

THE SIGNIFICANCE OF THE SYNOVIAL VILLUS AND
THE CILIARY PROCESS AS FACTORS IN THE
LOCALIZATION OF BACTERIA IN THE
JOINTS AND EYES OF RABBITS*

BY D. MURRAY ANGEVINE, M.D., AND SIDNEY ROTHBARD, M.D.

(From the Department of Pathology, Cornell University Medical College, New York)

PLATES 9 AND 10

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A metastatic infection in either the joints or eyes is a relatively common complication of various infectious diseases. It occurs frequently in gonorrhea, and less often in tuberculosis, undulant fever, cerebrospinal fever and typhoid fever. Ocular diseases, such as iritis and chronic uveitis, have also been described as complications of rheumatoid arthritis (1) and rheumatic fever (2, 3) although the infectious nature of these diseases is, of course, still open to question.

No adequate reason has been given to explain why the joints and eyes are affected in these conditions. Considerable importance has been attached to the fact that various bacteria have an affinity for certain tissues (4) and although this factor may be of importance, it seems that a more satisfactory explanation is necessary.

Because there is clinical evidence that both of these structures are involved in certain infections, a detailed study was made of the eyes and joints from 367 rabbits infected with various strains of streptococci, as well as with several other types of bacteria. Arthritis developed in many of these animals, and infections in the eye were produced (5). Similar results have been obtained with another group of 112 rabbits. The incidence of experimental lesions in the eyes of rabbits with arthritis was found to be about the same as in those with no arthritis.

Study of numerous sections of joints of rabbits with experimental streptococcus arthritis (6) showed that inflammation appeared first in the synovial layer of the joint capsule and was most conspicuous in the villi. As the inflammation increased, it usually involved the entire synovial membrane. Sections from infected joints were stained for bacteria, and isolated cocci were occasionally seen in the synovial tissue from rabbits with an arthritis of a few hours duration. It was difficult, however, to determine the exact site of localization of the bacteria.

The eyes of animals that received injections of various microorganisms intravenously

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were observed in the gross during life. The most satisfactory method of examination was by means of oblique illumination, using a strong, sharply focussed beam of light and a small hand lens of low magnification. In certain instances an intense injection, which appeared in the iris within a few hours, persisted for a period of from 24 to 48 hours and gradually subsided. Histological examination of the eyes of the animals showed that when infection occurred in the eye, the primary site of localization was usually in the ciliary process or ciliary body. Occasionally the entire uveal tract was involved; however, we seldom were able to demonstrate bacteria in the ciliary processes of these eyes even in appropriately stained sections.

During this study the similarity of the synovial villi of the joints and the ciliary processes of the eye, and the occurrence of the inflammatory processes in both suggested that the villus-like structures were the primary sites of injury.

To corroborate our earlier observations and to gain further insight into the mechanism by which bacteria are localized in the eyes and joints, we first studied the anatomy of the synovial villi and ciliary processes, and then attempted to demonstrate the presence of microorganisms in these structures following intravenous injections of streptococci.

Observations on Joints

The synovial membrane of a normal joint is smooth, glistening and yellow gray in color, and the villi are seen with difficulty. If the joint is submerged in water, the villi float and are more readily visible. They are slightly narrower at the base, frequently branched and are most numerous at the site of ligamentous attachments or at the junction of synovia and cartilage. There is a rich capillary network that is easily seen with a slight magnification. In many areas the synovial lining is arranged in folds which are not considered as true villi.

The joint capsule was removed from many normal rabbits, and sections were prepared. Although small folds and elevations of the synovial lining were frequently present, intact villi were rarely seen. When 1.0 cc. of Zenker-formol fixative was injected into the joint cavity of animals after death and allowed to remain for a few hours, the normal villi were well preserved and could be studied in histological sections. A villus from a normal rabbit is shown in Fig. 1; it is composed of a layer of synovial cells and a few small blood vessels.

