The career of Maclyn McCarty

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On January 2, 2005, the scientific community lost a valued colleague and friend. Maclyn McCarty, or "Mac," as he was better known, was perhaps most recognized for his part in the discovery of DNA as the carrier of genetic information. But McCarty's scientific career was long and fruitful, and his contributions to science were vast. This retrospective offers a look at some of Mac's other notable scientific achievements.

Maclyn McCarty was born in the Midwest in 1911, the second of four sons of a very close-knit and supportive family. His interest in investigative medicine arose in his early teens and led him to study Medicine at Johns Hopkins University, then the preeminent medical school. But first were his college days at Stanford, where he had the great fortune to study with Murray Luck, a pioneer in biochemistry, with whom Mac performed research on liver proteins. In 1933, he was admitted to Johns Hopkins, and there in addition to his medical curriculum he further enriched his biochemical background, working with Leslie Hellerman on a project in which he purified heparin from 20-pound batches of beef liver. This semiindustrial scale of biochemical work prepared him well for dealing with mass cultures of pneumococci and later streptococci. Mac trained for three years in Pediatrics at Harriet Lane at Johns Hopkins. He chose pediatrics in part because of the dawning of a new age in infectious diseases heralded in 1935 by the introduction of sulfonamide chemotherapy. In 1941, after a year at New York University, Mac arrived to work with Oswald Avery in the Rockefeller Hospital and soon applied his strong biochemical skills to bring to its stunning conclusion the program of identifying the chemical nature of the transforming principle. On February 1, 1944, there appeared in the *Journal of Experimental Medicine* the paper authored by Avery, Macleod, and McCarty entitled "Studies on the chemical nature of the substance inducing transformation of pneumococcal types" (1). It was the first of a series of three papers that together provided decisive evidence that DNA is the carrier of genetic information. This feat furnished heredity with a chemical basis, and thereby not only opened a new era in biology, but has fundamentally affected Western thought and culture. The *JEM* at the time of the 35th and 50th anniversaries of this groundbreaking finding published retrospectives recognizing and celebrating this achievement.

Mac was a physician-scientist of great distinction, and here we will highlight his other contributions to the biomedical sciences. A complete bibliography of Mac's work is provided at the end of the online version of this article. In 1946, the offer to leave the Avery laboratory and assume the leadership of another Rockefeller laboratory working on streptococci and rheumatic fever provided the opportunity to return to clinical disease-oriented research. For Mac the mystery of a child suffering with acute rheumatic fever as a result of a prior streptococcal throat infection was an irresistible challenge.

Over a period of several decades he led ground-breaking work that established the cell architecture of the Streptococcus and the nature and location of its important antigens. These studies were paralleled by a clinical inquiry of patients with rheumatic fever who were admitted to the hospital, and led to major advances in understanding the immune responses in this disease. Notable among these were his chemical and clinical studies of the serum protein C-reactive protein.

C-reactive protein

The work on C-reactive protein (CRP) was a direct outgrowth of his experience in the Avery laboratory. The protein had been discovered there in 1930 and over the years had become one of Avery's "Red Seal Records"—carefully rehearsed discourses by which he shared his thoughts on a scientific problem with colleagues and students (2). The pneumococcal transformation system required the addition of some components that were most readily obtained from human pathological pleural or abdominal fluids. It was known from the prior work in Avery's laboratory that CRP protein was an acute phase reactant, that its precipitation with C-polysaccharide of the pneumococcus (CPS) was calcium dependent, and that CRP was found in the albumin fraction of serum upon ammonium or sodium sulfate precipitation. The prior method for purification of CRP consisted of dialyzing the albumin fraction of serum against tap water or distilled water containing traces of calcium ion, which resulted in the precipitation of CRP. This property depended on the presence of some lipid. If the serum had been treated with alcohol or ether, this precipitation would not occur. Mac had available large volumes of two pathological fluids that had proved unable to support the transformation reaction. They were, however, very rich in CRP, and hence Mac decided to purify the protein. Once he had obtained the albumin fraction by ammonium sulfate precipitation, he noted that the protein did not come out of solution with calcium at low ionic strength. Therefore, he decided

