antigen, material prepared by these methods has not been adequately separated from extraneous antigens such as group-specific polysaccharide or nucleic acid. Hence, no reliable data are available on the chemical or physical nature of enzyme-liberated M proteins.

Alkaline extraction of cell walls has yielded M proteins of high molecular weight, ranging between 100,000 and 200,000 (54). This procedure employs buffers such as borate or hydroxylamine at pH 10 at 37 C for several hours or, alternatively, at 95 C for 10 min. The technique, as subsequent purification has borne out, appears to be less drastic than hot acid extraction and the high-molecular-weight M antigen is amenable to further purification. Sonic oscillation was used by Slade and Vetter (147) to disrupt streptococcal cell walls to a degree that solubilized the M antigen. Besdine and Pine (15) used the technique to obtain a crude M antigen with an estimated molecular weight of 300,000; no additional physical data have been published on M proteins obtained by sonication, no doubt owing to problems of subsequent purification.

Fractionation and Purification

As working definitions the following terms are used. (i) "Crude" M protein: extracted from whole cells, contains measurable amounts of extraneous protein, nucleic acid and, occasionally, group-specific polysaccharide; (ii) "partially purified" M protein: extracted from whole cells or walls, contains extraneous protein antigens, and frequently traces of nucleic acid. (iii) "Purified" M protein: usually extracted from cell walls, contains no measurable carbohydrate or nucleic acid, and may exhibit cross-reactions in immunodiffusion with heterologous absorbed typing sera. (iv) The term "M antigen" refers generically to the native material on whole cells or cell walls. It is also used broadly in reference to uncharacterized fractions capable of reacting with specific anti-M sera.

Most of the current methods for the preliminary fractionation and purification of M proteins evolved from the work of Lancefield and Perlmann (96). Their initial step consisted of hot acid extraction of whole cells, after which the neutralized soluble antigen was recovered in the supernatant fraction after centrifugation. Ribonuclease was added, and the mixture incubated at 37 C for 5 h or longer. The nucleotide products were removed by extensive dialysis. The M protein was recovered by ammonium sulfate precipitation: the fraction precipitating between 30 and 60% salt saturation contained the bulk of the partially purified antigenically active M protein. These procedures were modified by Fox and Wittner (54), who employed cell walls rather than whole cells as the starting extractable material. They observed that subsequent purification yielded a more antigenically homogeneous protein when the M antigen was extracted from walls rather than from whole cells. When whole cells were extracted, nonspecific cytoplasmic material was carried along through the final stages of fractionation. Various methods of obtaining cell walls have been described specifically for streptococci. Rapid oscillation or blending of bacterial suspensions in the presence of Ballotini beads has proved to be an efficacious method for nearly complete cell breakage (16, 103). The resulting soluble cytoplasmic contents and cell membrane fractions can be separated from the walls by differential centrifugation. Fox and Wittner treated washed cell walls with ribonuclease prior to acid extraction (54). The soluble crude M protein was precipitated by ammonium sulfate, and the protein precipitating between 30 and 60% salt saturation was collected. Immunodiffusion analysis of M proteins at this stage of purification revealed, with the aid of antiserum against whole homologous streptococci, a preparation contaminated with at least three extraneous antigens, including traces of group-specific polysaccharide.

Further purification by employing chromatographic techniques was shown for some serotypes to yield an antigenically homogeneous M protein. However, certain serotypes purified by these techniques yielded M proteins containing heterogeneous antigenic components which may be related structurally to M protein but which have not been completely characterized (176). Carboxymethyl cellulose columns with gradient elution by acetate and phosphate buffers was used to obtain purified M proteins from cell wall extracts after ribonuclease treatment and salt fractionation (43, 54, 65, 125). Hydroxyapatite (74), diethylaminoethyl cellulose (97), and Sephadex G-200 (13) have also been employed for M protein purification. A summary of the accumulated physical and chemical data obtained from analyses of M proteins purified by these various techniques is given in Table 2. No data are available comparing specific techniques for their efficiency in isolating purified M proteins. A comparative study of purification techniques using selected strains of streptococci under standardized conditions,

employing both whole cells and cell walls, would be worthwhile.

