Finally, we attempted to isolate the possible H_4folate intermediates formed when [3-^{14}C]serine was incubated with poison glands. 5,10-Methenyl-H_4folate, a rather stable intermediate, was isolated (i) by descending paper chromatography, which revealed a fluorescent, radioactive spot at R_F 0.5, corresponding to the R_F of standard 5,10-methenyl-H_4folate, and (ii) by column chromatography on Whatman cellulose CF-11 (*8*). The eluted fractions from the column whose absorbance ratios at 350 nm and 305 nm were higher than 1.6, characteristic of 5,10-methenyl-H_4folate (*8*), showed ^{14}C labeling (that is, originated from the [3-^{14}C]serine in the incubation medium). These fractions were pooled, lyophilized, and then reacted with adenosine diphosphate, inorganic phosphate, and a pure preparation of 10-formyl-H_4folate synthetase (E.C. 6.3.4.3.) from *Clostridium thermoaceticum* (*9*). The labeled compound isolated from the column was completely converted to [^{14}C]formic acid, demonstrating unequivocally that it was indeed 5,10-methenyl-H_4folate.

In view of these results, we suggest the following pathway of the biosynthesis of formic acid in the poison gland of *C. pennsylvanicus*. Serine, serving as the main precursor, contributes its C_2 and C_3 carbons to 5,10-methylene-H_4folate; this is subsequently converted to 5,10-methenyl-H_4folate and 10-formyl-H_4folate which is hydrolyzed to the final product, formic acid. In the last step H_4folate is regenerated and adenosine triphosphate (ATP) is produced (*6*); in turn, the ATP may be utilized for an energy dependent transport of formic acid through the glandular membrane into the gland lumen. Since the equilibrium between formic acid and 10-formyl-H_4folate is 1:20 (*6*), only small amounts of formic acid are produced in the glandular cells. For the acid to accumulate, it is therefore necessary to transfer it to a second compartment (the poison gland reservoir), which is insulated by a cuticular intima (*10*). Thus the biochemical equilibrium inhibits the accumulation of the acid in the glandular cells, and the insulated reservoir prevents it from diffusing out into the body cavity. When the reservoir fills up, the proposed formic acid carrier will be saturated, resulting in the accumulation of 10-formyl-H_4folate as well as ATP in the glandular cells, and inhibiting the biosynthetic pathway. This system would be readily reactivated as soon as the ants eject their reservoir contents in a defensive encounter.

The biosynthesis of formic acid in the ant poison gland deduced from our experiments is not different from that reported for bacterial and mammalian cells (*6*). The novelty of this system, however, is the adaptation of the biochemical characteristics of this pathway and the morphological compartmentalization of the gland for accumulating large amounts of this toxic compound.

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Adherence of Group A Streptococci to Pharyngeal Cells: A Role in the Pathogenesis of Rheumatic Fever

Abstract. *We used an assay in vitro to investigate the possible role of streptococcal adherence to human pharyngeal cells in the pathogenesis of acute rheumatic fever. There was no difference in adherence of rheumatic fever–associated and non-associated strains of group A streptococci to pooled pharyngeal cells of normal people. Likewise, streptococci not associated with rheumatic fever adhered equally well to cells taken from normal people and from patients with rheumatic heart disease. However, the pharyngeal cells of all nine rheumatic heart disease patients tested had increased avidity for adherence for a rheumatic fever–associated strain of streptococcus compared to the pharyngeal cells obtained from age- and sex-matched controls. Increased streptococcal adherence to pharyngeal cells of rheumatic fever–prone patients may play a role in the pathogenesis of rheumatic fever.*

There seems to be a special relation between pharyngeal infection with group A streptococci and the subsequent development of acute rheumatic fever. It is known that streptococci must be present in the pharynx for a prolonged period of time and must evoke an antibody response, and that treatment for less than 10 days may not eradicate the infection or prevent the occurrence of rheumatic fever (*1*). Streptococcal infections of other sites such as the skin do not lead to rheumatic fever (*2*). It is unclear why pharyngeal infection is necessary. Various postulates have been forwarded, including an antigenic change in the organism induced by the oral environment or the neutralization of some toxic or antigenic streptococcal products by the skin (*3*). There are also no currently recognized host or bacterial factors that fully account for the observation that less than 3 percent of individuals with untreated group A streptococcal infection of the pharynx develop acute rheumatic fever (*4*). Although it has been thought in the past that any strain of group A streptococcus is potentially rheumatogenic, epidemiologic studies have indicated that impetiginous strains may not have the potential to produce acute rheumatic fever even when they infect the pharynx (*5*). It is therefore unclear whether the most important factor in the pathogenesis of acute rheumatic fever is the strain of streptococcus or the site of infection or both.