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to use precipitation with CPS as a method for purification. The CRP–CPS precipitate was dissolved with citrate containing saline. When sodium sulfate was added, an amorphous precipitate developed which, over the course of a few days, turned into needle-like crystals. These were harvested by centrifugation, and the crystallization was repeated. With this material he obtained beautiful rhomboid plates after a few days. Subsequent recrystallization occurred much more rapidly. Chemical analysis of the purified CRP indicated that it contained 14.66% nitrogen and no detectable phosphorus. Since the CPS polysaccharide contained 5.21% phosphorus, it meant that the separation between the protein and the polysaccharide had been accomplished effectively. The purified protein was used to immunize rabbits to obtain antiserum that was able to detect the purified C-reactive protein at very low concentrations. The antiserum was also tested against an acute and convalescent serum from a patient with type 14 pneumococcal pneumonia and found to react only with the acute phase serum (3).

However, fluids obtained subsequently were rich in lipids and proved unsuitable for the previously described purification method. In collaboration with Harrison Wood and Robert Slater, a modification of the procedure was developed. This consisted of a very gentle extraction of the interfering lipids from the purified albumin fraction with chloroform. After this extraction, purification by precipitation with CPS and crystallization induced by sodium sulfate was performed readily as described before. The purified protein was characterized by free electrophoresis, and its isoelectric point was determined to be pH 4.82. Zone electrophoresis on starch-supported medium indicated that the protein behaved as a fast gamma globulin. Ultracentrifugation analysis indicated that the protein moved as a single sharp peak with a sedimentation constant at 7.5 (4).

The rabbit antiserum to crystalline CRP proved a much more sensitive way to detect the presence of CRP than precipitation with CPS. The CRP levels were determined by a new capillary precipitation method with the highly specific rabbit antiserum. Furthermore, it had the distinct advantage that there was no ambiguity with this test since normal sera gave a negative result. Mac, together with Wood, decided to study this test on patients. They reported a series of 45 patients hospitalized for rheumatic fever at the Rockefeller University Hospital, where these patients remained until convalescence. The findings confirmed that this test was more sensitive than the precipitation with CPS and that it was also much easier to execute. CRP elevation appeared to be the most reliable indicator of rheumatic inflammatory activity and responded more rapidly and reliably than the Westergren sedimentation rate (5). In a subsequent study with Slater that looked carefully at the sera of patients with viral hepatitis, it was found that only the antibody-dependent test, and not the CPS precipitation test, was sensitive enough to detect the presence of CRP. These studies led a New York company, Schieffelin and Co., to produce the antiserum commercially, and initiated the more widespread use of the CRP test in American medicine (6).

McCarty, 1942. Photo provided by the Rockefeller University Archive.

Of course the question of the biological significance and function of CRP had preoccupied Avery and continued to intrigue Mac. This prompted him, together with Harold Anderson, to follow up some as yet inconclusive evidence that the rabbit might produce a similar substance in response to injury. It was known that acute phase rabbit sera did not react with CPS, and therefore, they tested the possibility that some other polysaccharide might be reactive. They tested 10 type-specific or somatic polysaccharides derived from *Klebsiella* or pneumococci, and found that a pneumococcal CPS preparation that had been isolated and purified by a more rapid method was able to precipitate a protein from acute phase rabbit serum. Using this polysaccharide, the purification method using delipidated serum successfully yielded crystalline protein. They produced antisera in roosters and with these demonstrated that the time course of the appearance and disappearance of rabbit CRP was analogous to that seen in human beings (7).

In 1982, as the introductory speaker at a New York Academy of Sciences Symposium, Mac provided a very personal and comprehensive retelling of the whole story of the discovery of CRP in Avery's laboratory and the thought processes that led the early research of the biological response (8). Today we know that this protein is evolutionarily very old. In many species, CRP is an acute phase reactant; in others (such as mouse), it is not responsive to injury; and in the Syrian hamster, CRP production is an estrogen-controlled response. Although many functions have been attributed to it, none is as yet totally compelling. Further, there has been a renaissance of interest in CRP as a possible marker for coronary artery disease.