Physical and Chemical Analysis

Purified M proteins in two physical states will be considered (Table 2). The first, extracted from cells or cell walls by hot acid is, in all likelihood, a degraded protein which nevertheless retains both its antigenic specificity and immunogenicity. The second "variety" of M proteins is obtained from cells or cell walls by alkaline extraction, the action of surfactants, or enzymatic muralytic release. The latter are also antigenically and immunogenically reactive and may be more closely akin to the native antigen on the bacterial cell wall. Molecular weights of alkaline-extracted purified M proteins were between 120,000 and 180,000 when measured in an ultracentrifuge; this may be contrasted to the acid-extracted protein whose molecular weight was estimated to range from 20,000 to 40,000 (54). Sedimentation velocity and diffusion measurements were first carried out on acid-extracted, crude M proteins by Pappenheimer et al. (127) who estimated a molecular weight of 40,000 and an axial ratio of 25:1. This high degree of dissymmetry of the antigen has been confirmed for both high- and low-molecular-weight components (54). An intriguing aspect of the architecture of the M protein was the discovery of a multiple molecular structure of the acid-extracted antigen. Three purified M protein serotypes, 12, 14, and 24, exhibiting homogeneity by immunodiffusion, were each separated into at least four major peaks by gel filtration on Sephadex G-200 (51). The purified M proteins of each serotype were separated into 10 to 15 bands by acrylamide gel electrophoresis. These bands from individual serotypes were isolated, and all were shown to possess antigenic identity as well as similar, but not identical, amino acid content. Differences in electrophoretic mobility of these bands of M protein were attributed to small differences in net charge and polypeptide length. Homologous serotypes of M proteins from different sources gave nearly identical electrophoretic patterns in acrylamide gel. This was interpreted as a nonrandom and systematic chain cleavage resulting from the acid extraction. Alkaline-extracted M proteins of high molecular weights produced only one or two slowly migrating bands in gel electrophoresis (54). Attempts to dissociate the high-molecular-weight M protein into the multiple structure by electrophoresis in 8 M urea, 6 M guanidine, or by succinylation were unsuccessful, although heating briefly at pH 2 (as in acid extraction) partially degraded the high-molecular-weight structure to the multiple molecular "subunits" of the acid-extracted protein. Amino acid analyses and peptide maps of the large and small M proteins were very similar, suggesting that the multiple molecular forms were large fragments of repeating units of the more "native" structure. Antigenic analysis by quantitative precipitation and passive hemagglutination failed to demonstrate additional antigenic determinants associated with the high-molecular-weight M protein. These data were interpreted as demonstrating that the intact cellular antigen was composed of a subunit structure, dissociable by weak acid and heat, and was not a macromolecule with M-specific determinants or polypeptides covalently or physically associated with a carrier protein. The multiple molecular structure of M proteins has since been confirmed by Vosti et al. (164) and Beachey et al. (6). Further evidence for a subunit structure of M proteins was recently presented in a brief communication by Fischetti et al. (36) who employed a nonionic detergent to remove M protein from cell walls. When the purified M protein was subjected to electrophoresis in acrylamide gel containing sodium dodecyl sulfate, protein subunits with molecular weights estimated at 30,000 were obtained. Removal of the detergent resulted in the reaggregation of the subunits into a 90,000 molecular weight fraction. The nature of the association of the multiple units constituting the native antigen has not been elucidated, nor have the chemical or physical bonds by which the M protein is attached to the cell wall been determined, although removal by detergent suggests that the M protein is bound to the wall by noncovalent linkages.

As shown in Table 2, purified preparations of M proteins may be obtained with no detectable carbohydrate. Most determinations show various M proteins to contain between 14 and 16% nitrogen. Reported amino acid analyses agree that sulfur-containing amino acids are low; probably one or no cysteine residues are found for a molecular weight unit of at least 20,000. The value of 2.4% sulfur in the elemental analysis of Lancefield and Perlmann (96) possibly reflects both the incomplete purification of the antigen and the limitations of the analytical methods available at the time. Data from several laboratories reporting total amino acid analysis of a number of serotypes reveal a low content of aromatic amino acids, and this is reflected in the ultraviolet absorption spectrum.

TABLE 2. *Chronology of physical and chemical analyses of M proteins*

Serotype and source	Physical data			Chemical data	Remarks	Ref.
	Mol wt	Absorbancy (280/260 nm)	Axial ratio			
M-1		1.36		15% N	Electrophoretically heterogeneous	96
Whole cells, acid extract				2.4% S (?)	Immunogenic	
M-12		1.25		12.3% N	Carboxymethyl cellulose gradient elution	51, 54
M-14	38,000	1.15	15:1	12.1% N		
M-24		1.14		12.6% N	M-24: homogeneous in ultracentrifuge	
Cell walls, acid extract				Pentose, methyl-pentose, and hexosamine; all <0.2%	Immunogenic	
M-12	20,000 to 30,000	1.31	18:1	15.9% N	CM cellulose gradient elution	54
Cell walls, acid extract				No sugars detected	Multiple molecular structure Immunogenic	
M-12	120,000 to 180,000	1.15 to 1.22	26:1	14.6% to 16% N	Purified "native?" antigen	54
Cell walls, alkaline extract				No sugar detected	High molecular weight protein Immunogenic in rabbits in 1-μg doses	
M-12, M-30	50,000 to 200,000	1.1 to 1.25		<0.5% pentose	Single peaks in boundary electrophoresis; multiple peaks from Sephadex and DEAE cellulose chromatography	6
Whole cells, acid extract						
M-12; whole cells, acid extract; six other serotypes also were analyzed		1.1		N-terminal alanine predominates	CM and DEAE cellulose chromatography; multiple molecular structure	97, 154
M-12; whole cells, acid extract	32,000	1.30 to 1.37		N-terminal alanine C-terminal leucine No rhamnose, pentose, or deoxypentose detected	Hydroxyapatite chromatography; multiple molecular structure	164
M-6, M-12, cell walls; nonionic detergent extract	Subunits, 30,000 Aggregate, 90,000			N-terminus "blocked"	"Chemically, physically and immunologically homogeneous" subunits, aggregate in absence of detergent	36

