Relation of a Streptococcus to Epidemic Poliomyelitis

Studies in Etiology, Diagnosis and Specific Treatment

EDWARD C. ROSENOW, M.D., *Cincinnati, Ohio*

SUMMARY

The production in 1915 of herpes zoster or "posterior" poliomyelitis in animals with a streptococcus led to further research on the etiologic importance of streptococci in "anterior" poliomyelitis.

A specific streptococcus was demonstrated consistently in persons with poliomyelitis and in well persons having contact with them or merely inhabiting an area in which poliomyelitis was epidemic. That the organism was not present in areas remote from contact with the disease was likewise demonstrated.

The streptococcus has been isolated from filtrates of poliomyelitis virus and from the tissues and exudates which harbor the virus. It appears in the spinal fluid in the preparalytic stage of poliomyelitis and disappears from the spinal fluid during the severe stage of the disease.

Antibody and antigen prepared from the streptococcus were used to determine the presence of antigen and antibody indicative of streptococcal infection in many patients with poliomyelitis and in well persons. The intensity of reaction indicating specific streptococcal antigen was directly proportional to the degree of paralysis in patients; the reaction was greater in persons whose age, sex and previous isolation from the disease would normally indicate greater susceptibility. The test for antibody gave opposite results.

Specific agglutinins for the streptococcus and neutralizing antibody for the virus were present consistently in the serum of persons and monkeys during recovery from poliomyelitis.

Virus produced in vitro from the associated streptococcus caused all the clinical and pathologic features of poliomyelitis in monkeys inoculated with it, and the animals that recovered from the disease thus induced were proved to be immune thereafter to the natural virus.

Antistreptococcic serum prepared in horses was used to treat poliomyelitis. In a group of monkeys inoculated with the virus of the disease, 6 per cent of those receiving the serum before inoculation died of the disease; of the control group, 82 per cent. In a series of poliomyelitis patients treated with the serum the mortality rate was 8 per cent; in a control series, 21 per cent. In a series treated in all stages of the disease by the author, 10 per cent died; of those who did not receive the serum, 25 per cent.

An antibody has been prepared from the streptococcus which appears to prevent paralysis and otherwise mitigate poliomyelitis and to provide immunization from the disease.

The conclusion is reached that the virus of poliomyelitis is a form of the specific streptococcus, which is the agent in primary infections and in the development of the immunizing antibody.

PROOF that epidemic poliomyelitis is caused by a filtrable agent currently considered to be a virus is complete. That the clinical and pathological features of poliomyelitis occur in monkeys inoculated cerebrally with emulsions and filtrates of emulsions of the spinal cord of persons who died of poliomyelitis, and that these features can be reproduced in rhesus monkeys throughout a series of brain-to-brain transmissions is established beyond peradventure.

The influence so often exerted by current concepts in determining the nature and course of original research is strikingly illustrated in studies on the inciting agent of this disease. Bacteriologic studies prior to 1909 indicated that certain diplostreptococci that were isolated might have significance in the etiology of poliomyelitis. These studies were quite naturally dropped when in that year it was learned that the causative agent is filtrable; in consequence, forthright bacteriologic studies have not been generally used for more than forty years.

During studies on elective localization of streptococci in 1915,[14] the author produced herpes zoster or "posterior" poliomyelitis in rabbits and dogs with a streptococcus isolated from the nasopharynx, tonsils and spinal fluid of humans.[1] This result led to the concept that a specific type of streptococcus might have etiologic importance in "anterior" poliomyelitis regardless of the filtrability of the causative agent and might indeed be the source of the filtrable agent.

From the Department of Bacteriologic Research, Mayo Foundation, Rochester, Minn., and Longview Hospital, Cincinnati, Ohio.