Physician–scientist

Mac immersed himself in the study of patients with rheumatic fever on the

wards of the Rockefeller Hospital. He also took advantage of a remarkable collection of clinical histories, streptococcal isolates, serial throat cultures, and bleedings that were obtained at the Great Lakes Naval Training Center during WWII. In collaboration with Harold Anderson and Henry Kunkel, he performed a very detailed study of the immune response of recruits who were admitted for scarlet fever and were then followed for six weeks with weekly throat cultures and bleedings.

At that time the quantitative test to measure antibodies to streptokinase, a streptococcal-secreted protein, had only recently been developed and there was limited information on the human antibody responses to this enzyme. Thus, the primary aim of this study was to determine whether the level of the antistreptokinase antibody responses was correlated with the likelihood of developing rheumatic fever. These measurements were compared with gamma globulin levels and rises in antistreptolysin O (ASO) titer. The results showed that ASO, antistreptokinase titers, and gamma globulin levels were clearly higher in the group that went on to develop rheumatic fever. The next highest average ASO and streptokinase anybody titer (but not accompanied by increased gamma globulin levels) was seen in the group that had more than one type of group A Streptococcus isolated within the first two weeks of observation. The group with the lowest ASO and antistreptokinase titers was the group treated with penicillin with no subsequent isolation of group A streptococci. The authors emphasized that not all patients who developed rheumatic fever had higher titers of antistreptococcal antibodies than patients with uncomplicated streptococcal disease, but rather that the mean maximum titer of the rheumatic fever group was consistently higher than that of the patients who suffered no delayed complications. The authors concluded that the patients who went on to develop rheumatic fever had a general propensity to develop strong antibody responses to antigens (9).

DNase and the immune response to streptococcal products

At the same time that Mac was conducting the patient studies, he began analyzing the group A Streptococcus by chemical means. By 1948, several enzymatically active extracellular products produced by group A streptococci had been identified and partially characterized. Mac noted that the culture supernatants of streptococcal cultures had both DNase and RNase activity. He demonstrated that all of the 16 strains that he tested produced both activities and that the difference between strains was minor. The degree of DNase activity was very high compared with culture supernatant of pneumococcus, *Bacillus subtilis*, or *Escherichia coli* (10).

Mac developed a method to purify these enzymes and then asked whether antibodies to the enzyme would inhibit its activity. Indeed, he was able to show that this was the case and that with some human sera the inhibitory activity was extraordinarily high, being still evident at a dilution of 1:20,000. Using this test he studied the same set of 90 sera from recruits at the Great Lakes Naval base that had presented with scarlet fever. He found that in contrast with ASO or antistreptokinase antibodies, which the majority of recruits developed, only 38% showed a significant anti-DNase activity. However, some of those that responded had very high titers. The average anti-DNase response was again higher in the patients who developed rheumatic fever (11). Mac took note of the fact that fewer recruits responded to this antigen than to the other products studied. This observation led Lew Wannamaker, while he was still in Mac's laboratory, to characterize streptococcal DNase and discover that there were three immunologically distinct DNases (12).

From the studies showing that as a group individuals that develop rheumatic fever had a more exuberant immune response to several streptococcal antigens than those that did not develop rheumatic fever, the question arose whether these individuals were generally immunologically hyperactive or only hyperactive to streptococcal antigens. Mac tested this, together with William Kuhns, by determining the immune response to diphtheria toxin of normal volunteers compared with patients that had recovered from rheumatic fever. The group consisted of 245 individuals ranging in from 9 to 26 years of age. 132 of these individuals had a clear history of rheumatic fever. By using the Schick test—the intradermal injection of a minute quantity of

Rockefeller University, 1937. Photo provided by the Rockefeller University Archive.

diphtheria toxin that gives rise to a local skin reaction unless antitoxic antibodies are present—it was found that there was no significant difference between the two groups in the percentage of subjects with preexisting neutralizing antibodies to diphtheria toxin. Furthermore, kinetics and level of antibodies produced in response to immunization with toxoid showed no difference between the groups. This is perhaps the most incisive study, demonstrating that the capacity to develop rheumatic fever in response to streptococcal infection cannot be attributed to a generalized immunological hyperactivity (13).

