EXPERIMENTAL STREPTOBACILLUS MONILIFORMIS ARTHRITIS IN THE CHICK EMBRYO*

By G. JOHN BUDDINGH, M.D.

(From the Department of Pathology, Vanderbilt University School of Medicine, Nashville)

PLATES 3 AND 4

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Studies on the pathology and pathogenesis of the lesions which develop in rat bite fever caused by the *Streptobacillus moniliformis* have been confined to observations on the spontaneous or experimentally induced infections in rats and mice and occasional fatal cases of the disease in man. The history and literature concerning rat bite fever has recently been comprehensively reviewed by Brown and Nunemaker (1). They also presented a detailed account of the clinical characteristics of the disease in man and included important observations of their own on the bacteriology of *Streptobacillus moniliformis* and the experimental infection in mice. A review of the extensive literature on this subject will not be undertaken in the present report.

Van Rooyen (2) has demonstrated that the *Streptobacillus moniliformis* can be propagated in the chorio-allantois of the chick embryo. He did not extend his observations to a study of the membranal reaction or to a search for lesions in the embryo. An opportunity for investigating the experimental disease in chick embryos recently arose when a strain of *Streptobacillus moniliformis* was isolated by blood culture from a patient with rat bite fever. The results of these observations will be presented in this report.

**EXPERIMENTAL**

Source and Characteristics of the Strain of *Streptobacillus moniliformis* Used.—A 59 year old white woman was admitted to the Medical Service of Vanderbilt Hospital with a history of having been bitten on the upper lip by a rat 10 days previously. 4 days following the rat bite malaise, anorexia, chills, and fever developed. During the next 4 days the terminal joints of the little and index fingers of the left hand, the left elbow, and the right knee joint became painful, hot, and swollen. A maculopapular rash was noted over the soles of the feet, ankles, thighs, and the affected fingers. The entire course of her illness could not be followed because she left the hospital after 23 days. She remained febrile during this period, her temperature ranging from 99 to 103.4° with a daily maximum rise in the afternoon. The affected joints began to improve slowly during the last few days she was under observation. The *Streptobacillus moniliformis* was isolated on four separate occasions from the blood stream during the 1st 2 weeks she was in the hospital. A blood culture made during the 3rd week was sterile. Culture of fluid aspirated from the knee joint during the 2nd week was positive for the streptobacillus.

After 4 days of incubation at 37°C. in plain infusion broth, the admission blood culture

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showed the presence of "fluff balls." Smears stained by Wayson's method as recommended by Brown and Nunemaker revealed the typically pleomorphic streptobacilli in pure culture (Fig. 2). Subcultures of the microorganism were easily maintained in infusion broth to which sterile ascitic fluid (about 30 per cent by volume) was added as suggested by Brown and Nunemaker. The gross characteristics of this type of culture are pictured in Fig. 1. On infusion agar containing 30 per cent ascitic fluid small semitranslucent colonies which had a granular surface and irregular "fuzzy" edges developed in 24 to 48 hours (Fig. 3).

Infection of Chick Embryos.—By means of a capillary pipette a small drop from a 24 hour ascitic fluid broth culture was transferred to the exposed chorio-allantois of 12 and 14 day old chick embryos. The "cover slip" technique was used. Of 20 embryos inoculated on the 12th day of incubation 18 survived 24 hours, 3 survived 48 hours. None lived to 72 hours following inoculation. Of 26 embryos inoculated on the 14th day of incubation 25 survived 24 hours, 19 survived 48 hours, and 9 survived 72 hours. None was alive at 96 hours.

At 24, 48, and 72 hour intervals following inoculation living embryos and the chorio-allantois were fixed in Zenker's solution. Before fixation smears and cultures were made from the exudate on the chorio-allantois and from the heart blood of the embryos. Blocks from the membranes and in toto cross-section blocks from the embryos were embedded in paraffin. Sections were stained as routine with hematoxylin and eosin. Those showing lesions were also stained with phloxine and methylene blue and by Goodpasture's carbol-aniline-fuchsin method for better differentiation of the microorganism.