The first gross evidence of arthritis produced by the intravenous injection of streptococci is dilatation of the synovial vessels. In the knee joint this is first observed in the infrapatellar region and in the supracondylar fossae, both being sites in which the villi are prominent and numerous. The entire villus becomes edematous, and there is a moderate infiltration of the synovial tissue with polymorphonuclear leucocytes and lymphocytes. Although inflammation is observed elsewhere, the earliest microscopic evidence of inflammation is usually seen in the villi (Fig. 3).

Observations on Eyes

Davis (7) has written an excellent description of the gross and microscopic anatomy of the rabbit's eye. The ciliary body and ciliary processes are conspicuous structures, and when the eye is incised and the vitreous permitted to escape, they are well seen by magnification with the dissecting microscope. On section they appear as single branched processes (Fig. 2), or are attached to one another forming a fenestrated network. The processes are usually longer in the region of the ciliary body, and much shorter near the free margin of the iris. They are composed of a vascular plexus held in a loose stroma of fibrous connective tissue and are covered with a single layer of cuboidal epithelial cells.

Within a few hours after intravenous injection of bacteria that are pathogenic for rabbits, the iris becomes intensely injected. As previously described (5) the following organisms produced this change consistently: *Streptococcus hemolyticus* (strain NY 5), *Staphylococcus aureus*, pneumococcus Type II, *Bacillus coli communior*, *B. typhosus*, *B. paratyphosus* A, *B. dysenteriae* (Flexner), *Brucella abortus*, *Pasteurella leptisepticus* and *Proteus* X 19. This vascular dilatation subsides quickly or persists for several days. The fluid in the anterior chamber frequently becomes opaque. An almost uniform histological picture was found in sections prepared from such an eye. Eosinophilic material, apparently protein, is noted in both the anterior and posterior chambers, the ciliary processes are often enlarged and edematous (Fig. 4), the vessels are dilated, and in many instances there is complete necrosis and desquamation of the ciliary epithelium. The small vessels are often ruptured, and there are red blood cells in the tissues. When the inflammation is not extensive, the epithelium of a ciliary process may be stripped from the underlying tissue to form a vesicle that contains serum, fibrin and occasionally a few polymorphonuclear leucocytes (Fig. 4, insert). Lesions of this type occur frequently, and it is probable that they represent the initial site of injury to the ciliary process.

As inflammation in a joint or in an eye progresses, it is more difficult to determine the primary site of infection. In a few instances, the infection remained localized (Figs. 5 and 6) and a collection of lymphocytes and monocytes can be seen at the base of a synovial villus or in a ciliary process. There is a remarkable similarity in the inflammatory lesions in the two instances.

EXPERIMENTS

From the preceding observations, we concluded that when bacteria injected into the blood stream lodged in the joints or eyes, the synovial villi

and the ciliary processes were usually the sites primarily involved. Although we were occasionally able to demonstrate streptococci in these structures after intravenous injection, it seemed advisable to determine how soon they appeared, and how frequently they localized in other organs.

Method

Albino rabbits that weighed from 1800 to 2400 gm. were injected intravenously with 4.0 cc. of 18 hour broth cultures of relatively avirulent group A hemolytic streptococci, strain H (8) or strain AB 13 (9). They were killed at intervals varying from 3 hours to 5 days. The heart blood was usually cultured. In Experiment 1 the tissues were placed in nutrient broth and incubated in a sterile container for periods of from 3 to 24 hours. In Experiment 2 the joints were first injected with 1.0 cc. of either broth or saline, and together with tissues from other organs, were placed in a similar fluid and incubated at 37°C. for periods of from 12 to 24 hours. In a few instances the animals were killed, placed in the incubator at 37°C. for 18 hours and then autopsied. This was found to be the most satisfactory method.

All joints were injected with 1.0 cc. of Zenker-formol prior to fixation and were opened after about one hour. The same fixative was used for all tissues, which were stained for bacteria by the method of Brown and Brenn (10).