Attacking the streptococcal cell wall

Since very little was known about the cellular structure of the group A Streptococcus by the early 1950s, Mac began a series of systematic and elegant experiments to understand the nature of the cell wall and its relationship to the observed serological response to this organism. However, because the Streptococcus is a gram-positive bacterium with a very tough and highly resistant cell wall, this endeavor proved to be a challenge. Having a great deal of experience with enzymes and how to purify them, Mac elected to use an enzymatic approach to disrupt the bonds that hold the wall together. Since enzyme supply companies such as Sigma-Aldrich did not exist in those days, one needed to go to the source to isolate and purify the necessary enzymes. Using the soil organism *Streptomyces albus* as a source of cell wall–degrading enzymes, Mac successfully isolated a preparation from the growth supernatant of these organisms that had no proteolytic activity but could cause streptococcal lysis (14).

He planned to use this new enzyme preparation to disrupt the bonds that maintained the integrity of the streptococcal cell wall and thus release in solution the basic components of the wall. However, to accomplish this he needed to prepare isolated streptococcal cell walls to avoid contaminating his digest with cytoplasmic contents. Using a thick-walled Pyrex flask and stainless steel balls, he was able to grind a streptococcal powder sufficiently to crush the cells (14). Through a series of centrifugation steps he successfully separated the cell wall from the cytoplasmic contents. The dry crushing process was later replaced by an oscillating machine that shook a suspension of streptococci in the presence of fine glass beads and accomplished the same task, but more efficiently. Using this purified cell wall as a substrate for the Streptomyces enzyme, Mac isolated a soluble carbohydrate fraction of the streptococcal cell wall that was highly water soluble. Applying some elegant chemistry to this solubilized cell wall preparation, he determined that the streptococcal cell wall consisted of about one third protein and two thirds carbohydrate, with the carbohydrate portion composed primarily of *N*-acetyl-glucosamine and rhamnose. More importantly, he found that the serological reactivity of the soluble carbohydrate when tested with group A antistreptococcal antisera was comparable to the material isolated from streptococci by the Lancefield acid extraction technique and thus represents the group-specific antigen for streptococci. At the time, variant group A streptococci were available that did not react with the conventional group A typing antisera, so Mac explored the basis of this difference. Using the same techniques applied to the group A streptococcal cell walls, he discovered that the variant carbohydrate was reduced in the amount of total *N*-acetyl-glucosamine. To get a better handle on this difference, he isolated soil organisms by growing them in the presence of the group A or the variant polysaccharide as the only carbon source and selecting for the ability to degrade the serological reactivity of the antigens. Thus, he was able to isolate and purify enzymes that were able to destroy the serological activity of group A–specific and variant-specific antisera. He found that the variant-specific enzyme released rhamnose from the variant carbohydrate, whereas the group A–specific enzyme released *N*-acetyl-glucosamine. Furthermore, he found that treatment of the group A carbohydrate with the latter enzyme abrogated its reactivity with group A antiserum but made it reactive with the variant antiserum. His interpretation of these results was that the group A carbohydrate was composed of an undetermined number of rhamnose side chains with a terminal *N*-acetyl-glucosamine and that the variant carbohydrate lacked the terminal *N*-acetyl-glucosamine (14). The linkage to the rhamnose could be either α or β; however, this could not be resolved at that time. Six years later, using the azo antigen technique, in which *p*-aminophenyl-β-*N*-acetyl-glucosamine could be linked to egg albumin, he showed that this complex and not the α form was reactive with the group A antisera (15). He went on to show that the phenyl-β-*N*-acetyl-glucosamine and not the α form could induce the cleaving enzyme from the soil organism. From this he concluded that β-*N*-acetyl-glucosamine side chains represent the major antigenic determinant of group A streptococci. Decades later, his structural interpretation was proven to be entirely correct based on the NMR structure of these carbohydrates (16, 17).

