seen in humans who have received these emulsions. 3. The possible causes of this phenomenon and its possible relationship to the "pyrogenic" effects of intravenous fat are discussed.

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Production of Group A Streptococcal Cervical Lymphadenitis in Mice.*† (20032)

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(Introduced by C. G. Harford.)
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The fact that hemolytic streptococcal infections are implicated in the causation of rheumatic fever is well documented(1-3). Further, there is much evidence to suggest that rheumatic fever occurs as a result of an inappropriate immune response on the part of the host to the streptococcus or its products(4,5). Nevertheless, the pathogenesis of rheumatic fever remains obscure, its elucidation being seriously handicapped by the lack of a suitable experimental analog. The development, therefore, of an affection in experimental animals, bearing sufficient resemblance to rheumatic fever in man to satisfy the most stringent criteria, might well constitute a significant advance toward definition of the mechanism by which the human disease arises. One avenue of attack on this problem is represented by the attempt to simulate in experimental animals a sequence of events comparable to that which is assigned importance in human rheumatic fever; namely, repeated infections of the upper respiratory tract with group A streptococci(6). In order to pursue this par-

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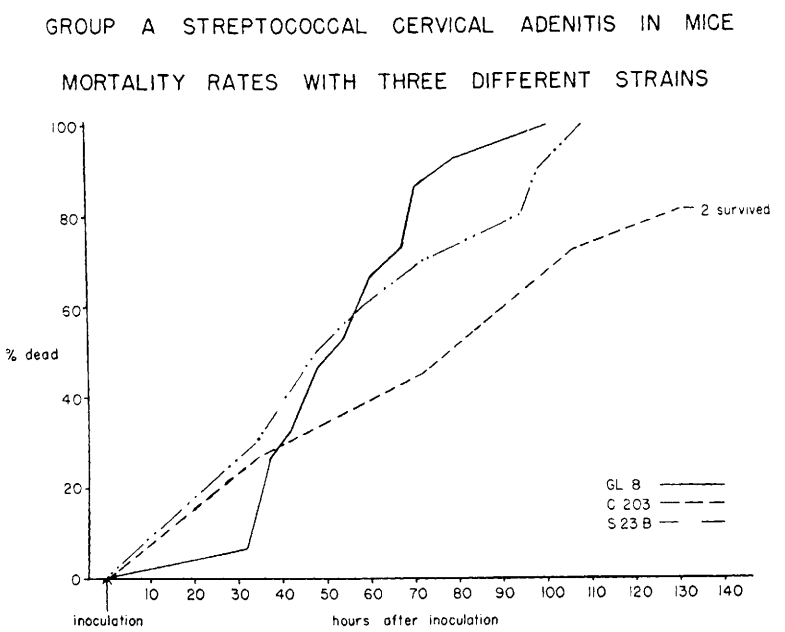


FIG. 1. Mortality curves for mice with cervical lymphadenitis. Ten animals were infected with S23B, eleven with C203 and fifteen with GL-8 (IGL-23).

ticular investigative approach, it is essential to develop a method by which group A streptococcal infection can be achieved via the upper respiratory route in an experimental animal.

The present paper describes the successful production of group A cervical lymphadenitis in mice by means of a modification of the intranasal technic described by Sonkin(7). In addition, the pathogenesis of the infection is delineated on the basis of systematic histologic observations.

Methods. Swiss mice of the Webster strain, weighing between 15 and 20 g, were injected intraperitoneally with 0.3 ml of a 2.5% solution of chloral hydrate, and then allowed to inhale ether until deeply anesthetized. Group A streptococci (strains C203, GL-8(IGL-23) or S23B)§ were grown in beef infusion broth containing 10% sheep serum. A 4-hour culture was centrifuged in the cold at 3000 RPM for 30 minutes, the supernate removed, and the organisms resuspended in gelatin-Locke solution. Intranasal inoculation was performed by introducing in turn, into each nostril, a blunt, smooth 27 gauge needle attached to a 0.25 ml syringe and delivering 0.01 ml of bacterial suspension.‖ The mice were allowed to recover from the anesthesia and were kept in groups of 5 or 6 in suitable cages. Several experiments were performed in which animals were inoculated with a given strain of streptococcus and observed until death, so that mortality curves could be computed. Other experiments were done in which mice were killed at given intervals, in order that systematic gross and microscopic observations could be made. In these pathogenesis studies three or more mice were sacrificed at the times indicated. The affected nodes were removed immediately, fixed in Zenker-formol solution, sectioned and stained by the Giemsa technic. Heart's blood was used for culture and streaked on blood agar poured plates.

Mortality. With the technics employed the infection induced was almost always fatal

§ All 3 strains were received from investigators at the Hospital of the Rockefeller Institute as follows: C203 from Dr. Rebecca Lancefield in 1943, GL-8(IGL-23) from Dr. Maclyn McCarty in 1947, and S23B from Dr. Sidney Rothbard in 1948.