The values of the ratios of absorbancy of 280 to 260 nm for a number of purified M proteins range from 1.1 to 1.3. Eighteen amino acids have been detected in the various serotypes analyzed. Glutamic acid, alanine, leucine, lysine, and aspartic acid are the five amino acids in highest concentration, although the molar ratios of these in homologous serotypes do not quite agree in the reports from various laboratories. All of the above amino acids reported by Fox and Wittner had the L configuration and therefore were presumed not to be contaminated with cell wall mucopeptide material (51). Vosti et al. (164) applied end group analysis to a purified preparation of acid-extracted type 12 M protein. They were able to detect only L-alanine as the N-terminal amino acid with minor amounts of glycine and leucine (isolated as dinitrophenyl derivatives). C-terminal amino acids were, in order of abundance, lysine, arginine, and histidine, with small amounts of leucine, serine, alanine, and glycine released by carboxypeptidases. Strauss and Lange (154) also carried out this type of analysis on M-12 protein and reported alanine as the N-terminal residue. In view of the probable fragmented multiple molecular structure of the acid-extracted M proteins analyzed by these procedures, a mixture of terminal amino acids would be more likely than a single N- and C-terminal residue for the entire protein mixture.

Several pieces of misinformation on M proteins have been carried for many years through the literature, including a few textbooks (e.g.,

160a). One reads from time to time that the M antigen is a "basic protein" (167) and is alcohol soluble. Neither of these statements is true: as seen in Table 2, the isoelectric point of purified M proteins is approximately 5.5, and purified M proteins are insoluble in neutral solutions containing more than about 50% ethanol.

ASSAY OF M PROTEIN AND TYPE-SPECIFIC ANTIBODY

Standard Procedures

Traditional methods for the assay of type-specific anti-M antibody include the capillary precipitin test, the in vitro bactericidal assay, the "long-chain" test, and passive mouse protection. The Lancefield capillary precipitin technique has long been the accepted procedure for detecting M antigen in crude extracts and for serotyping group A streptococci (95). Standard absorbed sera are required for positive identification of M types. Opsonic antibodies in humans and experimental animals are frequently detected in vitro by the indirect bactericidal assay (93, 106): heparinized whole human blood as a source of leucocytes (and possibly complement factors) is incubated with viable homologous streptococci and appropriate dilutions of antisera. After incubation, colonies from surviving organisms are counted on blood agar plates. A variation of the indirect bactericidal assay was described by Bergner-Rabinowitz et al. (12) who employed mouse peritoneal leucocytes; opsonization was measured by counting the number of intracellular streptococci on a fixed slide. Stollerman and Ekstedt (152) devised the "long-chain" test for assaying type-specific protective antibodies. This procedure is based on the observation that in the presence of homologous anti-M antibody, streptococci grow in long chains in comparison to growth in normal serum. The test results compare favorably with the bactericidal assay, but data obtained with low-titer sera frequently require statistical analysis for appraisal. The mechanism by which type-specific antisera induces streptococci to grow in long chains has not been elucidated. Passive mouse protection, whereby mice are injected intraperitoneally with 0.1 or 0.2 ml of antiserum and then challenged via the same route with dilutions of virulent streptococci, yields results in vivo usually analogous to the in vitro bactericidal assay (95). There is some question as to whether the mechanism of mouse protection is solely via opsonic mechanisms, because of the occasional demonstration of type-specific mouse protection with anti-M sera devoid of opsonic antibody measured in vitro (116).

Newer Techniques

The procedures summarized above have been the mainstay of immunological assays for M antigens and antibodies for many years. Methods for the detection of anti-M antibodies and M protein have been adapted to various micro and semimicro serological procedures developed more recently. An Ouchterlony immunodiffusion assay was described by Michael and Massell (115) in which M serotyping may be carried out with unabsorbed sera. The procedure, amplified by Rotta et al. (140), obviates the frequent problems encountered in the loss of type-specific antibody resulting from the absorption of sera with heterologous types of streptococci. In the assay, both type- and group-specific precipitin lines occur. The group-specific reaction is identified as a contiguous line of precipitation shared by adjacent wells, and the type-specific precipitin reaction is seen only with the sera and extracts of homologous types.

