SUPPRESSION OF THE ANTISTREPTOLYSIN O RESPONSE BY CHOLESTEROL AND BY LIPID EXTRACTS OF RABBIT SKIN*

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For many years the antistreptolysin O (ASO)[1] titer has been the most commonly used immunologic indicator of infection with group A streptococci. Within the last decade certain limitations in using the ASO response to define previous streptococcal infection have been recognized. The failure to demonstrate an ASO response in about 20% of patients with streptococcal infection of the upper respiratory tract has stimulated interest in antibodies to other streptococcal antigens (1, 2). Perhaps more importantly, and certainly more provocatively, reports from this and other laboratories suggested that ASO titers were often not elevated or only slightly elevated in children with streptococcal pyoderma when compared with children in the same or a similar population (3–5). A further study from this laboratory in which children were followed carefully with serial cultures and bleedings showed that in contrast to the antistreptococcal deoxyribonuclease B (anti-DNase B) response, children with streptococcal pyoderma generally exhibited a feeble ASO response (6). This study has been subsequently confirmed by others (7, 8).

Initially, several explanations for this observation seemed possible. A generalized immunological unresponsiveness to all streptococcal extracellular antigens was ruled out by the usually excellent anti-DNase B response in patients with streptococcal pyoderma (6–8). The possibility that the poor ASO response was due to low production of this antigen by the pyoderma strains seemed unlikely since no difference in in vitro production was demonstrated between strains recovered from the respiratory tract and those infecting the skin (6). With the failure to find support for these two possibilities, a third hypothesis was considered: that properties of the skin per se might be responsible for this peculiar disparity in the ASO response.

It has been known for some time that cholesterol is a potent inhibitor of two of

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[1] *Abbreviations used in this paper:* ASO, antistreptolysin O; DNase B, streptococcal deoxyribonuclease B; NADase, streptococcal nicotinamide adenine dinucleotidase; SO, streptolysin O.

the biologic properties of streptolysin O (SO), its hemolytic and its cardiotoxic effects (9–11). Cholesterol is found in abundance in skin (12, 13) and might exert an effect on SO produced during cutaneous streptococcal infections. We postulated that cholesterol and/or other related lipid compounds might also interfere with its antigenicity, thereby providing an explanation for the feeble ASO response observed in patients with streptococcal pyoderma.

In preliminary experiments (14), we demonstrated that chloroform:methanol extractable lipids from rabbit dermis and epidermis have the ability to block the hemolytic capacity of SO. Furthermore, these experiments suggested that such extracts can suppress the antigenicity of this streptococcal product.

The additional studies reported here provide definitive confirmation of these findings and answers to three further questions. Firstly, do lipid extracts of skin have the ability to inhibit the antibody response to other streptococcal extracellular antigens or is the suppression specific for SO? Secondly, will purified cholesterol, a prominent component of these lipid extracts of skin, also specifically inhibit the antibody response to SO? Thirdly, are lipid extracts of skin more or less effective than similarly prepared extracts from other tissues in blocking the hemolytic activity of SO?

Materials and Methods

Preparation of Streptococcal Extracellular Antigens. Rabbits were immunized with a crude mixture of streptococcal extracellular antigens obtained from the culture supernate of group A streptococcal strain C-203S[2] grown (except where otherwise noted) in Pfanstiehl broth (15) at 37°C for 18 h and partially purified by ammonium sulfate precipitation, dialysis of the redissolved precipitate against 10^{-3} M glycine, and Millipore filtration (Millipore Corp. Bedford, Mass.) using methods previously described (16). The material remaining inside the dialysis bags was analyzed for the content of SO, streptococcal DNase, and streptococcal nicotinamide adenine dinucleotidase (NADase) using standard methods (6, 17, 18).