Studies with streptococci, as well as

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other bacterial species, have indicated that bacterial adherence is an important first step in the pathogenesis of infection and that the virulence of organisms frequently correlates with their ability to adhere to particular sites (*6*). Group A streptococci adhere to epithelial cells by fimbriae composed of lipoteichoic acid and M protein. Strains that are rich in M protein tend to be more virulent and to adhere more avidly to epithelial cells (*7*).

Furthermore, studies with other types of infections have suggested that certain individuals may be prone to specific infections because their epithelial cells have increased avidity for adherence by pathogenic organisms (*8*).

Since pharyngeal infection is important in the pathogenesis of acute rheumatic fever, it seemed reasonable to determine whether strains of streptococci associated with rheumatic fever adhere more avidly than nonassociated (control) strains, and whether pharyngeal epithelial cells obtained from subjects who have had acute rheumatic fever have a special avidity for streptococcal adherence.

We used a variation of a recognized in vitro adherence assay (*9*). Epithelial cells were scraped from the posterior pharynx with a metal spatula. The cells were washed twice in phosphate-buffered saline (PBS) at *p*H 7.3 by differential centrifugation at 3.4*g* for 10 minutes and then adjusted to 10^5 cells per milliliter by hemocytometer count. The streptococci, which had been maintained on blood agar, were transferred to brain heart infusion broth and incubated for 16 hours at 37°C under CO_2. The organisms were washed twice in PBS. A suspension of organisms was passed three times through a 25-gauge needle to disrupt clumps and chains, and was then adjusted to an optical density of 0.6 at 570 nm, which corresponded to 3×10^8 organisms per milliliter as determined by colony counts. Then 0.5 ml of the streptococcal suspension was added to 0.5 ml of the epithelial cell suspension and was incubated for 30 minutes at 37°C in a shaking water bath. Unattached bacteria were separated from the cells by four differential centrifugations at 3.4*g* for 10 minutes each. The cells were then placed on a glass slide, air-dried, heat-fixed and stained with crystal violet. The slides were coded, the number of bacteria attached per cell was determined in a blind fashion in 50 consecutive cells, and the mean was calculated.

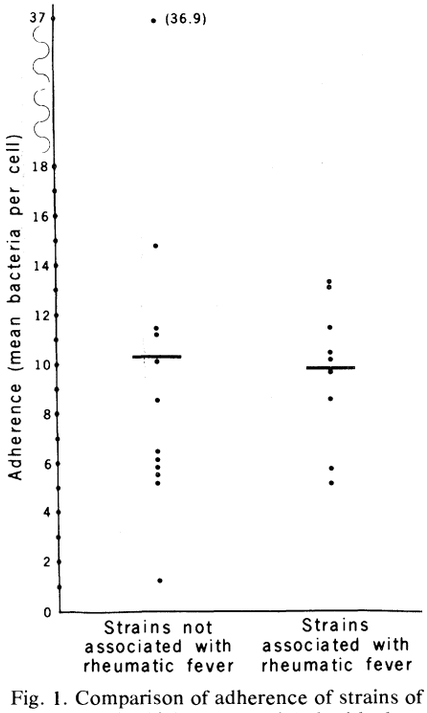

Fig. 1. Comparison of adherence of strains of streptococci which are associated with rheumatic fever and control strains to pooled pharyngeal cells of normal individuals. Horizontal bars indicate the means. There is no difference.