Passive hemagglutination (HA) by slight modifications of the Boyden (18) technique, with tanned erythrocytes "sensitized" with M proteins, is an extremely sensitive method for detecting anti-M antibody (28, 42, 135, 166, 184). Type-specific titers in hyperimmunized rabbits can often be measured in sera at dilutions of 10^{-5}. However, because of this sensitivity, cross-reactions among serotypes are invariably seen. The latter may be the result of shared antigenic determinants or the presence of heterologous antigens in the M protein preparations. These cross-reactions may be eliminated to some extent by absorption of sera with heterologous cells or crude cellular antigens (166). Generally, HA titers of immune sera parallel those seen in the indirect bactericidal test. Occasional sera exhibiting high HA titers are unexplicably devoid of opsonic activity in vitro (56). Because of the frequent cross-reactivity of sera assayed by HA, and the absence of bactericidal antibodies in certain sera with high HA titers, it has been suggested that the passive HA assay be considered only a persumptive test for a specific anti-M response in humans or experimental animals (53).

A microcomplement fixation assay was devised by Wittner and Fox (179) to measure the type specificity of anti-M antibody. There was a reasonable correlation between type-specific complement-fixing and bactericidal antibodies from immunized humans and experimental ani-

mals. Purified M protein was required (in nanogram amounts) to carry out the assay which was claimed to be more quantitative than the conventional bactericidal or long-chain tests. It is of interest to note that the IgG fraction of serum antibody is primarily responsible for the type-specific passive HA, bactericidal, and complement fixation reactions in immune animals and humans (52, 179), although Newcomb (123) has recently demonstrated type-specific HA activity in human immune serum IgA.

The primary antigen binding technique of Farr (35) was used by Grey (62) to detect micro quantities of anti-M antibody by means of 50% ammonium sulfate precipitation of soluble complexes of antibody and ^{131}I-labeled crude M protein. The method could detect nanogram amounts of antigen or antibody; the latter was obtained from hyperimmune rabbits and rheumatic fever patients. The specificity of the reaction employing crude M antigen may be questioned, owing to the precipitability of M protein in 40 to 50% ammonium sulfate. It was necessary for Grey to use only that portion of crude M protein remaining after first precipitating the antigen in 40% ammonium sulfate. The remaining soluble portion of the crude antigen may have been comprised of polypeptides of M antigen with limited specificity. Variations on the Farr primary antigen binding technique employing ^{131}I-labeled M protein and type-specific rabbit antibody have been described (1, 122). Soluble specific complexes were precipitated with sheep antirabbit IgG. During the course of rabbit immunization with several serotypes of heat-killed streptococci, type-specific anti-M antibody could be detected 20 days after injection, when no measurable bactericidal or precipitating antibodies were observed (1).

A semiquantitative assay for M antigen in crude acid extracts of group A streptococci was devised by Cohen and Pine (23), utilizing the capillary precipitin procedure. Measured amounts of type-specific serum and extracts were reacted, and after incubation the capillary tubes were centrifuged and the volume of packed precipitate was measured optically. Standard curves were linear in regions of antibody excess, although in antigenic excess the amounts of total precipitate diminished as would be expected in precipitin reactions covering a wide range of antigen and antibody concentrations. Another semiquantitative method for estimating M proteins made use of immunodiffusion in agar in which limiting dilutions of M protein solutions were titrated with antisera, the end point of the reaction being the highest dilution of antigen exhibiting a visible precipitin line (10). This method as well as the capillary assay depend upon the use of standardized sera for comparative results.

Streptococcal M protein on whole cells and subcellular fractions was measured with fluorescein-conjugated rabbit antisera (40, 41). After reaction of the particulate antigen and fluorescent type-specific antibody, the latter was eluted with dilute alkali and measured in a fluorimeter. The amount of labeled globulin eluted appeared to be a function of the amount of M protein on the cell walls or cytoplasmic membranes.

Newcomb (123) devised an insoluble immunoadsorbent coupled with anti-M immunoglobulin to purify M protein. The eluted M protein appeared to have most of the nonspecific antigenic material removed by the technique. Latex particles sensitized with partially purified M proteins were used for agglutination assays of type-specific sera (19, 131). Brighton (19) found that the agglutination titers of sera generally agreed with the results from mouse protection studies, although some of these same sera were negative in the bactericidal test.

In addition to the above procedures, several methods have been devised for typing group A streptococci by immunofluorescence (82, 134, 163) and immunodiffusion on cellulose acetate (11). A number of papers have also appeared describing procedures to improve the production and specificity of M-typing antisera by variations in immunization schedules and serum absorption (3, 8, 34, 63, 102, 134).