Rockefeller Hospital, 1946. Photo provided by the Rockefeller University Archive.

In collaboration with Richard Krause in Mac's lab, the same chemical and enzymatic experiments were performed with group C streptococci, which revealed a similar composition

of the cell wall polysaccharide to that found in the group A organisms. (18) However, in the group C polysaccharide, *N*-acetyl-galactosamine rather than *N*-acetyl-glucosamine predominated and was the basis of the serological specificity of the group C organism. In a related paper, Krause and McCarty identified group C streptococcal mutants that reacted with antiserum directed to the group A variant streptococci (19). Using the enzymes from the soil organism in experiments performed for the group A and A variant streptococci, the authors concluded that these group C mutants were similar to the A variant streptococci in that their polysaccharide was composed of rhamnose and lacked the terminal *N*-acetyl-galactosamine (18).

During the course of his serological studies, Mac found that extracts of several streptococcal groups and other gram-positive bacteria reacted with antisera directed to certain streptococci, indicating that the reactive substance was widely distributed in bacteria (20). Using these cross-reactive antisera as a reagent to follow the substance, he purified this common component to homogeneity from streptococcal extracts. Through qualitative and elemental chemical analysis he found that antigen was a simple polymer of glycerophosphate. This purified material reacted serologically with antiserum and was identical by infrared spectra with synthetic polyglycerophosphate. He further found that only certain gram-positive and not gram-negative bacteria contained this polymer. It was later discovered by James Baddiley (21) that the polyglycerolposphate belonged to a class of glycerol teichoic acids which contained ester-linked D-alanine. Based on this finding Mac went on to prove that the major antigenic determinant for the Ala-polyglycerophosphate was in fact the linked alanine (22).

McCarty and his colleagues proceeded to dig deeper into the cell wall of the Streptococcus to understand more of its basic components. In collaboration with Krause, they chemically extracted the group carbohydrate with hot formamide from both group A and A variant streptococci and found that this extract contained the expected carbohydrates for their respective groups. They also discovered that the residue of this chemical extraction was composed of *N*-acetyl-muramic acid, *N*-acetyl-glucosamine, and the amino acids alanine, glutamic acid, lysine, and glycine and represented the mucopeptide (later called peptidoglycan) of the cell wall. Since this residue was attacked by the phage lysin, the *Streptomyces albus* enzyme, and lysozyme, they concluded that the linkages split by these enzymes were found in the mucopeptide fraction of the cell wall and not the group carbohydrate.

Opaque versus blue colonies

In 1966, Mac attempted to explain a long standing phenomenon of group A streptococci grown on agar plates. Colony forms appeared which, when viewed under indirect lighting, appeared opaque in comparison to the blue appearance of the majority of the streptococci on the plate. Although there were several theories as to the reason for this opaque appearance, nothing definitive was known. To approach the problem, Mac ruled out the presence of new surface structures or molecules that were over- or underexpressed based on both serological and chemical analysis of the opaque and blue bacterial forms (23). As it is often in science, the answer usually reveals itself unexpectedly. During a routine examination of the opaque colony forms under the microscope, he found that the streptococci were in extremely long chains (hundreds of cocci per chain) compared with the short chains (~10 cocci per chain) produced by the blue colony forms. Further detailed experiments prompted by this observation revealed that the stability of these chains was not the result of the relatively weak interaction of proteins or surface carbohydrate but was likely due to the lack of separation of the peptidoglycans during cell division (23). Subsequently, in an elegant electron microscopy study of these unusual streptococci with John Swanson (24), it was clear that his conclusions were correct.