Preparation of Lipid Extracts of Rabbit Skin and of Other Organs or Tissues. The hair was shaved and the dermis and epidermis of sacrificed albino New Zealand rabbits were harvested. The subcutaneous adipose tissue was carefully removed. The skin was cut into small pieces, and lipids were extracted by adding 20 g skin to the mixing cup of a Sorvall Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.) in the presence of a mixture of two parts chloroform and one part absolute methanol. After grinding for 20 min, the supernate was removed and evaporated slowly over flameless heat. The remaining lipid material was redissolved in warm absolute methanol. The lipid solution was poured into boiling sterile physiologic saline to facilitate removal of the methanol by evaporation. The resulting lipid suspension was slowly centrifuged to remove large particulate matter. At each step in the procedure, aerobic and anaerobic cultures were taken to confirm bacterial sterility. The final suspension appeared to be white and cloudy; there was no red or pink color to suggest hemoglobin. The lipid suspension prepared from rabbit skin contained approximately 0.67 mg cholesterol per ml (14). Lipids were also extracted from samples of equal wet weights of the following rabbit tissues or organs: ventricular myocardium, liver, kidney, spleen, and lung. After removal from the animal, the tissues were cut into small slices and washed many times in sterile physiologic saline until the supernate was clear and contained no visible blood or hemoglobin. The extraction procedure for these tissues and organs was similar to that described for skin. In the hemolysis experiments, the final concentration of suspended lipid in physiologic saline was that amount of lipid extracted from 0.83 g (wet weight) of tissue per ml of saline.

Immunization Experiments. The initial experiment was designed to test the effect of lipid extracts of rabbit skin on the antigenicity of various streptococcal extracellular products. Two

[2] Kindly provided by Dr. Rebecca Lancefield, Rockefeller University, New York.

groups of albino New Zealand rabbits (15 in each group) were immunized intravenously twice weekly for 17 wk with a preparation of streptococcal extracellular products (see above) containing a sublethal dose of SO (33 U), streptococcal DNase (1,700 U), streptococcal NADase (1,100 U), and probably other streptococcal products of undetermined amounts. In the control group, 1 ml of the antigen preparation was mixed with an equal volume of physiologic saline for each injection. For the test group, 1 ml of the antigen preparation was mixed with an equal volume of a saline suspension of lipid extract of rabbit skin. Both groups of animals were bled before immunization and at the 2nd, 4th, 7th, 9th, and 17th wk of immunization. At the conclusion of the experiment, titers of ASO, anti-DNase B, and anti-NADase were determined on all sera from each animal by previously described methods (17–19).

The protocol for the second immunization experiment, designed to test the effects of cholesterol on the antigenicity of various streptococcal products, was similar to that of the first experiment. The albino New Zealand rabbits were divided into two groups and were injected twice weekly for 9 wk. One group (control) received at the time of each injection 1.5 ml of a partially purified preparation of antigens containing similar amounts of SO and streptococcal NADase (obtained as described above)[3] to those used in the first experiment and mixed with 0.5 ml of physiologic saline. The second group received 1.5 ml of the same partially purified antigen preparation mixed with 0.5 ml of a suspension of cholesterol (greater than 99% pure; Sigma Chemical Co., St. Louis, Mo.) in physiologic saline. The saline suspension of cholesterol was prepared by dissolving 100 mg cholesterol in 10 ml absolute methanol and then pouring the solution into 20 cm^3 of boiling sterile saline. It was found that 0.5 ml of the resulting suspension (containing 2.5 mg cholesterol) was required to neutralize the hemolytic capacity of the previously determined sublethal dose (33 U) of SO.

In the second immunization experiment, the animals were bled before immunization and at the 3rd, 6th, and 9th wk after the beginning of immunization. At the conclusion of the experiment, ASO and anti-NADase titers were simultaneously determined on all sera obtained from each rabbit.

In Vitro Inhibition of Hemolysis Experiments. Experiments were carried out to compare lipids extracted from several different rabbit organs or tissues with respect to their capacity to block hemolysis by SO. Lipids were extracted from rabbit skin (dermis and epidermis), lungs, myocardium, kidney, and liver using a 2:1 mixture of chloroform and methanol as described above.

Suspensions of extracted lipid(s) were diluted serially in saline (undiluted, 1:10, 1:25, 1:50, 1:100, 1:200). 1 ml of each dilution of the various lipid suspensions was mixed with 0.5 ml of buffer containing 10 U of reduced SO (Difco Laboratories) for a total volume of 1.5 ml, and the mixture was incubated for 20 min. To each tube 0.5 ml of a washed 5% suspension of rabbit erythrocytes was added. After incubation again for 20 min, the tubes were centrifuged at 2,000 rpm for 2 min, and the supernates were visually examined for evidence of hemolysis. For each experiment, appropriate SO and erythrocyte controls were run concomitantly, i.e., one tube contained only SO and erythrocytes and the other tube contained only lipid extract and erythrocytes.