M ANTIGENS AND IMMUNOLOGICAL INJURY

Toxicity

At the present time there is no evidence (reviewed by Ginsburg [60]) that M proteins are inherently toxic to humans or experimental animals, regardless of the mode of administration. However, like other bacterial antigens, M proteins are capable of inducing adverse immune responses, such as delayed and immediate allergic reactions (6, 56), as well as complement-mediated immune phenomena (7). Fox and Dorfman (unpublished data) found that approximately 75% of 420 normal adults exhibited delayed cutaneous hypersensitivity to a 1-μg intradermal dose of one or more of the common serotypes of purified M proteins. Over 90% of these persons had type-specific anti-

bodies with titers greater than 1:100 measured by passive HA. It is assumed that previous clinical or subclinical exposure to these or antigenically related serotypes was responsible for the skin reactivity and serum antibody. Beachey and Stollerman (7) observed in normal human heparinized blood to which M protein was added that platelets aggregated, became surrounded by polymorphonuclear leukocytes, and the total aggregate of cells eventually lysed. The authors suggested that this process resulted from the presence of anti-M antibody plus complement in the system. Further evidence for a complement-mediated immune reaction in vitro was their finding that M protein added to polymorphonuclear leukocytes and platelets in Tyrode's solution or heated plasma did not initiate the clumping or lytic phenomena. Beachey et al. (6) claimed that purified M proteins, types 6, 12, and 30 (about 60-μg doses), uniformly produced delayed cutaneous reactions in normal, outbred adult guinea pigs and also inhibited the migration of adult guinea pig peritoneal macrophages, although both of these reactions were minimal in young animals. They speculated that the reactivity of their normal guinea pigs to M proteins could be the result of the presence of minor contaminants in their M preparations. These allergenic contaminants could cross-react with antigens from group C streptococci which guinea pigs frequently carry asymptomatically.

Normal human infants under the age of 24 months only infrequently exhibit delayed cutaneous hypersensitivity to purified M proteins (56). Pachman and Fox (126) also observed that inbred adult guinea pigs not previously exposed to M protein vaccines did not exhibit cutaneous hypersensitivity nor did their peritoneal leucocytes respond to M proteins in vitro. On the other hand, infants or guinea pigs immunized with alum-precipitated M proteins did exhibit a delayed cutaneous response and, in vitro, their leucocytes in the presence of M proteins responded by increased thymidine uptake (i.e., deoxyribonucleic acid (DNA) synthesis) or decreased migration in capillary tubes (126). These in vitro responses were also seen in cultured lymphocytes of persons who exhibited delayed cutaneous hypersensitivity and circulating antibody to a number of common M protein serotypes to which they may have been clinically exposed. I. M. Lyampert's current monograph (in Russian) discusses much of the evidence relating streptococcal antigens to the immunopathology of poststreptococcal sequelae (100). From a private translation obtained by this reviewer, it must be concluded that Lyampert has found no additional evidence than that which is presented here. Adverse effects of crude or partially purified M antigen vaccines will be discussed subsequently.

Do M Proteins Share Antigenic Determinants with Human Tissue?

The autoimmune hypothesis of the etiology of poststreptococcal sequelae was originally proposed by Cavelti (21); Kaplan and coworkers (80, 81) gave credence to this idea by demonstrating that an antigen extractable from the cell walls of selected strains of group A streptococci could induce in rabbits antibodies capable of reacting with the sarcolema and subsarcolema of human and other mammalian cardiac myofibers. Moreover, antibodies in sera of many rheumatic fever patients were shown to react with mammalian heart muscle as demonstrated by immunofluorescence techniques; streptococcal cell wall antigens were capable of absorbing out these heart-specific antibodies (81). The discovery of a cell wall antigen cross-reacting with cardiac myofibers by Kaplan et al. has been confirmed by other laboratories (101, 124), although several investigations claim to have disassociated the M antigen from the heart-specific antigen (44, 124). Zabriskie and Freimer (182) subsequently found an antigenic fraction of group A streptococcal cytoplasmic membranes cross-reacting with mammalian myofibers. Although the latter membrane antigen did not contain or cross-react with M proteins, Kaplan (79) claimed that the cross-reacting antigen of streptococcal walls was a distinct substance chemically or physically associated with M proteins. In partially purified M preparations obtained by acid extraction, the heart-reactive fraction, upon immunoelectrophoretic analysis, migrated more rapidly toward the anode than M protein (81). Fox and Grossman (44) employed Kaplan's indirect immunofluorescence technique to detect heart-specific antibodies in the sera of rabbits and human infants immunized with M protein vaccines. No antibodies specific for human or rabbit cardiac myofibers were found in the sera of the 35 rabbits and 42 infants immunized. In addition, the sera of 150 acute rheumatic fever patients were surveyed for heart-specific antibodies detectable by immunofluorescence, and about one-half of those sera reacted with human and mouse cardiac myofibers. Antigenic excesses of M protein added to the sera failed to absorb the heart-specific antibody. The authors concluded from these results that purified M

proteins were unrelated to the antigens of heart tissue that are shared with group A streptococci.