Mac's thoughts on rheumatic fever

Because of the scientific prominence of Mac's studies of streptococcal biology and his interest in the clinical aspects of rheumatic fever over the years, he was repeatedly asked to summarize his thoughts about this problem. Thus, by reading the reviews that he wrote over a period of two decades, one can see the evolution of his thoughts. In 1951, in a presentation at a meeting at the New York Academy of Medicine (25), Mac takes it as a given that preceding streptococcal infection is seen very commonly in rheumatic fever: "Whether the disease can be initiated by any other event cannot be excluded, remains a very difficult question, but is not supported by any evidence. To further complicate the issue are the recurrences and recrudescence of rheumatic fever." He proceeds to review the main hypotheses for the causation of the disease existing at that moment in time. He mentions the deleterious effects of streptolysin O on membranes of isolated frog heart cells and that this may be a promising line to pursue. He makes mention of hyaluronidase as a potential agent of disease because it affects the extracellular matrix that is the location of the streptococcal infection. Furthermore, although streptococci grown in vitro often do not produce hyaluronidase, Mac reasoned that it must be produced in vivo during infection because antihyaluronidase antibodies are at least as common as ASO antibodies after infection. He takes note of the fact that rheumatic fever has been classified by many as a collagen disease. He suggests that this is a misleading name, and notes that the fibrinoid found in Aschoff bodies or subcutaneous nodules is not collagen, but rather degrading myofibers. His own view was that immune causation is the most probable explanation for disease. Favoring this hypothesis is that an immune response to streptococcal antigens is almost invariably seen, the latent period of about three weeks is in keeping with the kinetics of an immune response, patients

with rheumatic fever have a more pronounced immune response, and finally that effective treatment of the infection with penicillin prevents the onset of rheumatic fever. In this review, Mac recognizes that the chronic nature of the inflammatory disease presents a challenging problem. However, he suggests that it is possible that certain streptococcal products have physical and chemical properties that could account for the long persistence in human tissues. Although he does not state it explicitly in this review, it is likely Mac was already thinking of the cell wall. Speaking of the treatment of the disease, he takes note that although ACTH, cortisol, or salicylates are certainly helpful in controlling the acute inflammatory aspects of the disease, there is actually no data whether they prevent the chronic valvular disease (25).

In 1956, Mac again discussed in detail the various hypotheses for the etiology of rheumatic fever (26). Although recognizing the complexity of the problem, he notes that rheumatic fever, as a disease, is more amenable to study than the other so-called collagen diseases where no underlying cause was known at the time and, for the most part, remains unknown today. He discusses the possibility that live streptococci are involved in this disease. Mac, although he did not subscribe to this hypothesis, felt that it could not be definitively excluded with the data available at that point in time. Again, his own thinking leaned toward an immunological hypersensitivity reaction. He notes that it is quite clear that on average the immune response to streptococcal antigens is higher in the group that proceeds to development of rheumatic fever. However, on an individual basis the level of the immune response obtained does not have predictive value. This raises the question of whether this population exhibits a general hyperreactivity to antigenic stimulation. Mac points out that the study of the immunogenicity of unrelated antigens, and in particular to diphtheria toxoid in normal controls and in individuals who have had rheumatic fever, indicates clearly that the there is no generalized hyperreactivity. Since it is the response to streptococcal products in general that is aberrant, the difficulty becomes to identify a particular streptococcal product in the etiology of rheumatic fever. At this point, Mac's interest was aroused by the streptococcal cell wall, based on the fact that no known mechanism operative in human tissues had been identified that was capable of degrading this highly resistant structure. In this 1956 review, he does not overlook the fact that there must be some genetic basis for the susceptibility to this disease because only a subset of those infected with streptococci develop the disease (26).