Results

Inhibition of Antigenicity. In the initial experiment reported here, the effects of the lipid extract of rabbit skin on the immune responses to SO, DNase B, and NADase were examined. One group of animals was immunized with the preparation of extracellular antigens mixed with a saline suspension of extracted skin lipids, and the second group was immunized with identical amounts of the same preparation of antigens mixed with physiologic saline.

Fig. 1 is a scattergram comparing the ASO titers of sera obtained at various intervals from the two groups of animals. Although there was some overlap, the distributions of ASO titers for the two groups were different at each bleeding; the geometric mean ASO titer of the group immunized with antigen plus saline

[3] In this experiment, in which Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) was used instead of Pfanstiehl dialysate broth, the yield of DNase B was low.

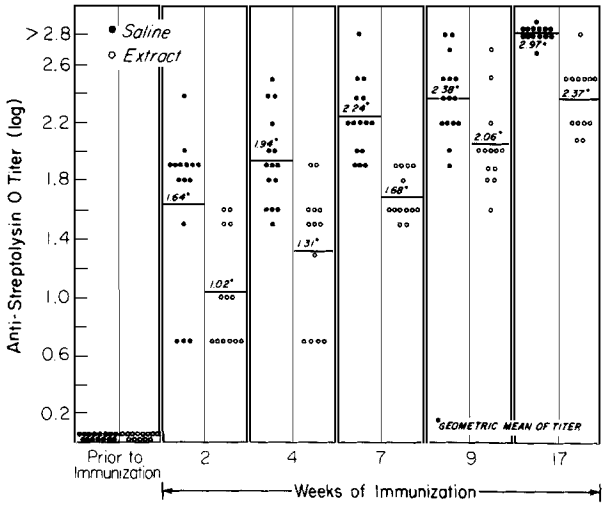

FIG. 1. Scattergram showing the effects of skin lipid extract on ASO response in rabbits immunized with streptococcal extracellular products. (Geometric mean titers for each group are represented by horizontal bars.) On the left of each panel are shown the titers of these animals immunized with extracellular products mixed with saline; on the right are the titers of those animals immunized with the same dose of extracellular products mixed with lipids extracted from rabbit skin (see text).

was significantly higher at each interval than that for the group immunized with antigen plus lipid extract. In contrast, for both anti-DNase B (Fig. 2) and anti-NADase (Fig. 3), there was essentially no difference between the two groups in the distributions of individual titers and in the geometric mean titers. In this experiment the most rapid rise occurred in the ASO titers. There was a delay in the rise of anti-DNase B titers, and the geometric mean titers for antibody to this enzyme were lower than those for ASO and anti-NADase.

The purpose of the second immunization experiment was to determine whether cholesterol is also capable of affecting the antigenicity of SO. In one group the animals were immunized with streptococcal extracellular antigens plus saline and in the other group with antigens plus a saline suspension of cholesterol.

The distribution of antibody titers and the geometric mean titers for ASO and anti-NADase in this experiment are shown in Figs. 4 and 5. The distribution of ASO titers (Fig. 4) was generally higher, and the geometric mean ASO titers were greater in rabbits immunized with the antigens mixed with saline than in animals immunized with antigens mixed with cholesterol suspended in saline ($P < 0.05$). At the 9-wk bleeding, the geometric mean ASO titer was only slightly higher for the saline group; because of the death of several rabbits, the numbers were small and the difference was not significant (P $0.05 < P < 0.10$). There was no striking difference in distributions of the anti-NADase titers (Fig. 5) for the two groups of rabbits nor was there a significant difference in the geometric means.

In Vitro Inhibition of Hemolysis. In the hemolysis inhibition experiments, lipids extracted from several different organs or tissues were compared with

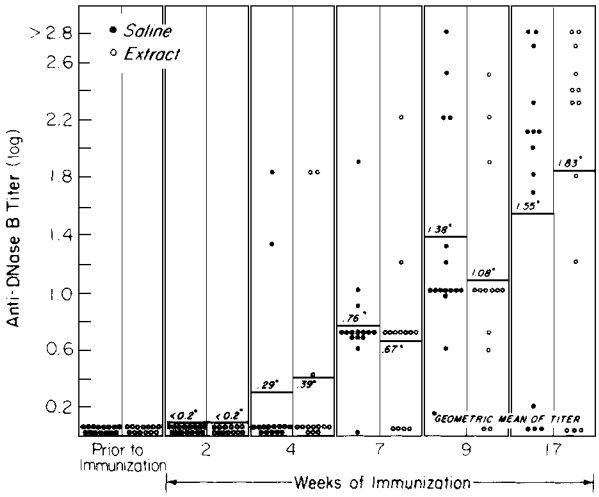

FIG. 2. Anti-DNase B titers for the same animals shown in Figs. 1 and 3.