OBSERVATIONS

Smears and Cultures from the Chorio-Allantois and the Embryo Heart Blood.—At the 24, 48, and 72 hour intervals following inoculation smears of the exudate on the chorio-allantois (Fig. 4) stained by Wayson's method revealed streptobacilli in abundance. The microorganisms exhibited extreme pleomorphism. At 24 hours numerous extremely long filaments of irregular width and staining capacity predominated over the more definitely short slender bacillary forms (Fig. 5). Numerous red blood cells, a few mononuclears, and an occasional polymorphonuclear were present. At 48 hours the filaments presented a ragged appearance, bead-like swellings were prominent in the bacillary forms, and numerous granules of irregular shape and size were encountered. The number of red blood cells and inflammatory cells had diminished. At 72 hours the filaments had largely disappeared; swollen irregular bacillary forms were numerous, and the granular forms appeared to predominate although a few well staining rods were still present. The inflammatory cells had almost entirely disappeared. Cultures made from the exudate at the intervals stated were positive in every instance. Films of the embryo heart blood at these intervals stained by Wayson's method and Wright's stain did not conclusively demonstrate the presence of the microorganism. Occasional slender rod-like forms were discerned but they could not be definitely excluded as artifacts. The cultures made at each of these periods were positive for the streptobacillus.

Microscopic Sections. The Chorio-Allantois.—At 24 hours (Fig. 6) focal accumulations of microorganisms mixed with numerous red blood cells and necrotic inflammatory cells were present on the surface of the membrane. At this period these masses were already assuming an amorphous hyaline-like character. In some areas, ulceration of the ectodermal epithelium had developed. At these points masses of the microorganism extended into the mesoderm and were apparently proliferating in the immediate vicinity of the blood vessels. Destruction of the vessel walls indicated the
manner of invasion of the embryonal circulation. It was difficult to determine the exact morphology of the microorganisms in these situations. They presented a dense, uniformly staining mass composed either of closely packed granules or intricate skeins of irregularly staining filaments. Definite bacillary forms were not observed. Edema of the mesoderm was present and a moderate number of "histiocytes" and fibroblasts had proliferated in the immediately adjacent areas. Extensive extravasation of red blood cells occurred where vessel walls had been destroyed. Walling off, or abscess formation around the masses of microorganisms did not develop. The 48 and 72 hour lesions in the membrane were chiefly characterized by the development of large areas of necrosis of the entire membrane, presumably resulting from thrombosis and destruction of blood vessels. In these areas of necrotic cellular debris dense masses of microorganisms were present. On the surface of the intact membrane hyaline necrotic masses of red cells mixed with degenerating microorganisms were present. Evidence of intracellular growth of the microorganisms within the constituent cells of the membrane was not observed at any of the stages of the reaction.

**The Embryo.**—Cross-sections of the embryos included all the organs, body cavities, and the principal joints. Except for the fact that the 12 day old embryos did not survive longer than 48 hours they did not differ in their reactions from 14 day old embryos. The description of the lesions which developed applies to embryos of either age. Save for two exceptions to be noted later all the lesions in the embryo were confined to the joints.

Embryos sacrificed 24 hours following inoculation of the chorio-allantois revealed the beginning of focal lesions which were confined strictly to the synovial lining of the joints. At one or more points the synovial cells stood out sharply because of their swollen appearance and deeper staining (Figs. 7 and 8). In the immediate vicinity of these areas a few mononuclear cells had wandered into the joint space. Carbolaniline-fuchsin stains of these sections showed these synovial cells to be greatly swollen. Their cytoplasm was distended and filled with densely packed microorganisms. It was difficult to determine whether the intracellular microorganisms were exclusively made up of fine granular forms or consisted of a mixture of fine granules and irregularly staining short granular rods. The microorganisms were not all confined intracellularly; masses were also present within intercellular spaces. Along the outer border of the synovial cells a thin layer of granules and fine rods was also present (Fig. 12). The outstanding character of these lesions was their sharp limitation to the environment in and directly adjacent to the synovial lining of the joint. At no other point in the joint structure was there any localization of microorganisms.