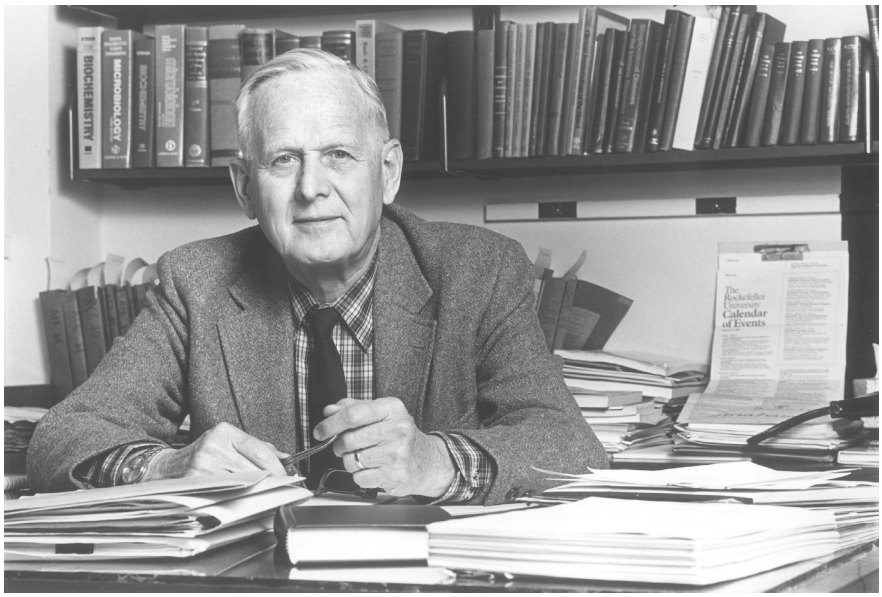
In a 1964 review, he tries to come to grips with what might be the unique properties of group A Streptococcus that is involved in rheumatic fever (27). Mac points out that this really is an abbreviation of the more complex question: "On the assumption that rheumatic fever results from the tissue response of a susceptible human being to some constituent of the Streptococcus, what components of the host response and what constituents on the microorganism are involved, and how do they interact to produce the disease?" This question had led to broad efforts directed toward a detailed analysis of the group A Streptococcus, and these studies had yielded a very rich body of knowledge about this species. Mac notes the intriguing possibility that cell wall–less L-forms that are totally resistant to penicillin are a theoretical possibility for the pathogenesis of this disease. The richness of antigens to consider and the diversity of unexplained aspects of the disease make the identification of the pathogenesis extremely daunting. He illustrates this complexity by following the fate of one of his own favorite hypotheses: the role of the group A cell wall carbohydrate. This substance is an attractive candidate because it is the defining antigen of group A Streptococcus. It is an insoluble portion of the cell wall, a structure that is difficult to digest by human degradative processes. Its antigenic signature is terminal *N*-acetyl glucosamine residues. This sugar certainly occurs in many human tissues and may be exposed in the terminal position either during biosynthesis or degradation. However, he notes that tests of this hypothesis indicated that

Colin MacCleod, Maclyn McCarty, and Detlev Bronk, 1965. Photo provided by the Rockefeller University Archive.

the human immune response to group A polysaccharide was not dramatic or consistently produced. Furthermore, numerous attempts to identify a human tissue component where the *N*-acetyl glucosamine is situated in a linkage that provides a cross-reactive antigen were unsuccessful. Nevertheless, Mac expresses his confidence that further characterization of the basic biology of the Streptococcus is ultimately necessary for the solution of the problem (27).

In a 1972 review, he notes that his views on the problem of the pathogenesis of rheumatic fever had changed little over the years and that this was due to the fact that there had not been any substantive changes in the information available (28). At this time, one school of thought focused on the possibility that the toxic molecules of the Streptococcus were involved. For instance, the disease could be due to a toxic manifestation in response to the nonimmunogenic streptolysin S, which was known to have the ability to attack a wide variety of cell membranes. Mac comments that the lack of an immune response could explain the recurrent nature of the disease. The streptolysin O is also a potent toxin, which had been shown to have deleterious effects on cardiac myocytes in vitro (29). However, since antibodies to it are almost always engendered, many workers had found it difficult to accept a causative role because of the well-known propensity for the disease to recur in the absence of penicillin prophylaxis. Some held that the immune response causes the toxin to circulate in the form of immune complexes that will lodge in tissues. This view suggests that the slow degradation of the complexes might release the toxin and cause damage. This variant hypothesis would explain the latent nature of the disease, and the immune response would actually be a contributing factor (28, 29).