‖ Tenfold dilutions of the resuspended culture were made, and blood agar plates were poured with 1.0 ml aliquots of the 10^{-6}, 10^{-7}, 10^{-8} dilutions. The inoculum was calculated to contain approximately 5 x 10^6 chains.

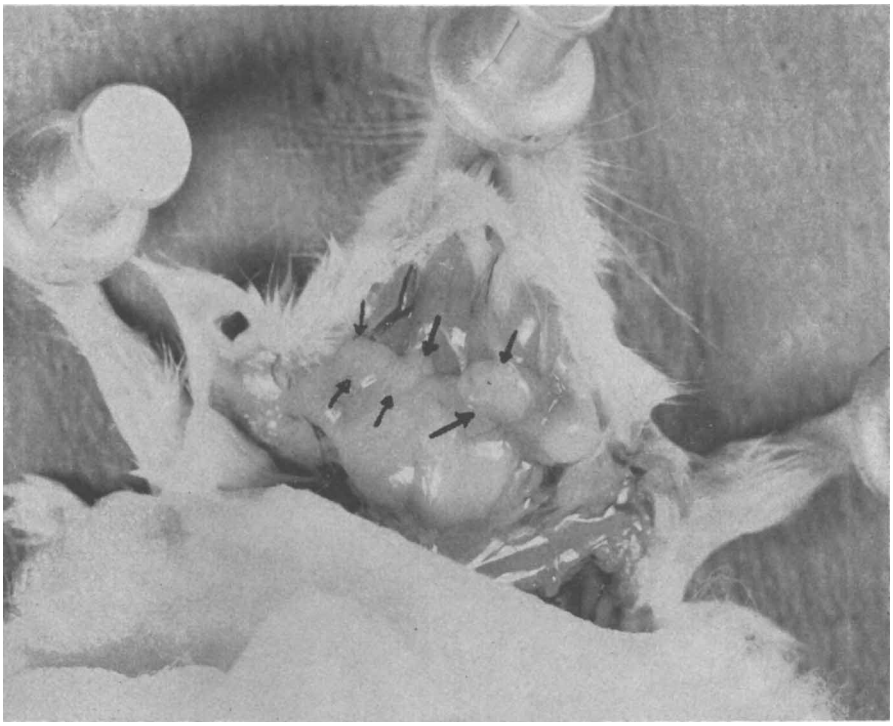


FIG. 2. Gross appearance of involved cervical lymph nodes (arrows). Animals sacrificed forty-eight hr after intranasal inoculation.

within 120 hours. In Fig. 1 composite data from three mortality experiments, employing 10, 11, and 15 mice respectively, are shown.