At the 48 hours stage the affected joints showed the involvement of the entire synovial membrane by extension of the proliferating microorganisms within and between the synovial cells (Fig. 9). No extension of the infection into the underlying cartilage or along the periosteum of the bone had taken place. A marked inflammatory exudate, consisting chiefly of mononuclears and a few polymorphonuclears, had accumulated within the joint space. At this stage numerous free bacillary forms of the microorganism were observed to be scattered throughout the exudate (Fig. 10).

The reaction at 72 hours showed a marked increase in the amount of inflammatory cells in the joint space. There was an increase in the destruction of the synovial lining. The necrotic cells and microorganisms formed a thick, coagulated, amor-
phous, hyaline-like layer in which few if any morphological details could be discerned. No microorganisms could be found free among the inflammatory cells. All appeared to be clumped in deep staining, hyaline masses scattered throughout the exudate (Fig. 11). It appeared as if the streptobacilli were no longer proliferating and were rapidly degenerating. The self-limiting nature of the process was further brought out by evidences of regeneration of the synovial lining cells at the edges of the areas of ulceration.

Lesions in the various stages of development just described have been observed in the mandibular, shoulder, hip, and knee joints of embryos infected with the streptobacillus by way of the chorio-allantois. In some embryos only one joint, in others two, and in a few all the movable joints which could be included in the cross-sections were involved. In one instance involvement of one of the intervertebral articulations was noted.

Sections through the liver at the 48 and 72 hour stages showed rather marked dilatation of the sinusoids. At several points small accumulations of mononuclears, polymorphonuclears, red blood cells, and thrombocytes appeared to be attached to the sinusoidal walls. Within and around these cellular clumps a small number of bacillary forms of the streptobacilli were present. There were no evidences of degeneration or necrosis of the neighboring hepatic cells.

One embryo sacrificed at the 72 hour stage presented a small focus of ulceration of the endocardium. At this point a vegetation consisting of a clump of inflammatory cells and numerous streptobacilli had formed. No involvement of the underlying myocardium was noted.

**DISCUSSION**

It is generally agreed that the most common lesion or complication in human rat bite fever due to *Streptobacillus moniliformis* is an acute purulent arthritis. Involvement of the joints is also one of the outstanding features in the spontaneous and experimental infection of rats and mice (1, 3, 4). In the chick embryo an acute disease of short duration, in which a bacteremia or septicemia is most likely the cause of death, develops as a result of the introduction of the streptobacillus onto the chorio-allantois. Nevertheless, within the short period of 72 hours definitely localized pathological lesions confined almost exclusively to the joints develop. This has provided opportunity for a study of the development of the early stages of the pathogenesis of the arthritis which seems to be so characteristic of the disease. The streptobacillus invades the embryonic circulation within a short period after proliferation has begun in the chorio-allantois. Within the embryo the parasite exhibits an adaptation which limits its localization specifically and almost exclusively to the synovial membranes. Its cells and their immediate environment are apparently preeminently suited to the needs of the streptobacillus. The evidence suggests that in the initial stages the microorganism behaves as a facultative intracellular parasite within the cytoplasm of the synovial lining cells. This phase of the infectious process is quite likely of extremely short duration. In the earliest lesions ob-
served, the microorganisms are predominantly intracellular but small numbers
are also found apparently proliferating within the immediately adjacent inter-
cellular spaces. For a relatively short period of time following the establish-
ment of the infection in the synovial lining of the joints there are favorable con-
ditions for the free growth of the microorganisms within the joint fluid and the
fresh inflammatory exudate. With the degeneration of the inflammatory cells
unfavorable conditions prevail and the microorganisms no longer proliferate
but are found in tightly packed clumps, which appear to be undergoing
hyalinization.

It is evident that the arthritis of *Streptobacillus moniliformis* infections de-
velops on the basis of fundamental host cell–parasite relationships in which
environmental and nutritive factors peculiar to the growth of the microorganism
are most advantageously balanced within and in the immediate environment
of the synovial lining of the joints. The balance in favor of the microorganism
is maintained only temporarily thus accounting for the self-limiting nature of
the infectious process.