A hypothesis that bridges direct toxicity and immune responses was put forth by Schwab and Cromartie who demonstrated that cell wall fragments obtained by sonication, when injected subcutaneously into rabbits, produced a nodular and relapsing skin lesion. When injected intraperitoneally, focal lesions were seen in the heart and other organs (30, 31). The activity was dependent on the particulate nature of the material and was probably a response to the peptidoglycan. With the recognition in the last dozen years of Toll-like receptors and their capacity to recognize microbial components, such as peptidoglycan, and stimulate immune responses, revisiting this model might be very instructive.



McCarty, 1982. Photo provided by the Rockefeller University Archive.

The other theories for the causation of this disease at that time relied on concepts of autoimmunity. Mac was particularly impressed by the remarkable number of instances of molecular mimicry of human antigens by the Streptococcus, a concept developed in great part by John Zabriskie while working in Mac's lab. The hyaluronic capsule of streptococci is chemically identical to the hyaluronic acid in the extracellular matrix. Indeed, immunological cross-reactions of group A polysaccharide and heart valve glycoprotein have been described. M protein and streptococcal cell membrane components have strong cross-reactions with the sarcolemma of cardiac muscle. Heart-reactive antibody levels rise in patients with rheumatic fever and diminish during convalescence. Particularly striking is the fact that they rise again with rheumatic recurrences (32). Cross-reactions with histocompatibility antigens have also been described. Mac emphasized that the absence of an animal model makes it extraordinarily difficult to verify or reject any of the various theories of pathogenesis (33).

Finally, in reviewing the immunological basis of rheumatic fever in 1980 Mac finds several aspects that render the evidence for molecular mimicry by Streptococcus very strong. In addition to the arguments presented previously, he cites the then recent finding that patients with chorea have antibodies reactive with subthalamic and caudate nuclei of the brain (34). Over the years it had become evident that the patients with rheumatic fever produce an immune response to over a dozen secreted proteins, to cell wall polysaccharides, and to several antigens of the streptococcal cell membrane. Both humoral and cellular responses had been documented. But it had not been possible to inculpate the immune response to any particular antigen as the pathogenetic basis of the disease. Mac, however, felt that this multiplicity of im-

mune responses to different antigens might be the explanation for the diversity of the clinical manifestations of the disease. He was thinking of separate immune mechanisms for the causation of, for instance, valvulitis compared with myocarditis (35).

Thus, as new data accumulated from many laboratories over the years, very often led by individuals that he trained, there was a shift in Mac's focus and emphasis in his writings about rheumatic fever. The earlier papers focused on justifying and cementing the role of group A infection. They were also very focused on enzymatically active extracellular products and the immune response to them. His own work added one important new component to this set, the DNase. As his work on the cell wall anatomy and immunochemistry matured, he took as a given the streptococcal causation of disease, and moved to a view that began to include aberrant immune reactions. Very careful studies indicated that quantitatively there is an unusually strong immune response to streptococcal antigens in patients with rheumatic fever. However, the response of diphtheria toxin, an unrelated antigen, was not out of normal bounds. As the immunochemical dissection proceeded further, much of it by members of his own lab or from a handful of investigators strongly influenced by his methodology, the findings emerged that there are a surprising number of streptococcal antigens that cross-react serologically with human tissue antigens. His view of the pathogenesis was eventually cognizant of these mimetic relationships (36). However, in this 1979 article he recognizes that the field is rich in interesting and innovative theories for the causal link between streptococcal infection and the nonsupurative sequelae. But he also points out that the field unfortunately lacks any credible animal model and hence is bereft of methodology to verify any particular hypothesis.

This recounting of the achievements of Mac as a scientist only touches upon the highlights of his scientific contributions. His enormous impact as a mentor, a model of professional integrity, and a statesman for science should be the subject for another biographical retrospective. Mac was quintessentially a physician-scientist. Being a member of the trio that established DNA as the chemical basis of heredity did not cause him to deviate from his goal of applying his scientific prowess to patient-oriented research. He lived a long and very fruitful life, and medicine and science have benefited from his manifold contributions.

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