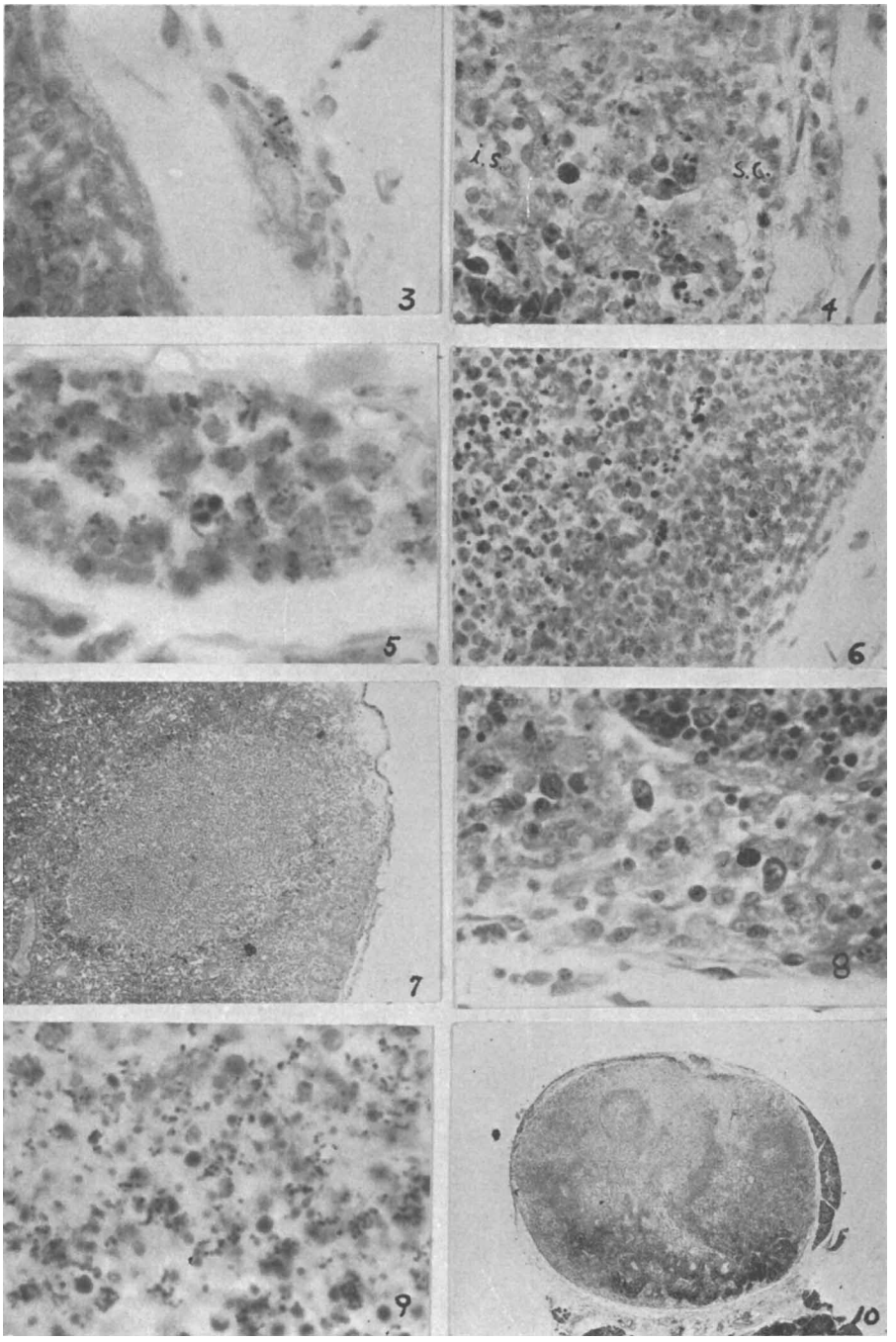
Gross observations. In almost all instances the superficial cervical lymph nodes were primarily affected, often symmetrically. In 12 to 20 hours after inoculation the nodes were enlarged, although maximum increase in size usually occurred between 24 and 48 hours. The degree of enlargement was variable, but often the infected nodes were 2 to 4 times normal size (Fig. 2). Swelling of the nodes was visible, and they could be palpated easily through the intact skin. The nodes of animals dying of the infection were frequently friable, and in some instances frank abscess formation was apparent. Extension of the inflammatory process to the contiguous tissues or to the lungs did not occur.

Microscopic observations. Some variation was encountered in the histologic appearance of nodes taken from animals sacrificed at the same interval after inoculation. These observations, therefore, constitute a summary of the usual pattern of changes found at a given time.

No alteration was found in the nodes 6 hours after inoculation, although streptococci were found within lymphatic vessels in the capsule (Fig. 3). These channels presumably represented the afferent pathway by which the organisms reached the node. Within 12 hours after inoculation bacteria had reached the subcapsular sinus, and were entering the intermediary sinuses. A polymorphonuclear exudate was present, and there was active phagocytosis of streptococci by the leucocytes (Fig. 4).

Twenty hours after inoculation, the organisms in the subcapsular sinus were often completely phagocyted by the neutrophilic leucocytes (Fig. 5). The bacteria had advanced farther into the substance of the node, where a leucocytic response was provoked (Fig. 6).

Streptococcal invasion and polymorphonuclear leucocytic exudation often involved much of the node within 30 hours, and markedly altered its architectural pattern. In some



Photomicrographs of sections of cervical lymph nodes from mice inoculated intranasally with Group A streptococci. The sections were stained by the Giemsa technic.

FIG. 3. Streptococci in a capsular lymphatic of a cervical lymph node six hr after intranasal inoculation. × 667.

FIG. 4. Bacteria and polymorphonuclear leucocytes in the subcapsular (s.c.) and intermediary (i.s.) sinuses 12 hr after induction of infection. Phagocytosis of streptococci by leucocytes is already apparent. × 400.

FIG. 5. Phagocytosis of streptococci in the subcapsular sinus by neutrophilic leucocytes 20 hr after inoculation. × 1133.

FIG. 6. Section of the same node shown in Fig. 5. Although the bacteria in the capsule have been phagocyted, other organisms have invaded the substance of the node, and polymorphonuclear leucocytes have appeared in the newly involved areas. × 400.

FIG. 7. Replacement of a portion of a node by an abscess containing neutrophilic leucocytes and bacteria 30 hr after onset of infection. × 67.

FIG. 8. Macrophages in an area in which the active infection has been controlled 48 hr after its initiation. × 400.

FIG. 9. Large numbers of streptococci in another portion of the same node shown in Fig. 8. Here the cellular defenses have lagged. × 1020.