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Opportunity to put this idea to test was presented in Rochester, Minnesota and New York City during the epidemic of 1916. Guinea pigs and rabbits were inoculated with the streptococcus isolated from the nasopharynx and spinal cord of persons who were ill with or had died of poliomyelitis. Flaccid paralysis (often fatal); atrophy and deforming contractures of muscles in animals that survived severe paralysis; death from respiratory failure; edema; hemorrhage and degeneration of nerve cells in the anterior horns of the spinal cord—the all-important occurrences in poliomyelitis—were produced in high incidence.[15] Such symptoms and lesions almost never occurred in animals inoculated with streptococci similarly isolated in studies of other diseases. In short, the specific streptococcus, when injected intravenously into animals, tended to localize electively and to cause systemic lesions like those occurring in patients from whom the organism was taken.[14] Mathers[6] and also Nuzum and Herzog,[8] using the same methods, reported similar results in studies of epidemic poliomyelitis in Chicago. The demonstration of pleomorphic cocci and diplostreptococci in the lesions of the spinal cord in poliomyelitis,[15, 1, 2, 16] the occurrence of streptococcal antibodies in convalescent serum,[16, 17, 18, 7, 4] and the production and use of antistreptococcic serum in treatment soon followed.[19, 20, 9]

Convinced of the epidemiologic and etiologic importance of the streptococcus, and recognizing that no practical means for specific prevention and treatment had resulted from the purely viral studies, the author continued making further streptococcal and viral studies of epidemic and experimentally produced poliomyelitis, as opportunity was afforded, from 1916 to the present. This research will be briefly reviewed with citations of the original publications in which the methods used are described. Corroborative results obtained by other investigators will be indicated. The reasons for omitting certain phases of the author's work are set forth in a monograph on poliomyelitis.[20]

I. *Isolation and Microscopic Demonstration of the Streptococcus in Poliomyelitis*

In seventeen epidemics studied, a specific type of nonhemolytic or green-producing, often pleomorphic, streptococcus was isolated consistently, by the special methods employed, from a number of significant sources: (a) from the very tissues and substances that harbor the virus, such as the nasopharynx and the feces of persons with the disease; (b) from the nasopharynx of persons who had had contact with others who had the disease, as well as from the nasopharynx of persons who had had no such contacts (and at the same time it was proved absent during winter in areas remote from epidemics of poliomyelitis); (c) from the spinal fluid, in the very early stages of the disease, of persons stricken during epidemics and of monkeys inoculated with the virus; (d) from the brain and spinal cord, after death, of victims of epidemics, and of rhesus monkeys and mice that had been inoculated with material containing the virus; (e) in significant incidence, from serial dilution cultures of the "bacteria-free" filtrates of highly potent virus.[20] The streptococci isolated from the nasopharynx of persons who had the disease, from the brain and spinal cord of persons who had died of epidemic poliomyelitis and of monkeys that died after inoculation of emulsions and filtrates of the virus, were remarkably alike in appearance, in staining reactions, in virulence, in electrophoretic velocity and in agglutinability. In general, the isolation of streptococci from emulsions and from filtrates of emulsions of brain and spinal cord of monkeys that died of poliomyelitis was accomplished with steadily diminishing frequency on successive transmissions. This was especially true in mice.

The streptococcus was proved absent from the spinal fluid of rhesus monkeys before inoculation of emulsions and filtrates of highly potent virus, and during the asymptomatic period of incubation, but as fever and other symptoms occurred the organism appeared in high incidence and in great numbers on microscopic examination of Gram-saffranin-stained films of centrifuged sediment. Cultures in freshly prepared dextrose-brain broth grew pure colonies in many instances as fever, tremors and staccato voice developed in the subjects after inoculation with different virus strains. The organism diminished in the spinal fluid or disappeared during severe paralysis but was observed in stained films of fluid from the edematous, hemorrhagic anterior horns of the spinal cord; it was isolated from the spinal cord and seen in the lesions, especially of the anterior horns, in monkeys killed by anesthesia after severe paralysis had occurred and in monkeys that died of respiratory failure from rapidly progressing paralysis.

In order to rule out the possibility of chance occurrence of unrelated streptococci or so-called secondary invaders in spinal fluid, the filtrate of the highly potent MV. strain of poliomyelitis virus, which had been transmitted through many monkeys over a period of years, was injected intracerebrally into fourteen monkeys immediately after spinal tap. Cultures of the filtrate of the virus in dextrose-brain broth yielded a pure growth of the streptococcus, whereas culture in dextrose broth and on blood-agar proved sterile. Microscopically examined, the sediment of the spinal fluid of all fourteen monkeys was seen to be free of cells and streptococci, and cultures in dextrose-brain broth produced no growth from spinal fluid extracted before inoculation or from that extracted 48 hours later when symptoms were absent and the temperature of all the animals was normal. At 96 hours, when fever, tremors and staccato voice (but not paralysis) had developed, the streptococcus and lymphocytes and polymorphonuclear leukocytes were readily found in the stained sediment, and pure growth of the streptococcus was isolated in dextrose-brain broth culture from each of the monkeys, while aerobic cultures on blood-agar remained sterile. Two days later, after severe paralysis had developed, the streptococcus was seen