Hirata and Terasaki (67) reported that allogeneic antisera specific for the HL-A antigens of human lymphocytes were no longer cytotoxic after absorption by type 1 M protein. Two to 5 mg of M protein per ml were required to completely absorb these tissue-typing sera. Six other serotypes of M proteins did not absorb the HL-A antibodies. The authors suggested from these data that type 1 M protein shared determinants with HL-A antigens. Pellegrino et al. (128a) pursued these studies further on the relatedness of HL-A antigenic determinants and type 1 M protein. Cultured peripheral lymphocytes from adults (all of whom had circulating antibodies to M-1 protein, as measured by passive HA) were stimulated to DNA synthesis in the presence of purified M-1 protein. HL-A antisera, without regard to phenotype, were capable of inhibiting the reaction, presumably by specific binding to antigenic determinants of M-1 protein.

Somewhat at variance with these results (67, 128a) were the experiments of Fox and Peterson (48) who also studied antigenic relationships between M proteins and HL-A phenotypes. They demonstrated that hyperimmune rabbit and human antisera specific for M types 1, 2, 12, and 49 were without complement-dependent cytotoxic activity against lymphocytes obtained from a panel of donors known to possess all of the characterized HL-A histocompatibility antigens. In addition, none of the sera from 97 acute rheumatic fever patients (about one-third of whom had antibodies against type 1 group A streptococci) exhibited cytotoxicity for any of the lymphocytes of the panel of donors whose cell types comprised the complete HL-A spectrum. Fox and Peterson concluded that no relationship could be established between streptococcal M proteins and human histocompatibility antigens. Indeed, in contradiction of Hirata's and Terasaki's suggestion, no serological basis could be established between the induction of rheumatic fever and the presence in patients' sera of anti-HL-A antibodies.

Glomerulonephritis

A number of experimental models of poststreptococcal glomerulonephritis, based on the induction of proteinuria and occasional histological findings have been devised (60). One such system employing membrane chambers (Millipore) containing live streptococci implanted intraperitoneally in rats was shown by Lindberg et al. (99), and in the same laboratory by Vosti et al. (165), to be an effective technique for inducing a nephrotoxic syndrome with certain strains of group A streptococci. The authors demonstrated the presence of bound gamma globulin, β-1-C globulins, and M antigens in the region of the glomerular basement membranes of rats 30 to 60 days after implantation of the chambers. Although the general syndrome occurred most frequently with "nephritogenic" strains of streptococci, a group C organism also induced proteinuria and deposits of globulin on the basement membrane of the glomerulus. With rats, as in the human disease, the roles of a so-called "nephrotoxic" substance or M proteins have not been established. Moreover, other investigations have excluded M antigens in favor of streptococcal membrane antigens cross-reacting with human and mammalian glomerular basement membranes as etiologic agents in poststreptococcal glomerulonephritis (69, 103, 161, 181).

M PROTEIN VACCINES IN CLINICAL TRIALS

Many laboratory and clinical observations (95) have established that immunity to group A streptococcal infection is in response to the M antigens. Circulating antibodies against one or more of a plethora of other somatic and extracellular antigens such as streptolysin O, fibrinolysin, erythrogenic toxin, etc. may alter the symptoms and duration of infection, but anti-M antibodies in all likelihood determine immunity. Although ill-defined factors such as "natural resistance" may play a role, no other parameters have been recognized in resistance to streptococcal infection in humans. Consequently, attempts at human immunization against group A streptococci carried out during the past 15 years have utilized M proteins or cellular fractions containing these antigens. Reviews of this subject have been written by Gill (58) and by Stollerman (149), covering the literature to 1967, and Lyampert's review (100), in Russian, supplements these to 1969.

Some of the more important factors to be taken into account for the successful development of a streptococcal vaccine for human use include the following. (i) The M protein antigens must be nontoxic and immunogenic in relatively small doses. (ii) A polyvalent vaccine should contain the majority of M protein serotypes endemically associated with specific geographical areas. (iii) The M proteins of serotypes in question must be readily obtained in a purified state from characterized, stable strains

of group A streptococci. Various attempts to prepare streptococcal vaccines have encountered some of these problems, which will be reviewed for investigations carried out in recent years.