Experiment 1.—20 rabbits were killed at intervals of 3, 6, 18, 24 or 48 hours, and of 3 and 5 days after an intravenous injection of 4.0 cc. of broth culture of hemolytic streptococci. The knee joints and eyes were incubated for periods varying from 3 to 18 hours. Although there was moderate disintegration of some of the tissues following incubation, the anatomical structures were easily recognized. There was evident multiplication of bacteria shown by readily discernible colonies of streptococci in the sections of tissues that had been incubated for periods of 8 hours or longer. These colonies were most conspicuous in tissues that had been incubated for 18 hours, and this period was considered to be the most satisfactory for our purposes. Streptococci were demonstrated in the synovial membrane in 8 of 20 rabbits, but in only one instance were they localized in a synovial villus. Bacteria were present in the vessels of the eyes of 4 rabbits, and in 2 instances they were observed in the ciliary processes. If the tissues from all the animals had been incubated for at least 12 hours, there would have been greater multiplication of bacteria. There was considerable inhibition of growth of streptococci in tissues immersed in saline compared with those in broth. On the other hand, when the tissues were placed in broth, there was frequently a heavy growth of contaminating organisms.

Experiment 2.—To determine how frequently bacteria could be demonstrated in other organs as well as in the eye and joint, 20 rabbits received an injection similar to those in Experiment 1 and were killed after intervals of 4, 8, 24 or 48 hours, or of 3, 4 or 5 days. The following tissues from these animals were incubated at 37°C. for from 12 to 24 hours: joints, eyes, heart, lung, liver, spleen, kidney, femoral bone marrow, and rectus femoris muscle. In a few instances the bone marrow, spleen or liver underwent complete autolysis so that it was impossible to prepare sections. The frequency of the localization of bacteria in the various organs is given in Table I. Streptococci were observed in the joints and eyes more frequently than in any other site with the

exception of the spleen. They were present in the synovial tissue in 18 of 20 rabbits (Table I), and in the vessels of the ciliary processes or choroid in 13.

TABLE I
Distribution of Streptococci in Various Tissues Following Incubation at 37°C. for Intervals of from 12 to 24 Hours

Time killed after injection	Number of rabbits	Joint	Spleen*	Eye	Bone marrow*	Lung	Liver*	Kidney	Heart	Muscle
<i>hrs.</i>										
3-8	4	4	4	2	2	4	1	1	0	1
24-48	11	10	9	10	7	6	7	6	6	3
72-120	5	4	2	1	0	0	1	2	1	0
Total.....	20	18	15	13	9	10	9	9	7	4
Per cent.....		90.0	83.3	65.0	56.0	50.0	50.0	45.0	35.0	20.0

* Tissues that underwent autolysis during incubation: spleen, 2; bone marrow, 4; liver, 2.

TABLE II
Incidence of Localization of Streptococci in Joints and Eyes in Relation to Time after Injection

Time killed after injection	Number of rabbits	Localization of streptococci			
		Joints	Synovial villus	Eyes	Ciliary process
<i>hrs.</i>					
3	2	2	0	2	0
4	2	2	0	2	0
6	2	2	0	0	0
8	2	2	0	0	0
18	3	1	0	0	0
24	14	6	2	7	3
48	7	6	2	5	1
<i>days</i>					
3	2	1	0	0	0
4	2	2	0	1	1
5	4	2	0	0	0
Total.....	40	26	4	17	5

COMMENT

The results of these two experiments (Table II) show that streptococci injected into the blood stream appeared in the joints of 26 of 40 rabbits and were demonstrated in the synovial villi of 4. They were observed in

the eyes of 17 of 40 animals, in 5 of which they were in the ciliary processes. Bacteria were observed (Table II) most frequently in the villi and ciliary processes of animals killed 24 or 48 hours after injection when arthritis usually appears. Of the animals killed at this time, there was localization of streptococci in the synovial villi in 4 of 12 joints in which bacteria were demonstrated, and in the ciliary processes in 4 of 12 eyes.

These figures would have been higher had all the tissues been incubated for more than 12 hours. Of the 21 rabbits killed at 24 or 48 hours after injection, streptococci were present in sections of only 2 of 10 synovial tissues that had been incubated for less than 12 hours, whereas they were present in 10 of 11 synovial tissues that had been incubated for 12 or more hours. The period of incubation bore a similar relationship to the multiplication of bacteria in the eyes (Table III).