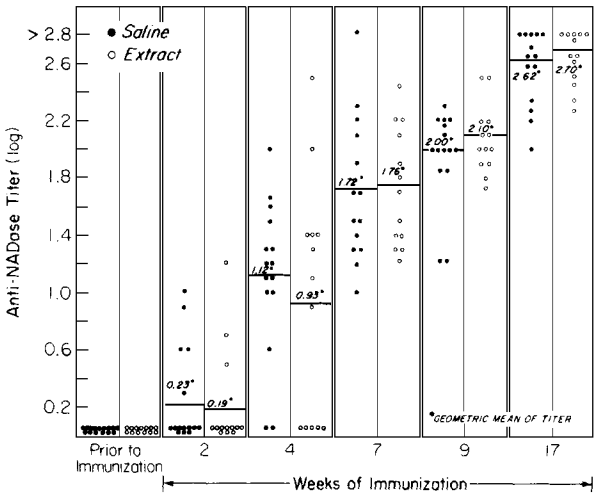

FIG. 3. Anti-NADase titers for the animals described in Figs. 1 and 2.

respect to their ability to block hemolysis of erythrocytes by SO. For these experiments, samples of equal wet weights of each of the rabbit organs or tissues (skin, ventricular myocardium, lung, spleen, kidney, and liver) were extracted with a chloroform:methanol mixture. Suspensions of the lipid preparations were diluted in sterile physiologic saline and tested for their ability to block hemolysis of rabbit erythrocytes by a constant amount of SO. The results of these experiments are shown in Fig. 6. These photographs are typical of several experiments with extracts prepared from each organ or tissue.

The top left panel of Fig. 6 illustrates the results of an experiment using lipids extracted from rabbit dermis and epidermis. The original concentration of lipid extract from skin could be diluted 100-fold (1:100) before the inhibitory capacity of the skin lipid was sufficiently reduced to allow significant hemolysis to occur.

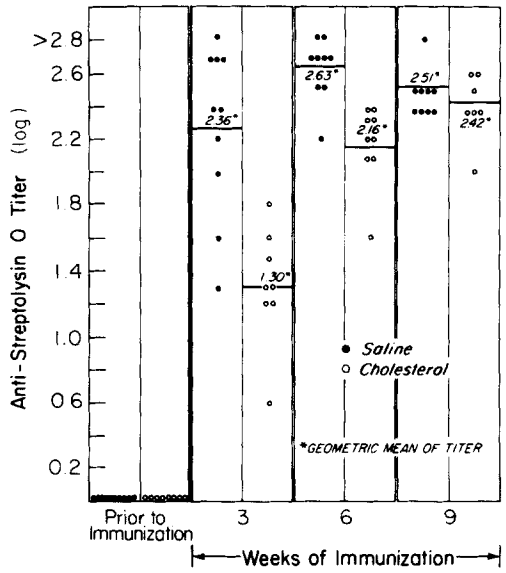

FIG. 4. A scattergram showing the effect of cholesterol on the ASO responses in rabbits immunized with streptococcal extracellular products (see text).

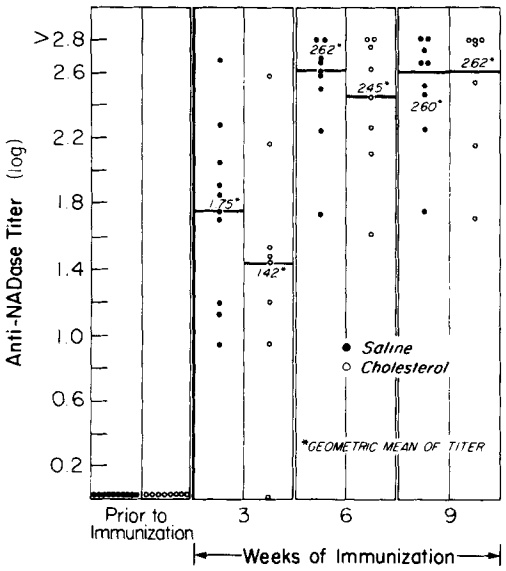

FIG. 5. Anti-NADase titers for the same animals shown in Fig. 4 (see text).