microscopically in specimens from nine of the monkeys, and cultures from but four revealed streptococci. On the eighth day, streptococci were microscopically apparent in two monkeys, and cultures from all remained sterile. The streptococcus was isolated, in dextrose-brain broth, from the spinal cord of each monkey that died from rapidly progressing paralysis. All strains isolated from the spinal fluid were agglutinated specifically by the poliomyelitis antistreptococcus serum, and the distribution curve of cataphoretic velocity was bimodally neurotropic.

Gram-staining pleomorphic diplococci, sometimes in short chains, were found consistently in the lesions of the spinal cord, medulla and brain of persons who had died of epidemic poliomyelitis and of monkeys and mice that died or were killed by anesthesia during the acute stage of poliomyelitis after inoculation with either emulsions or filtrates of the virus; but on investigation of points remote from these lesions the diplococci were proved to be absent.[20]

A distinct tendency to elective localization had been noted in certain streptococci: The streptococcus isolated in studies of epidemic and experimentally induced poliomyelitis tended to localize in the brain and spinal cord and to produce flaccid paralysis, while the streptococcus isolated in studies of arthritis tended to localize in joints and to produce arthritis. In the hope of determining a cause for this phenomenon, large numbers of streptococci of these types were killed by heat and injected intracerebrally in parallel manner into rhesus monkeys and rabbits. The results were remarkable. The streptococcus of poliomyelitis remained in the cerebrospinal fluid and spinal cord, producing great weakness or flaccid paralysis, but no lesions were produced in muscles or joints, nor was the streptococcus demonstrable in the knee joint fluid. The streptococcus of arthritis disappeared promptly from the cerebrospinal fluid and appeared in large numbers in the knee joint fluid while pain and stiffness became evident and lesions of muscles and joints developed. The cause of these examples of specificity or tropism in the dead streptococci is considered similar to or identical with that involved in the well-recognized specific pharmacological action of drugs, chemicals and bacterial toxins.

Of the monkeys subjected to the previous experiment, all were sacrificed for postmortem examination except four that had received the dead streptococci of poliomyelitis and two that had received the dead streptococci of arthritis. These remaining animals were inoculated intranasally with highly potent poliomyelitis virus ten days after the previous intracerebral inoculation. The four monkeys that had received the dead streptococci of poliomyelitis remained well, while typical poliomyelitis developed in the two that had received the dead streptococci of arthritis.

The importance of the streptococcus in poliomyelitis was indicated further in the course of experiments with many different virus strains which had been preserved for periods ranging from several months to six years, at 10° C. in 50 per cent glycerin, in specimens of brain and spinal cord of monkeys that had died of experimentally induced poliomyelitis. Emulsions of these virus strains were injected intracerebrally into a total of 298 monkeys and were also cultured in dextrose-brain broth. Emulsions which yielded pure growths of streptococcus in culture caused a much higher incidence of typical poliomyelitis in the monkeys than those emulsions which on culture yielded no streptococci.[14]

II. *Serologic and Antigenic Specificity of the Streptococcus and Diagnostic Cutaneous and Precipitation Reactions*

In addition to having distinctive distribution curves of cataphoretic velocity and characteristic localizing and disease-producing properties, the streptococcus isolated from and noted in the lesions of poliomyelitis was found to be specific in its serological reaction. It was agglutinated specifically in high titer by antiserum produced in the horse and differentially by serum obtained from convalescent subjects.[18, 20]

The presence of specific streptococcal antigen was demonstrated consistently by the precipitation reaction at the interface between the poliomyelitis antistreptococcus serum and extracts, in sodium chloride solution, of nasopharyngeal swabbings of persons who had poliomyelitis, of persons who had had contact with poliomyelitis patients, and of persons who had had no such contacts but were in the area during epidemics in the summer. The presence of the antigen could not be demonstrated by identical test in winter in places remote from epidemics, except in the serum of persons and monkeys in the active stage of poliomyelitis.[20, 21]