TABLE III

Effect of Time of Incubation on the Demonstration of Streptococci in the Joints and Eyes of Rabbits Killed 24 or 48 Hours after Injection

Time of incubation <i>hrs.</i>	Number		
	Rabbits	Joints	Eyes
3-7	10	2	2
12-24	11	10	10

We believe that these results are significant because they are in accord with our earlier observations that the synovial villi and ciliary processes are the primary sites of damage when infections are observed in either the joint or the eye.

A few animals, not included in the experiments described, received intravenous injections of more virulent strains of hemolytic streptococci. Many colonies of bacteria were present in the vessels of the synovial villi (Fig. 7) of one of these that died with bacteremia. This section demonstrates very well the vascularity of a synovial villus.

It is noteworthy that streptococci were present in the myocardium of 7 of 20 rabbits that were incubated after death, and in many instances in the blood clot within the chambers of the heart. Sections were made through the aortic leaflet of the mitral valve of all the animals, but in no instance were bacteria seen in the tissues of the valve.

CONCLUSIONS

1. Histological examination of sections of the eyes and joints of large numbers of rabbits injected with hemolytic streptococci has clearly demon-

strated that when arthritis or cyclitis occurs, the synovial villi and ciliary processes are the most frequent and usually the primary sites of inflammation.

2. By special methods for demonstration of bacteria, it has been shown that bacteria which found lodgement in either an eye or a joint were demonstrable first in the vessels of ciliary processes or synovial villi.

3. A localized synovitis or iridocyclitis is brought about by the localization of bacteria in the synovial villus and ciliary process.

4. These experiments, which give a clearer insight into the pathogenesis of infectious arthritis and iritis, explain why both may occur in association with certain infectious diseases.

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EXPLANATION OF PLATES

PLATE 9

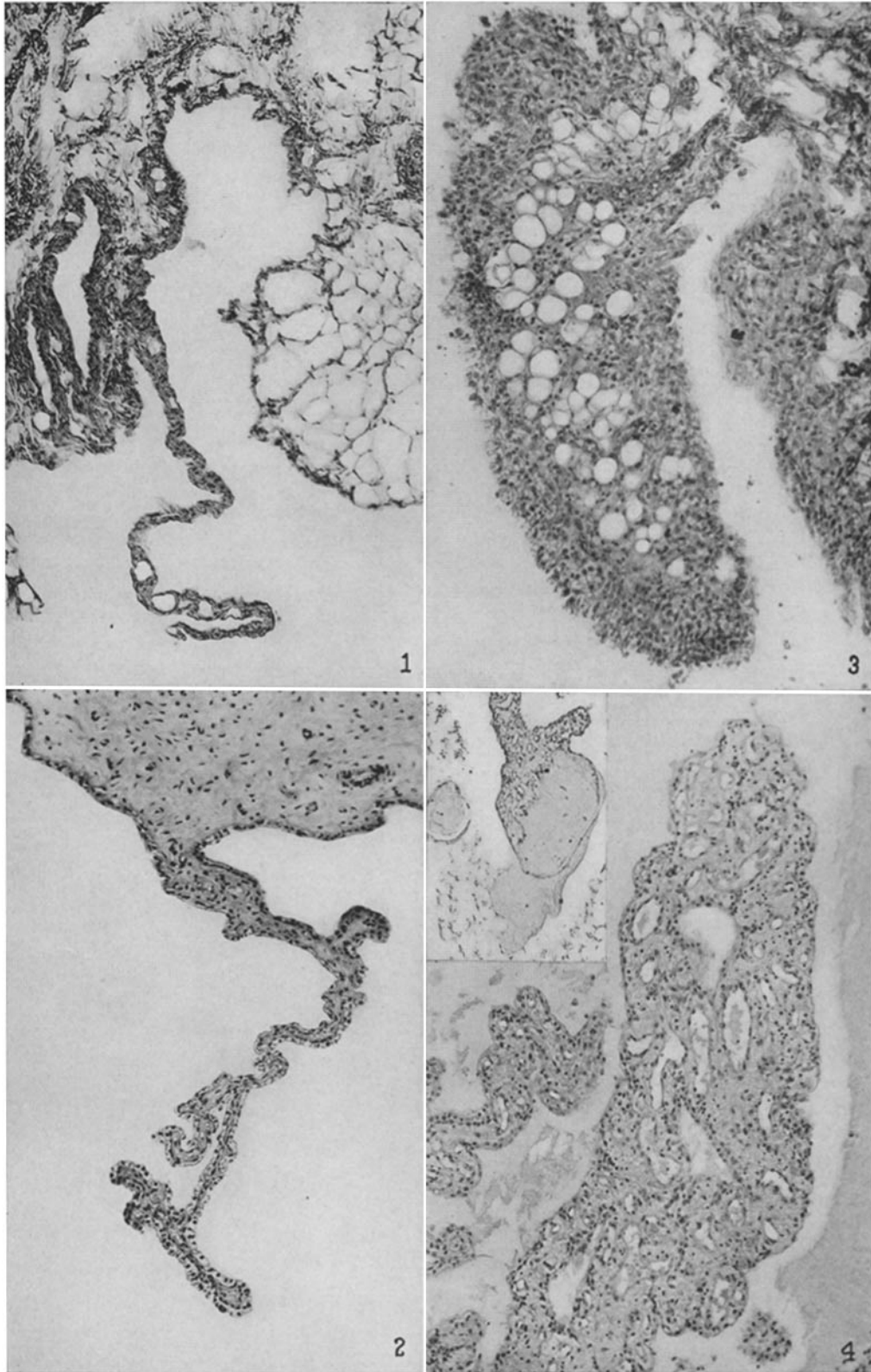
FIG. 1. A section of synovia from the knee joint of a normal rabbit to illustrate the villous structure. Hematoxylin and eosin. $\times 90$.