The remaining panels are photographs of results of similar experiments using lipid extracts prepared in an identical manner from rabbit myocardium (heart), lung, liver, spleen, and kidney. In contrast to lipids extracted from skin, lipids extracted from each of these tissues showed no significant effect in blocking the hemolytic capacity of SO. Even in the undiluted preparation, there is no evidence of inhibition by lipid extracts of heart, lung, and spleen, and there is only slight inhibition in the lower dilutions of extracts prepared from liver and

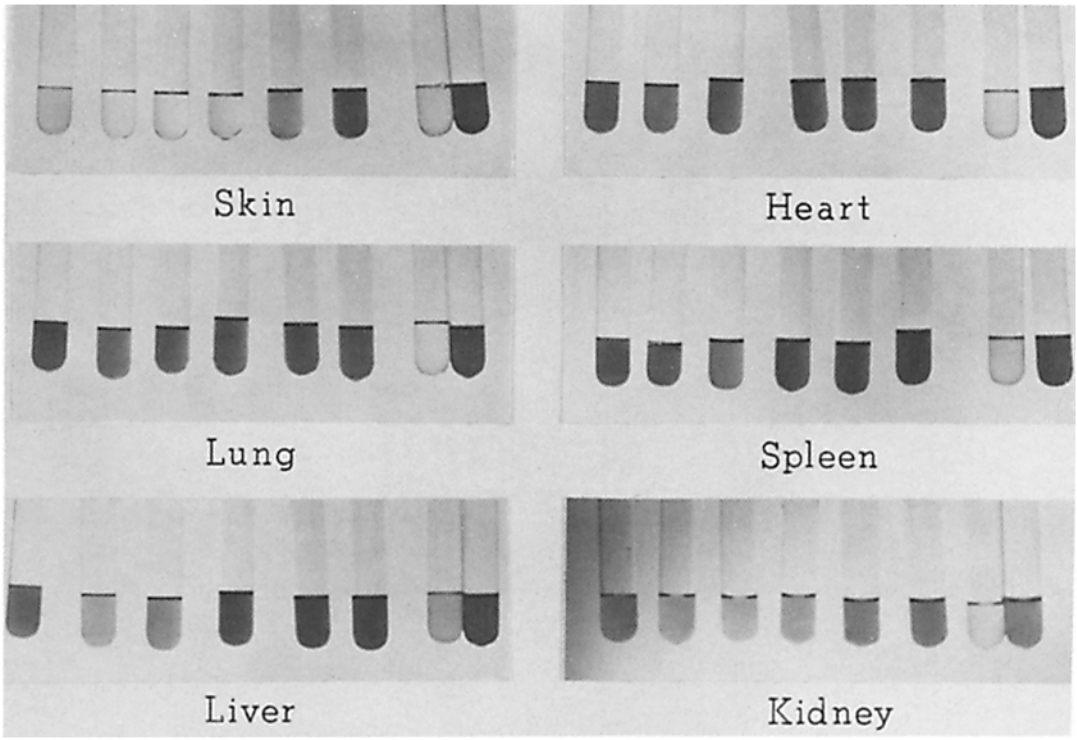

FIG. 6. Effects of lipid extracts of various rabbit tissues and organs on hemolysis of rabbit erythrocytes by SO. The results of the in vitro hemolysis tests are shown in the six panels of this figure. In each panel are shown eight tubes. Beginning on the left, the tissue (as labeled) lipid extracts are shown in the following dilutions: undiluted, 1:10, 1:25, 1:50, 1:100, and 1:200. The two tubes on the right of each panel are the erythrocyte and SO controls. (No erythrocyte buttons are visible because the supernate has been removed from the tubes with incomplete hemolysis to prevent further hemolysis during photography.)

kidney. Experiments using the detergents lecithin and polyoxyethylene sorbitan monooleate (Tween 80; Fisher Scientific Co., Pittsburgh, Pa.) to make a fine suspension of the lipids extracted from skin did not significantly alter the results.