Crude M proteins as well as those in various stages of purification, especially those administered with adjuvants, readily induced type-specific immunity in experimental animals (42, 52, 96). In humans, several clinical trials with partially purified M protein vaccines and cell wall preparations likewise resulted in type-specific antibody responses as assayed by the bactericidal test (58, 149). However, it became evident that M proteins administered as cell wall fractions or crude extracts were impractical because of intense local and systemic reactions frequently encountered (133, 143, 180). In retrospect, the work of Schwab and colleagues (145, 146), demonstrating the highly toxic and dermonecrotic action of the group A streptococcal cell wall-carbohydrate complex, serves to emphasize the potential hazards in the use of crude soluble and particulate M antigen vaccines. Schmidt (143) prepared a partially purified type 19 M protein vaccine. The soluble antigen (several hundred micrograms in three doses) was administered to seven adults, two of whom produced bactericidal antibodies, but local and systemic reactions to the injections were evident in a number of instances. The same antigen precipitated with alum was administered to 22 children and several developed type-specific antibodies. No systemic symptoms were reported, although local reactions were "moderate and of 48-h duration." Wolfe et al. (180) prepared vaccines of type 14 streptococcal cell walls suspended in saline or combined with adjuvants of alum or mineral oil emulsions. Subjects were selected who exhibited minimal skin reaction to an intracutaneous test dose of antigen. Vaccinees received up to 40 mg of cell wall material in a series of injections. In some subjects whose serum contained low levels of bactericidal antibody prior to inoculation, secondary responses were observed in those who received cell walls in oil emulsion, and in about one-half of 19 subjects the vaccine also appeared to evoke a primary antibody response. Local inflammatory reactions to the various vaccine preparations were reported in over one-half of the subjects, with the result that some were withdrawn from the study before the second or third dose was administered.

Potter et al. (133) and Stollerman (149) were successful in eliciting secondary responses in humans by using types 5 and 12 cell wall preparations and partially purified M proteins in mineral oil emulsions. Most of these subjects had previous streptococcal infections, resulting in bactericidal levels of type-specific antibody which had diminished to low, but detectable, titers at the time of vaccination. In several children these vaccines produced sterile abscesses at the injection sites several weeks after the vaccines were administered. The adverse reactions were most likely due to repository adjuvant combined with the M antigen to which the children exhibited delayed hypersensitivity.

A partially purified M protein vaccine from type 3 streptococcus was used by Massell et al. (105) to immunize a group of 21 children. Total dosages ranged from 0.35 to 6 mg of soluble antigen administered subcutaneously in gradually increasing amounts at weekly intervals for a series of 18 to 33 injections. Some of the children whose sera contained detectable levels of antibody exhibited secondary responses, and 13 of 14 of the subjects showing no type 3 antibody prior to immunization exhibited a presumed primary response to the vaccine. Approximately one-half of all the subjects reacted to the various dosages with local erythematous induration at the injection sites, accompanied by mild systemic reactions including fever and malaise. The most significant and controversial aspect of this clinical trial was the report of subsequent bouts of rheumatic fever in three of the vaccinees. In a follow-up report Massell and colleagues described these cases (104). During and after the vaccination of the children, occasional group A streptococcal infections (not type 3) were observed preceding these attacks of rheumatic fever. The authors suggested that the vaccine "sensitized or otherwise altered the tissues of these children so that subsequently their response to streptococcal infection differed from what it might have been had the vaccine not been given."

In regard to the results of Massell and colleagues several interpretations can be made, including their suggestion that a "sensitized" state may have been induced. A description of their methods of preparing the type 3 M protein from whole cells indicates that the antigen was at best only partially purified and therefore could have contained a putative "rheumatogenic" factor. On the other hand, statistical analysis of a cohort of 21 subjects compared with their control population may be subject to an alternative (i.e., random) interpretation of the distribution of poststreptococcal sequelae. Regardless, the results do emphasize, as the authors stress, "the need for extreme caution in

conducting studies with streptococcal vaccines in human subjects."

More recent attempts to immunize humans with M protein vaccines have been carried out by Fox et al. (47, 56). These clinical trials evolved from extensive laboratory animal protection studies with purified M proteins from a number of the more common serotypes (2, 42, 52). Initial experiments showed that purified M proteins administered to rabbits in a few doses totaling 100 to 400 μg, combined with a variety of adjuvants, could induce bactericidal and, in some cases, precipitating type-specific antibody. These results, including the observations that no local or systemic reactions were induced to the M proteins in hyperimmunized rabbits, lead directly to the first human testing of the vaccines (56). Ten micrograms of type 12 M protein precipitated with alum was used to induce a secondary response in adults. Fifteen subjects in a panel of 22 responded with elevated antibody titers, as measured by passive hemagglutination, and five subjects exhibited significant rises in bactericidal antibody levels. No local or systemic reactions to the vaccine were encountered, probably due to the fact that none of the subjects displayed a delayed hypersensitivity reaction to a 1-μg intracutaneous injection of the vaccine in a screening before vaccination. It was also found that delayed cutaneous reactions in hypersensitive persons could be minimized by the use of "attenuated" purified M proteins produced by partial enzymatic degradation of the antigen (56).