Solutions were made of the euglobin fraction of the serum of horses that had been immunized with the streptococcus[20, 21, 22] and with the heat-produced antibody prepared in vitro from the streptococcus. These solutions were used to test well persons in areas of poliomyelitis epidemics, both those who had and those who had not had contact with persons who had the disease. The presence of specific streptococcal antigen in the skin or blood of these well persons was demonstrated, and corresponding streptococcal infection in throat, intestinal tract or elsewhere was thereby indicated. Again, the application of the test in winter in places remote from epidemic areas demonstrated the absence of infection with the streptococcus.[23, 24, 25] Conversely, the soluble antigen of the heat-killed streptococcus, similarly used, demonstrated the presence of the streptococcal antibody in the skin or blood of persons with poliomyelitis and of well persons in areas of epidemics.[20, 21, 22]

Since these tests—with the thermal antibody for the antigen and with the antigen for the antibody—were found to be invariably harmless and non-sensitizing, and since they yielded such precise information, they were applied, in an etiologic and epidemiologic study, to determine the presence of the specific streptococcus in a total of 432 patients with poliomyelitis and a larger number of well persons in

epidemic areas in summer and, in winter, in places remote from epidemic areas. The results were illuminating. The intensity of reaction indicating specific streptococcal antigen was directly proportional to the degree of paralysis in patients with the disease; the reaction was significantly greater in well persons that had not previously been exposed to poliomyelitis than in persons previously exposed, and in males, the sex more susceptible to poliomyelitis, than in females. In striking contrast, the reaction indicating the presence of antibody was greatest in persons with slighter paralysis or none, least in persons severely paralyzed, greater in females than in males, and greater in persons previously exposed to poliomyelitis than in persons not previously exposed. The intensity of reaction indicating the presence of streptococcal antigen in well persons during epidemics, both in those who had had contact with diseased persons and in those who had not, was roughly proportional to age and somewhat greater in males. In well persons, the test for antibody produced a somewhat greater reaction in females than in males and uniformly a greater reaction than was produced in persons severely paralyzed by the disease. Evidence was obtained in these studies which indicates that immunity to poliomyelitis in proportion to age, and immunity following mild cases or following exposure to epidemics in temperate climates in summer, is due to a long-lasting streptococcal antibody response that is accelerated on subsequent exposure to the organism, especially in summer.

A close parallel was noted among three phenomena: (1) The incidence and degree of reaction indicating streptococcal antigen following intradermal injection of antibody; (2) the agglutination titer of the serum during convalescence; and (3) the viral-neutralizing antibody titer of the serum in convalescence proportional with the severity of the illness, as reported by Jensen.[3] Moreover, all these reactions were observed to occur in inverse proportion to the degree of paralysis in the subject.

Two other tests were made, with notable results, on monkeys inoculated with filtrates of highly potent virus. Daily intradermal injection of saline solution of the euglobin fraction of poliomyelitis antistreptococcal serum caused immediate erythema and edema of the skin. A precipitate occurred in 24 hours at the interface between clear unconcentrated antistreptococcal serum and the serum of the monkeys obtained daily. Neither test produced results before the monkeys were inoculated or during the period of incubation; the results described were observed on the first day of symptoms and throughout twelve to eighteen days, and could no longer be produced after recovery.[20]

The degree of cutaneous erythema that occurred eighteen to 24 hours after intradermal injection of suspensions of the heat-killed streptococcus was used as a gauge of the susceptibility of humans to poliomyelitis. Little or no erythema was produced in persons who had recovered from poliomyelitis either a short or a long time before. Among persons who had not had the disease, in general the reaction was less in older than in younger subjects. Patients recovering from the disease had successively less pronounced reaction as recovery progressed. Moreover, when well students at a college where an epidemic of poliomyelitis occurred were tested, each a number of times, the degree of reaction diminished sharply during the epidemic and for six weeks thereafter; there was no change in reaction to control injection of heat-killed streptococci isolated in studies of arthritis, and no reduction in reaction to the poliomyelitis streptococcal suspension in students tested as controls at a neighboring college where poliomyelitis had not occurred.[20]