Discussion

Striking contrasts between group A streptococcal infections of the throat (pharyngitis) and of the skin (pyoderma) have been documented in epidemiological studies reported in recent years (20–22). Among the most fascinating of these differences are those relating to the immune response (6–8) and to the occurrence of nonsuppurative complications (22). The experimental findings reported here may bear on both of these observations.

Analysis of the influence of the site of infection on the streptococcal antibody response and on the occurrence of nonsuppurative sequelae is complicated by the fact that in patients with cutaneous infections, group A streptococci may also be recovered from the upper respiratory tract, and these streptococci may be of the same M-type as those found in the skin lesions of the patient (3, 4, 23, 24). The

appearance of group A streptococci of the same serological type in the upper respiratory tract is a late development in patients with pyoderma (25), and in patients in whom the infecting streptococcus is isolated from both sites, suppression of the ASO response may be absent or less evident (6). Moreover, although recent studies have emphasized differences in serological types between "skin" and "throat" strains (21, 26), certain strains, notably type 49, have the capacity to produce clinical infection of either the upper respiratory tract or the skin (27–30). Studies of an outbreak of type 49 infections permitted documentation of differences in ASO response after recovery of streptococci from different sites under conditions in which strain and other variations were minimal (6). The strains isolated from different sites during this outbreak showed no significant differences in in vitro capacity to produce SO. The findings of this epidemiological study suggested that it is the site of propagation per se rather than the infecting strain which influences the ASO response and pointed up the need to examine this question in the laboratory, which was the purpose of the studies reported here.

This communication presents experimental evidence that (*a*) lipid extracts of rabbit skin but not other organs are effective in blocking the in vitro lysis of rabbit erythrocytes by SO. (*b*) both cholesterol and lipid extracts of skin, when injected intravenously with a preparation containing several different extracellular antigens of group A streptococci, significantly suppress the antibody response to SO but not to other streptococcal products.

The inhibition of the ASO response by skin lipids and cholesterol is only partial and becomes less striking with time. Thus, in this model, all of the available antigen may not be bound ("antigen excess") or may not remain bound to lipid, or the model may involve only one of several mechanisms which may influence the suppression of the ASO response. However, in this regard, it is of interest that suppression is not always complete after natural skin infection in humans (6–8).

The findings in this study support the view that the feeble ASO response following streptococcal pyoderma may be due to a local factor in the skin capable of specifically inhibiting the antigenicity of SO. This inhibitor is present in high concentration in lipid extracts of rabbit skin and is most likely cholesterol which is known from previous studies to inhibit other biologic and toxic effects of SO and, as shown here, inhibits its antigenicity as well. The results of experiments comparing the relative ability of lipid extracts from several rabbit tissues (Fig. 6) to block the hemolytic effect of SO are intriguing, but as yet not fully explained. The excellent ability of free cholesterol to neutralize SO has been recognized. In fact it is thought that because most cholesterol in serum is not in the free state, serum itself (in the absence of antibody) is a poor inhibitor of SO unless the serum is contaminated by bacteria that produce an enzyme such as cholesterol esterase or possibly a proteolytic enzyme which might free the cholesterol or alter the spatial relationship of cholesterol in lipoprotein fragments (31). Liver, as well as others of the tissues tested, does contain cholesterol in several forms, including free cholesterol. We do not yet have a detailed chemical analysis of these tissues or data which will allow assignment of the relative importance of the free and bound cholesterol in these tissues in inhibiting hemolysis. Our data only indicate that when one extracts lipid from equal

wet weights of these rabbit tissues, those present in skin (known to contain a relatively large amount of free cholesterol) appear most effective in preventing hemolysis of rabbit erythrocytes by SO. Preliminary experiments searching for an inhibitor present in these tissues, which might interfere with the inhibitory effect of cholesterol and/or related lipids on hemolysis of erythrocytes by SO, have been suggestive but not conclusive. Additional efforts are underway in our laboratory.