FIG. 10. Low power view of the node from which the sections in Fig. 8 and 9 were taken. Note the destruction of the architectural pattern. × 18.

areas abscess formation was noted (Fig. 7).

Where the infection was controlled, as evidenced by the disappearance of bacteria and neutrophilic leucocytes (Fig. 8), macrophages predominated. Usually such sites were in the periphery where the acute changes first occurred. Frequently within the same node, however, the presence of great numbers of streptococci, outside the phagocytes, indicated that the infectious process was uncontrolled (Fig. 9), a fact further substantiated by the destruction of nodal tissue (Fig. 10). *Bacteriologic findings.* Although in the pathogenesis studies bacteremia was demonstrated to have occurred as early as 12 hours after inoculation, blood stream invasion usually began 18 to 24 hours after induction of infection. Terminally all the animals had positive blood cultures.

Discussion. Laboratory animals are not naturally subject to infections with group A streptococci. Although it has been known for many years that rabbits are susceptible to hemolytic streptococcal skin infections(8,9), it has recently been demonstrated that such infections may be induced by group A strains (10). The production of pneumonic infections in rats with group A streptococci has now also been reported(11). Previous attempts to cause upper respiratory infections in small laboratory animals with group A organisms have been unsatisfactory(12), but this study indicates that cervical lymphadenitis in mice can be induced consistently with group A strains by a modification of the technic previously described by Sonkin(7). Despite the fact that the method employed in the present study is less accurate in regard to the inoculum delivered than in Sonkin's, it is nonetheless adequate for the production of infections of reproducible virulence. The data presented in Fig. 1, compiled from mortality studies with 3 different strains of streptococci, as well as unpublished data for mortality curves for the same strain used in various experiments, support this conclusion.

The inocula employed in this study caused a rapidly progressing fatal infection. In order to study the response to a non-fatal infection, or to evaluate the effects of repeated infections, modification of the inoculum will be necessary unless an effective chemotherapeutic agent, such as penicillin, is to be used.

Intranasal inoculation of mice with streptococci consistently results in grossly detectable lymph node involvement. Symmetrical lymphadenitis, however, is not achieved with uniformity, a fact which limits to some extent the systematic microscopic study of the infection. This situation differs significantly, therefore, from that encountered by Smith and Wood in their study of type I pneumococcal popliteal lymphadenitis in the rat(13), where a single node drained the site of inoculation. Nonetheless, the pathogenesis of group A streptococcal lymphadenitis in mice and of type I pneumococcal lymphadenitis in rats is similar in the following respects: 1) earliest involvement occurs in the subcapsular sinus; 2) polymorphonuclear leucocytes appear promptly in the involved areas and persist in the portions of the node in which bacteria are present; 3) phagocytosis of bacteria by neutrophilic leucocytes¶ becomes ap-

¶ Because this phenomenon occurs within 12 hours after induction of the infection—a period too brief to allow for the appearance of circulating antibody (14)—it is considered to represent another example of the important host defense mechanism, surface phagocytosis(15).

parent as soon as the polymorphonuclear cells appear in the infected areas; 4) as the inflammatory process subsides, macrophages, which herald the onset of the repair process, become the predominant cell in the involved areas; and 5) fibrin formation does not occur.

In contrast to Smith and Wood's observations with type I pneumococci, the following features were noted in this study: 1) there is destruction of nodal architecture by the infectious process with formation of frank abscesses;** and 2) streptococci persist in the involved nodes for many hours. It should be pointed out that in these regards type III pneumococcal infections bear more similarity to group A streptococcal infections than do those produced by type I pneumococci (11,17).

That the infection was confined initially to the lymph nodes was evidenced by the fact that bacteremia usually did not occur until 18 or more hours after the animals were inoculated. Terminally blood stream invasion was encountered in all instances. Examination of contiguous structures and of the lungs failed to show streptococci outside the involved nodes, even in the most fulminating infections. This finding contrasts with that report by Sonkin when group C streptococci were used. With the latter organism, under the conditions employed, cellulitis of the areas surrounding the involved nodes was common.

Summary and conclusions. The study described herewith affords a demonstration of the fact that a small laboratory animal can be infected via the upper respiratory tract with group A streptococci by a simple reproducible method. The lymphadenitis resulting from group A streptococcal infection bears significant resemblance in its histologic pattern to that produced by pneumococci, particularly by type III strains.

** The necrotizing property of group A streptococci(16), a property not common to pneumococcus, probably explains this particular difference.

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Adrenal Weight and Ascorbic Acid Concentration in Alloxan-Injected Rats. (20033)

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A reduction in adrenal ascorbic acid concentration and/or an increase in wet or dry weight of the adrenal glands are commonly regarded as a good index of increased adrenocortical activity. However, the relationship of adrenal ascorbic acid concentration to adrenal steroid production is still a controversial subject(1-3). Though the literature intimates