III. *Experimental Production of the Poliomyelitis Virus From Neurotropic Streptococci*

Attempts to reproduce the typical clinical and pathologic features of poliomyelitis including the period of incubation by means of the associated streptococcus resulted in failure, as did attempts to produce the "virus" in vitro and in vivo with various cultures of the streptococcus, until a medium was used which did not become acid from growth of streptococci. This medium consisted of "infantile" tissue—that of 19-day hatching chicken eggs. The eggs, including the shells, were reduced to a mash which was mixed with seven parts of distilled water, infused at 10° C. for 24 hours, placed in tall containers, sterilized in an autoclave and sealed with a film of liquid petrolatum. This medium was found very favorable for the isolation, rapid growth and maintenance of viability of the streptococcus. As the cultures aged, smaller and even submicroscopic filtrable forms developed.

After inoculation of mice and monkeys with the older cultures and the filtrates from them, symptoms and lesions of encephalomyelitis developed. On serial brain-to-brain transmission in mice of a strain highly susceptible to encephalitis virus and resistant to poliomyelitis virus, the virus became extremely potent but remained encephalitic. After numerous transmissions, brain emulsions were prepared from the latest mice in the series and injected into rhesus monkeys of a strain highly resistant to encephalitis virus and highly susceptible to poliomyelitis virus. Encephalopoliomyelitis resulted from this inoculation and also from inoculation with the aged chick-embryo culture and with filtrates of cultures of the streptococcus isolated directly from poliomyelitis virus; but on serial brain-to-brain transmission in the monkeys, the virus became poliomyelitic.[29, 30, 31]

The clinical and pathologic features of the poliomyelitis caused by the experimentally developed virus were indistinguishable from those caused by the natural virus. Monkeys that recovered from poliomyelitis produced with the experimental virus were found to be immune to natural virus, and vice versa; the serum of monkeys convalescent from natural virus neutralized the experimental virus and vice versa. When animals were inoculated with emulsions and filtrates of emulsions of spinal cord of monkeys that had received the experimental virus, the streptococcus appeared in the spinal fluid and

in the lesions in the anterior horns of the spinal cord in the same manner as in infection with the "natural" virus, and could be isolated from spinal cord only by the special methods used for isolation of the natural virus.

Diplococci, varying greatly in size, sometimes grouped in short chains and circles, were seen with the electron microscope at 12,000 diameters in filtrates of both natural and experimentally produced virus without staining or shadowing; after special staining the larger forms were seen with the light microscope at 1300 diameters. It was estimated from the number of particles, ovoid in shape, some diploid and some in short chain formation, that 400 million were present per milliliter of the Berkefeld N filtrates examined. In films of dextrose brain-broth cultures of the streptococcus isolated from poliomyelitis virus, prepared with Gram-saffranin stain and fixed with formalin, extremely minute diplococci, both free and in radial orientation, were seen, but only with the electron microscope.[32] The diplococcal chain formations were more conspicuous in a second study with the electron microscope of filtered highly potent virus (unpublished data).* Proof now appears complete that the virus of poliomyelitis is particulate, spherical or elongated, and grouped in diploid or in short filamentous chain formation, as evidenced in electron micrographs by Loring, Schwerdt and Marton[5] and, most convincingly, by Reagan, Schenck and Brueckner.[11] The observations of these investigators further strongly suggest that the virus and the streptococcus are related. The recent reports by others of electron micrographs of particles of various sizes, spherical or ovoid and in diploid or short chain formation, in the viruses of herpes zoster, mumps, encephalitis and influenza—the treatment of which remains an unsolved problem—may be taken to indicate that the viruses of these diseases may likewise be related to the respective specific streptococci which the author has isolated by special methods in these diseases and with which the lesions characteristic of the diseases have been reproduced or closely simulated.

IV. *Protection of Monkeys Against Virus and the Serum Treatment of Epidemic Poliomyelitis*

Poliomyelitis, occurring in epidemics or experimentally produced in animals, has been treated in a large number of cases with the antistreptococcal serum prepared in horses. A total of 282 rhesus monkeys were actively immunized with the serum, subjected to tests which gave presumptive evidence that the virus would be neutralized, and then inoculated with poliomyelitis virus. Of these monkeys 103 or 36 per cent died of the disease. In sharp contrast, of 293 normal control monkeys also inoculated, 240 or 82 per cent died of the disease.[20] The serum was made available for treatment of patients with poliomyelitis by Eli Lilly and Company and was distributed to physicians working independently. Of 710 patients treated with the serum, 60 or 8 per cent died. During the period of this trial, 2,737 other cases (in which the serum was not used) were reported as occurring in different epidemics under similar conditions, and 583 or 21 per cent of the patients died.