Cholesterol is found in abundance in human skin (12, 13). SO appears to bind firmly to cholesterol in vitro (32). Cholesterol has also been known for some time to possess the capacity to inhibit two of the biologic properties of SO: its hemolytic and cardiotoxic effects. It therefore is of interest that this lipid could be shown to modify yet another of the biologic properties of SO, its antigenicity. In its effect on the antibody response, cholesterol, like the lipid extract of rabbit tissue, also appears to be specific for SO. This observation is of further interest since Fehrenbach has suggested (33) that SO and streptococcal NADase may be the same. Our data would not support his contention. The effect of cholesterol and/or other skin lipids in modifying the ASO response and not the anti-NADase response indicates at least one significant difference. Other data from this laboratory also suggest that these two antigens are different (E. D. Gray, personal communication), as do the studies of Shany and colleagues (34).

The data presented in this paper raise questions regarding the mechanism(s) by which cholesterol and possibly other lipids which are present in abundance in skin modify the antigenicity of SO. Alouf and co-workers (35, 36) have postulated the presence of two antigenic sites on the SO molecule, one of which is responsible for fixation of SO to the erythrocyte membrane, and the other responsible for subsequent lysis of the cell. It has been suggested that these two properties of the molecule have different requirements for optimal activity (35). If both these sites are antigenic and result in the production of two different kinds of neutralizing antibody, the attachment of cholesterol and/or other lipids to the "fixation site" might modify this site in such a way as to suppress its antigenicity but may fail to modify the antibody response to the second site. Preliminary studies in our laboratory, in which human sera from patients with streptococcal infections were repeatedly absorbed with SO preincubated with erythrocyte stroma, revealed that the ASO titer can be significantly reduced but not eliminated. Further studies are needed to characterize the mechanism of inhibition of antigenicity by cholesterol and to examine critically the postulated existence of two antigenic sites on the SO molecule (only one of which may be suppressed by cholesterol) which could result in qualitative as well as quantitative differences in the neutralizing antibodies for SO produced after infection at different sites.

The earlier epidemiological studies and the experiments reported here may also permit some cautious speculation about the elusive pathogenetic mechanisms involved in the development of one of the nonsuppurative sequelae of group A streptococcal infections, rheumatic fever. Several observations are perhaps relevant: first is the clinical and epidemiologic information indicating that, in contrast to acute poststreptococcal nephritis which may follow either skin or upper respiratory tract infection, acute rheumatic fever does not follow streptococcal pyoderma (20); second is the known toxicity of SO for myocardial

tissue (10, 37); and third is the reported relationship of the attack rate of rheumatic fever to the magnitude of the antibody response to this antigen (38).

The necessity for infection with group A streptococci to occur in the upper respiratory tract in order for acute rheumatic fever to develop and the various possible explanations for this curious phenomenon have been discussed in some detail elsewhere (22). We shall review here only the clinical and epidemiological evidence supporting this observation and discuss the possibility that a local inhibitor of SO (cholesterol and/or possibly other lipids) may play a role in preventing the development of this complication in patients with impetiginous infection due to group A streptococci.

For many years clinicians have made casual observations suggesting that infections of the skin do not lead to acute rheumatic fever. Interpretation of these observations has been clouded by inadequate documentation of the exact nature and intensity of the infectious challenge and the constancy of follow-up in such patients. Thus, uncertainties existed as to whether the streptococcus is the primary infecting agent in lesions from which staphylococci are often also recovered, whether the streptococci are regularly group A, whether they produce M protein, whether these skin infections provoke a significant immune response to streptococcal antigens, whether the streptococcus infecting the skin may originate from the upper respiratory tract, and whether unrecognized intervening infection may have occurred at some other site (20).

Detailed bacteriologic, serologic, and epidemiologic studies from several different laboratories have provided assurance that the primary infecting agent in the thick-crusted type of impetigo is a group A streptococcus which produces M protein, that these skin infections result in a vigorous antibody response to certain streptococcal antigens other than SO and that these strains producing impetiginous or pyodermatous lesions of the extremities do not enter via the respiratory tract nor do they commonly produce signs or symptoms related to this site when they (in some patients) finally arrive there some weeks after the initiation of skin infection. Thus, the streptococci producing primary infection of the skin possess many of the qualities associated with streptococci infecting the upper respiratory tract. They also often result in repeated infections, a condition which may be important in the pathogenesis of acute rheumatic fever. Although the strains isolated in these skin infections are usually of a different serological type than those commonly infecting the throat (20, 21, 26, 30), no biological differences have so far been detected which can account for their failure to result in rheumatic fever. The feeble antibody response to SO does not appear to be related to differences in production of this antigen, at least as judged by in vitro studies (6), but rather to reflect a difference in host response related to the site of infection.