FIG. 2. A section from the eye of a normal rabbit to illustrate a ciliary process. Hematoxylin and eosin. $\times 96$.

FIG. 3. A section from the knee joint of rabbit 8-74 to show early inflammation in a synovial villus. The animal received 1.0, 2.0 and 2.0 cc. of a broth culture of *Streptococcus hemolyticus* (strain H) intravenously on 3 successive days and was killed a few hours after the last injection. Hematoxylin and eosin. $\times 120$.

FIG. 4. To illustrate an early cyclitis characterized chiefly by edema of the ciliary process. The rabbit (No. 9-79) had been sensitized by repeated intradermal injections of heat-killed hemolytic streptococci. Following an intravenous injection of 2.0 cc. of living culture the iris became injected, and there was clouding of the anterior chamber. The animal died 8 days following this injection. Hematoxylin and eosin. $\times 96$.

The insert on the left upper corner illustrates a vesicle on the free end of a ciliary process. It is composed of fibrin and a few polymorphonuclear leucocytes. The rabbit (No. 8-10) had received 4.0 cc. of a broth culture of hemolytic streptococci and died within 24 hours. Hematoxylin and eosin. $\times 70$.



(Angevine and Rothbard: Localization of bacteria in joints and eyes)

PLATE 10

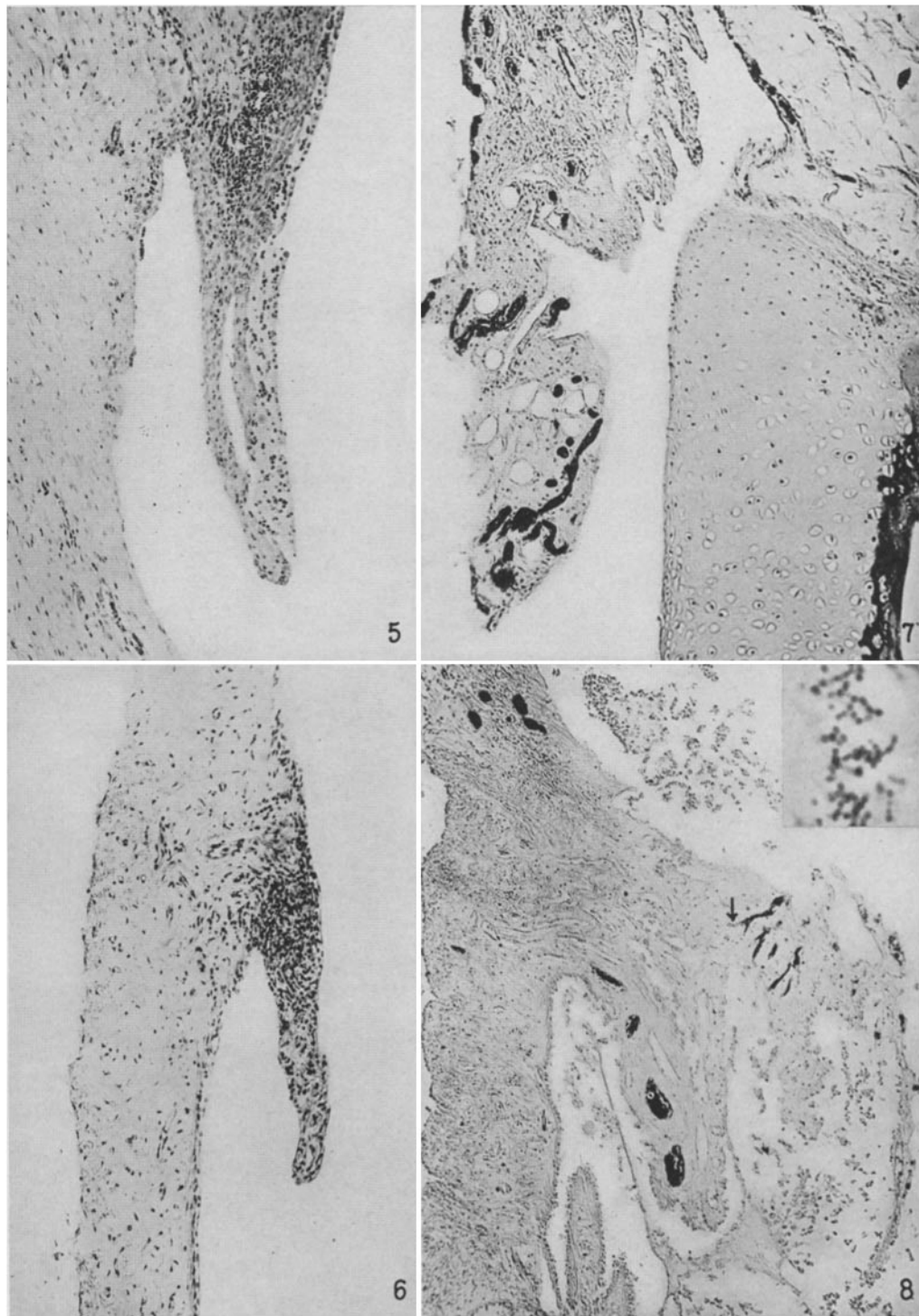
FIG. 5. A section of a synovial villus to show an early subacute arthritis from the same rabbit described in Fig. 4. Part of the region occupied by synovial fluid has been opaqued for the sake of comparison with Fig. 6. Hematoxylin and eosin. $\times 90$.

FIG. 6. A section through the iris and ciliary body of rabbit 10-86 to illustrate an area of inflammation at the base of a ciliary process. The animal received an injection of 4.0 cc. of hemolytic streptococci (strain AB 13) into the right Fallopian tube and was killed after 28 days. Hematoxylin and eosin. $\times 90$.

FIG. 7. Colonies of streptococci in the vessels of a synovial villus of a knee joint of rabbit 9-22 that received an intravenous injection of 2.0 cc. of hemolytic streptococci and died after 6 days. Stained by the method of Brown and Brenn. $\times 90$.

FIG. 8. Colonies of bacteria in the vessels of the ciliary body and ciliary processes of rabbit 8-08 that received 4.0 cc. of hemolytic streptococci (strain H) intravenously and died 3 hours later. $\times 70$.

Insert in upper right hand corner shows streptococci in area denoted by arrow. Stained by the method of Brown and Brenn. $\times 1320$.



(Angevine and Rothbard: Localization of bacteria in joints and eyes)