Cases in which the author and co-workers used the antistreptococcal serum were divided into three groups according to the length of time that had elapsed from onset of disease to beginning of treatment. (The diagnosis was established by studies of spinal fluid in all cases in which paralysis had not already occurred and in nearly all other cases.) The results were as follows: Of 487 patients who received the first injection of serum before onset of paralysis, 16 or 3 per cent died, and of 460 of the remainder who were adequately followed, only 9 or 2 per cent had severe residual paralysis. Of 696 patients who had slight or moderate paralysis at the time of the first serum treatment, 42 or 6 per cent died, and of 621 of the rest of them who were followed, 20 or 3 per cent had severe residual paralysis. Of 771 who were severely paralyzed before the first serum treatment, 134 or 17 per cent died and of 635 of the remaining patients who were followed, 150 or 24 per cent had severe residual paralysis. Of the total number of patients in these three groups, 10 per cent died and 10 per cent had severe residual paralysis. Of the control patients who did not receive the serum, 25 per cent died and 33 per cent had severe residual paralysis. Equally favorable results were independently obtained in 1917 by Nuzum and Willy[9] in the treatment of epidemic poliomyelitis with antistreptococcal serum prepared in the horse.

The antistreptococcal serum is not now available; it deteriorates rapidly in storage, and outbreaks of the disease are seasonal. However, studies on the production in vitro of antibody from streptococci and other bacteria [24, 25] have resulted in the development of non-sensitizing and more stable solutions of heat-treated antibody from streptococci isolated in studies of a number of diseases including epidemic poliomyelitis. In such solutions the antibody prepared from the streptococcus of poliomyelitis has neutralized with significant frequency a virus potent in the mouse;[26] it specifically agglutinates the streptococcus in extremely high titer and notably accelerates the destruction of the streptococcus on intraperitoneal injection into mice.[24] Subcutaneous or intramuscular injection of this antibody in therapeutic amounts in persons with poliomyelitis causes abrupt diminution of antigen and increase in antibody (as determined by reaction to intradermal injections of antibody and antigen);[20] the treatment appears to prevent paralysis and otherwise affect favorably the clinical course of the disease[26, 33, 10, 12] and, prophylactically used, to prevent transmission within family groups.

CONCLUSIONS

On the basis of the facts reviewed in this presentation, it is concluded that epidemic poliomyelitis is due to infection by a specific streptococcus

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which in the "virus" phase becomes minute and filtrable and perhaps thus penetrates the blood-brain barrier to invade the central nervous system from the primary site of infection in the nasopharynx or the intestinal tract. During or after the filtrable phase, the organism reverts to streptococcal size in which it is cultivable, toxicogenic and causative of lesions, fever, tremors and paralysis. During the subsequent course of the disease both the viral and the coccal forms propagate in parallel in varying proportions and are virtually inseparable even in filtrates of highly potent "virus."

Immunity following the course of the disease would seem to be due mainly to the effects of the large, cultivable form of the organism in its toxicogenic-antigenic phase. With serum containing the organism in this phase, monkeys have been immunized against the effects of the virus; antistreptococcal sera and antibody have been prepared with the streptococcus, although these preparations could not be made with the minute, filtrable form.

That the large cultivable streptococcus is also the form of the organism in primary infection is evidenced by the facts that (1) the "viral" form cannot propagate except in or on the susceptible living cells of a susceptible host, as has been shown in studies; (2) the virus has been produced experimentally from neurotropic streptococci; and (3) the streptococcal flora indigenous in man and in animals tend to become neurotropic in summer in temperate climates.

The use in adequate dosage of the non-toxic, non-sensitizing heat-produced antibody prepared from the specific type of streptococcus whose specificity was maintained is strongly indicated for the treatment of epidemic poliomyelitis. This material can readily be prepared, by methods previously described, from the streptococcus as isolated from the nasopharynx of persons who have the disease during epidemics.

Longview Hospital, Cincinnati, Ohio.

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