SO has been known for some years to be a cardiotoxic agent in the isolated perfused heart of the frog (39) and of the rabbit (40). Halbert and co-workers (10) have demonstrated that intravenous injection of an activated, partially purified preparation of SO produces profound electrocardiographic changes in the rabbit. SO also has a profound effect on beating mammalian heart cells in vitro (37). Thus, SO may play an essential role in damaging myocardial and other tissues affected by rheumatic fever, perhaps providing the initial insult, with immunologic mechanisms involving cross-reacting antibodies (41) or cellular immune

reactions (42) which result in the full-blown pathologic picture. Ginsburg (43) has postulated that by causing myocardial cell injury, SO may "prepare" these tissues for subsequent immunologic injury by a mechanism involving one of the so-called "cross-reactive" antibodies. Halbert (32) had earlier outlined an hypothesis which proposed a primary role for SO in the production of rheumatic fever. If SO is involved, cholesterol or other lipids in the skin might interfere by at least two different mechanisms: (*a*) inhibition of the immune response to SO or (*b*) inhibition of direct toxicity of SO (22).

An exaggerated immune response to SO has been noted in patients who develop rheumatic fever after acute streptococcal pharyngitis as compared with patients who do not develop this complication (1, 2, 44). A similar relationship has been reported for other streptococcal antibodies (45, 46), but the most extensive documentation on this point concerns the antibody response to SO. Indeed, in patients with streptococcal sore throat, the magnitude of the ASO rise correlates well with the risk of developing rheumatic fever, for both initial and recurrent attacks (37, 47). In an experimental animal model, Kirschner and Howie (48) have reported that the frequency and severity of heart lesions are related to the magnitude of the rise in ASO titer but not to the antihyaluronidase response. Therefore, it seems possible that by suppressing the ASO response, cholesterol or other lipids in the skin may influence the risk of developing rheumatic fever in patients with superficial cutaneous infections (pyoderma) caused by group A streptococci.

An alternative hypothesis, not involving antibody, is that local factors in the skin may directly affect the toxicity of SO. Cholesterol is an effective inhibitor of the toxic effects of SO in the intact animal (9), in the isolated perfused heart (40), and in beating mammalian heart cells (37). Thus, it is possible that cholesterol or other skin lipids may have a direct influence on the toxicity of SO by binding with this toxin at or near the site of its production in superficial skin lesions. According to this theory, the antibody response to SO would not contribute to the pathogenesis of rheumatic fever but would merely reflect the extent of local binding of this toxin.

Further studies are needed to determine which, if either, of these two hypotheses (an exaggerated antibody response or a direct toxic effect of SO) plays a role in the pathogenesis of rheumatic fever, and to evaluate the concept that a local inhibitor of SO is responsible for the odd absence of rheumatic fever after group A streptococcal pyoderma.

Summary

Lipids extracted from rabbit skin block the hemolytic capacity of SO and also suppress the neutralizing antibody response to this streptococcal extracellular antigen in rabbits immunized intravenously. The modification in antibody response is specific for SO; the antibody responses to streptococcal DNase B and to streptococcal NADase are not affected. Cholesterol, a lipid present in abundance in skin, has a similar specific effect on the antigenicity of SO and may be the component responsible for the demonstrated effects of these lipid extracts of skin. In vitro experiments indicate that lipid extracts of rabbit skin have a greater capacity to block the hemolytic capacity of SO than do lipid extracts of rabbit heart, kidney, lung, liver, or spleen.

These data support the view that the feeble ASO response observed in patients with streptococcal pyoderma is a result of the abundance of a local lipid inhibitor, such as cholesterol, in the skin. They may also bear on the pathogenesis of rheumatic fever, a complication which apparently does not occur following group A streptococcal pyoderma. Two possible explanations for this remarkable epidemiologic observation, both related to the presence of a local inhibitor, are considered: (*a*) suppression of the ASO response, the magnitude of which has been correlated with the risk of developing rheumatic fever after streptococcal infection of the throat, and (*b*) inhibition of the toxicity of SO, which has been shown to have a direct toxic effect on the mammalian heart and on isolated beating myocytes.

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