We obtained rheumatic fever–associated streptococci from three sources: (i) strains isolated in 1973 from patients with acute rheumatic fever provided by the Pyramids Free Rheumatic Heart Center in Cairo, Egypt, (ii) strains isolated in 1949 from patients with acute rheumatic fever in Cheyenne, Wyoming (*10*), and (iii) strains also isolated in 1949 during the rheumatic fever outbreak at Warren Air Force Base, Cheyenne, Wyoming (*11*). These strains had all been preserved by lyophilization or desiccation. Control strains of streptococci, not known to be associated with rheumatic fever, were obtained from patients with pharyngitis. Included were lyophilized isolates provided by the Center for Disease Control and Wannamaker (*11*), and fresh isolates from our clinic. We verified that all streptococci belonged to group A by using specific antiserum.

For subjects we identified nine rheumatic heart disease patients and nine sex- and age-matched controls who had no history of rheumatic fever. There were six females and three males in each group. Ages ranged from 20 to 47 years.

There was no difference when we compared the adherence of rheumatic fever–associated strains of streptococci and control strains to pooled pharyngeal cells taken from a normal population (see Fig. 1). These results must be interpreted with caution, however, since the control strains were fresher isolates and less often lyophilized. It is possible that the lyophilization process and the length of time they were kept in the laboratory may have decreased the adherence ability of the rheumatic fever–associated strains (*12*).

We did find a striking increased adherence to pharyngeal cells taken from rheumatic heart disease patients compared to pharyngeal cells from paired normal individuals when these were tested with a rheumatic fever–associated strain of streptococcus. This was significant at $P < .005$ by the Student's *t*-test. When the same epithelial cells were compared for their avidity of adherence to a control strain of streptococcus in a simultaneous experiment, no difference was found (see Fig. 2).

These results suggest that certain individuals may be prone to develop rheumatic fever because strains of streptococci capable of producing rheumatic fever adhere avidly to their pharyngeal cells. Other individuals may be protected because of the lower avidity of their epithelial cells. This hypothesis could help explain why less than 3 percent of individuals with untreated streptococcal pharyngitis develop acute rheumatic fever.

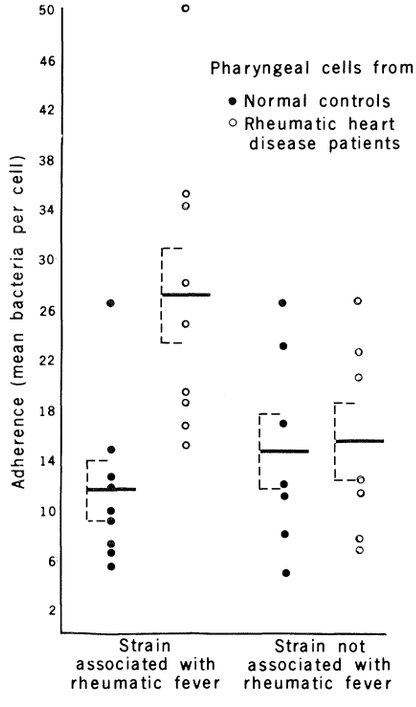

Fig. 2. Comparison of adherence of pharyngeal cells taken from rheumatic heart disease (RHD) patients and age- and sex-matched controls. There is increased adherence of pharyngeal cells from RHD patients when a rheumatic fever–associated strain of streptococcus is used, but no difference in adherence when a control streptococcal strain is used. Horizontal bars indicate means. Standard error of the mean is indicated by dashed lines.

Since the pathogenesis of acute rheumatic fever is largely unexplained, the significance of this increased adherence remains conjectural. Increased adherence may be necessary to prolong infection or to promote colonization with a critical mass of bacteria, factors that may be necessary for the induction of acute rheumatic fever. Since bacterial adherence is a cell surface phenomenon, the increased adherence of pharyngeal cells of certain individuals may reflect a relation between surface components of host cells and of streptococci, which in turn, may be important in the immunologic cross-reactivity which has been implicated in the pathogenesis of acute rheumatic fever. Certain similarities of cell surface components of patients with acute rheumatic fever, namely HLA (histocompatibility) and blood groups, have been investigated with equivocal results (*13*). Perhaps another cell surface component, yet to be identified, is important in this regard.