Primary immunization has been achieved in infants with a type 12 alum-precipitated vaccine without provoking local or systemic reactions (47). Groups of infants (51 in all), aged from six months to three years and without type-specific skin sensitivity or circulating antibody, were given a series of three subcutaneous injections at monthly intervals. The vaccine dosages ranged from 10 to 40 μg of protein combined with one to three mg of aluminum hydroxide. Type-specific antibodies measured 1 month after the final injection were significantly elevated in all of the infants when assayed by passive HA. In the groups of infants who received higher dosages of M protein, about 75% of 44 subjects produced bactericidal antibodies. Nearly all of the infants were converted to a mild type-specific cutaneous hypersensitivity when skin tested after the vaccination schedule was completed. Although passive HA titers persisted for at least 6 months after the final dose, bactericidal antibody levels generally waned to low levels 1 year later. Serotypes 3 and 6 were also used to vaccinate smaller groups of adults and infants (179). The authors reported that all subjects responded to the series of injections of the alum-precipitated M proteins (measured by passive HA), and about one-half of the subjects' sera were bactericidal for the homologous streptococci. No reactions to the vaccines were encountered in the infants, all of whom had been screened for a negative delayed cutaneous hypersensitivity before the clinical trials, although several adults exhibited mild local reactions to the vaccines.

In a protective study, adult volunteers were immunized with purified type 1 M protein administered subcutaneously as an aluminum hydroxide precipitated antigen (49). To test the efficacy of the immunization, vaccinees and control subjects were challenged with a virulent strain of type 1 streptococci applied to the pharynx. Thirty to 50 days after the last injection, the vaccinees and control subjects (19 men in each group) were infected with the streptococci, and surveillance was maintained to evaluate the extent of acquired streptococcal infection. Throat cultures, leukocyte counts, temperatures, and physical signs and symptoms were monitored daily. All subjects received penicillin no later than 6 days after inoculation with the culture. Illness was judged by the appearance of exudative pharyngitis and cervical adenopathy accompanied by a positive throat culture. It was observed that nine of the 19 placebo control subjects and one of the 19 vaccinees became ill with a streptococcal upper respiratory infection. No residual illness or clinical complications were reported after the penicillin treatment. It was concluded that the alum-precipitated M protein vaccine afforded a measurable degree of protection against an upper respiratory streptococcal infection.

CONCLUDING REMARKS

In surveying the current literature on streptococcal M protein, one must conclude that our knowledge of this antigen is far from adequate. Structurally, the native antigen is probably an aggregation of subunits. However, the size, configuration, quaternary arrangement, and mode of attachment of the protein to the cell wall await elucidation. The primary structure of M proteins and the relationship of amino acid sequence to differences in antigenic specificity between serotypes remains to be solved. Functionally, M proteins serve to protect the microorganism from phagocytosis; this has been known for years, and the newly discovered effect

of adherence to epithelial surfaces adds further to the teleological argument for the persistance of the M proteins in the antigenic armamenteria of group A streptococci.

Group A streptococci possess two unique characteristics in addition to the group-specific carbohydrate: they produce the M antigen and they have the capacity to induce rheumatic fever or acute glomerulonephritis. Hence, it has been tempting for some investigators to devise experiments implicating the M antigens in the etiology of poststreptococcal sequelae, and until the mechanisms are determined by which these diseases are manifest, the M proteins will in some measure continue to be suspect. However, in terms of an autoimmune hypothesis of the etiology of poststreptococcal sequelae, research on other antigens, particularly those of the bacterial cytoplasmic membrane, has diminished the probability of a role for the M proteins in these disease processes.

Epidemics of acute glomerulonephritis caused by pyoderma-associated strains of group A streptococci present a difficult challenge to the microbiologist. The paucity of M protein in many of these strains and its relatively poor antigenicity in clinical or experimental infections demonstrate that immunological approaches to the study of upper respiratory infections may not be as readily applied to the prevention of impetigo. This is true in terms of serological typing (by the M precipitin test) of the pyodermic streptococci and for prospects of immune prophylaxis. In contrast, research and clinical trials are progressing with the use of M protein vaccines to prevent upper respiratory group A streptococcal infections. Small-scale clinical testing and protective studies in humans indicate that polyvalent M protein vaccines have a potential for becoming useful prophylactic measures in medical practice. This, of course, depends on the outcome of field trials yet to be conducted on populations at risk. One can only conclude that the immunobiology of the group A streptococcal M proteins remains a fertile ground for continuing investigation.

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