There is also some evidence from these observations that the presence of the
streptobacillus in the blood stream may have an injurious effect on endothelial
cells. Focal accumulations of clumps of inflammatory cells and streptobacilli
on the walls of the liver sinusoids occur in the infected embryo with some
regularity. This type of lesion has been previously observed and described
in mice by Levaditi (4). In one instance a vegetative endocarditis was en-
countered in the present observations. Blake (5) and Stuart-Harris et al. (6)
have described the occurrence of streptobacillus vegetative endocarditis in man.

Other investigations from this laboratory concerning either experimental
virus or bacterial infections of the chick embryo have placed emphasis not only
on the adaptability of this experimental host for the culture of various patho-
genic microorganisms but also on the fact that it presents a convenient method
for the demonstration and elucidation of host or host cell–parasite relationships
intimately concerned in the pathogenesis of various infectious processes (7, 8).
The exquisitely specific adaptation of the *Streptobacillus moniliformis* to syn-
ovial membranes and their immediate environment as demonstrated in these
studies adds further support to this emphasis.

**SUMMARY**

1. A strain of *Streptobacillus moniliformis* isolated from a case of rat bite
fever in man has been found to produce infection of the developing chick em-
bryo following inoculation of the chorio-allantois.

2. The disease in embryos is characterized by invasion of the blood stream
and an almost exclusive localization of the infectious process to the synovial
lining of the joints.

3. In the early stages of the development of the joint lesions the *Strepto-
_Streptobacillus moniliformis_ behaves as a facultative intracellular parasite within the cytoplasm of the synovial lining cells. Conditions favorable for the growth of the microorganisms are maintained only temporarily. The infection appears to be self-limiting in nature.

**REFERENCES**


**EXPLANATION OF PLATES.**

**PLATE 3**

Fig. 1. Ascitic fluid broth culture of _Streptobacillus moniliformis_, 37°C for 48 hours, showing characteristic "fluff balls." Approximately actual size.

Fig. 2. Smear from 24 hours ascitic fluid broth culture stained by Wayson's method showing extreme pleomorphism and characteristic bead-like swellings. × 1400.

Fig. 3. Colonies on ascitic fluid agar, 48 hours, showing characteristic granular surface and wavy edges. × 30.

Fig. 4. Chorio-allantois of 13 day embryo 24 hours after inoculation. Approximately actual size.

Fig. 5. Smear of exudate from reaction in chorio-allantois 24 hours after inoculation. Note extremely long tangled filaments and occasional fine granules. Wayson's stain. × 1400.

Fig. 6. Chorio-allantois, 13 day embryo. Section of 24 hour reaction. Note masses of exudate on surface. The darker staining mass between the two large veins is composed almost entirely of microorganisms. Phloxine-methylene blue stain. × 140.
(Buddingh: Streptobacillus moniliformis arthritis in chick embryo)
PLATE 4

Fig. 7. Knee joint. 24 hour infection. The dark staining of the synovial lining is due to the presence of the microorganisms. There is beginning inflammatory reaction in the joint space. Carbol-aniline fuchsin stain. × 100.

Fig. 8. Hip joint 48 hour infection. Showing marked proliferation of streptobacilli within and along the synovial membranes. Carbol-aniline fuchsin stain. × 100.

Fig. 9. Shoulder joint 48 hour infection. Showing extensive involvement of the synovial lining and inflammatory reaction within the joint cavity. Carbol-aniline fuchsin stain. × 140.

Fig. 10. Inflammatory exudate within joint cavity. 48 hours. Showing presence of streptobacilli among the inflammatory cells. Carbol-aniline fuchsin stain. × 1800.

Fig. 11. Hip joint. 72 hours. Showing joint spaces packed with purulent exudate. The microorganisms have formed into amorphous hyaline masses along the synovial lining and in dark staining hyaline clumps within the exudate. Carbol-aniline fuchsin stain. × 60.

Fig. 12. High power of synovial lining cells, 24 hour infection showing microorganisms within the cellular cytoplasm and extending out from the cell surface into the joint space. Carbol-aniline fuchsin stain. × 1800.
(Buddingh: *Streptobacillus moniliformis* arthritis in chick embryo)