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Genomic Masking of Nondefective Recombinant Murine Leukemia Virus in Moloney Virus Stocks

Abstract. *HIX virus cloned from Moloney leukemia virus stocks is a nondefective, leukemogenic, and amphotropic murine oncornavirus with a recombinant-type major glycoprotein. Although Moloney leukemia virus stocks generally contain little or no free amphotropic virus, dilution analysis of several virus stocks and the examination of virus progeny from individual foci revealed that HIX virus is present and functionally coated with ecotropic Moloney virus envelopes. Because most mice have serum factors that inactivate recombinant viruses, masking may represent a general survival mechanism for HIX as well as other analogous recombinant viruses.*

Moloney murine leukemia virus (M-MuLV) passed in mouse cells can contain at least three kinds of nondefective murine oncornaviruses: typical ecotropic M-MuLV, xenotropic murine oncornavirus (MuX), and an amphotropic recombinant virus (HIX) that shares M-MuLV and MuX information (*1*). HIX virus breeds true in both mouse and nonmurine cells, and contains antigenic determinants of the group-specific antigen (gag) region, which are type-specific for M-MuLV (*1, 2*). The major glycoprotein (gp71) is neither eco-, xeno, or wild mouse amphotropic type as analyzed by interference properties, specific neutralization, and peptide maps. Partial relationships of HIX gp71 to both MuX and M-MuLV were observed by the above procedures (*1–3*). HIX virus was cloned by multiple cycles of focal and limiting dilution isolation, was grown in feline embryo fibroblast (FEF) cells, and was inoculated into newborn mice and cats. In several strains of mice, lymphomas were observed 2 to 3 months after inoculation. On the basis of the clinical syndrome and histopathology, these lymphomas were indistinguishable from the original M-MuLV–induced disease (*4, 5*). Extracts of leukemic organs contained high titers of virus with amphotropic properties. Further analysis of envelope properties and the genetic content of many individual infectious units of tumor-derived virus revealed that the only detectable virus in tumors was HIX (*6*).

Because leukemogenic HIX was isolated from M-MuLV stocks, we reexamined several questions. (i) Do M-MuLV–induced lymphomas contain HIX virus? Previously M-MuLV stocks were found to contain little or no detectable amphotropic virus (*1, 2, 6, 7*). (ii) Can HIX virus arise regularly by de novo recombination? (iii) Is the recombinant virus the actual leukemogenic agent in M-MuLV stocks? A similar virus has been isolated from lymphomas of AKR mice, and appeared to be a recombinant of AKR-MuLV and MuX; it was postulated to be the actual leukemogenic virus of AKR mice (*8*). Because this recombinant virus (MCF) could not be isolated in free form from AKR-MuLV stocks, the answers to the above questions could contribute to our understanding of virus participation in murine leukemia. Our examination of several lymphoma- and tissue culture–derived stocks of M-MuLV revealed that HIX virus was present in uncloned virus populations, but in a masked form. Single infectious units of HIX could be coated with ecotropic envelopes, giving the impression on assays that no virus with xenotropic properties existed in such stocks.

Several stocks of M-MuLV from NIH Swiss and BALB/c mouse lymphomas induced by the IC isolate (termed IC) of M-MuLV were reexamined for the presence of virus that could be detected in nonmurine cells (*4*). Assays for murine ecotropic and xenotropic viruses were performed in mouse or cat sarcoma-positive leukemia-negative (S+L−) cells (*9, 10*). Previously BALB/c lymphoma–derived M-MuLV (ICXB) had no detectable HIX-type virus. Because HIX virus was very susceptible to virus-inactivating factor found in normal mouse serums (MSF), it appeared possible that recombinant virus was already inactivated (*1, 11, 12*). The NIH Swiss strain of mice was unusual in that no MSF was detectable in the serum (*12*). Virus from extracts of NIH Swiss mouse tumors induced by the IC isolate of M-MuLV (ICXN) more than 8 years ago was also reexamined (*4*). The ICXN isolate was used to generate lymphomas in BALB/c mice. Both tumor-derived virus stocks had high titers of ecotropic M-MuLV (Table 1). ICXN had obvious xenotropic

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