

A STUDY OF EXPERIMENTAL NON-HEMOLYTIC STREPTOCOCCUS LESIONS IN VITALLY STAINED RABBITS.

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Within the last 15 years much has been written about the streptococcus in rheumatic fever, and emphasis has been laid on the bacteriological rather than on the pathological aspects of the disease. The reasons for this were twofold: (1) the low mortality in rheumatic fever made the material for pathological study scanty; (2) the frequently reported occurrence of streptococci in the various lesions of rheumatic fever cases stimulated so much interest in the bacteriology of the disease that other phases of the problem have been neglected.

HISTORICAL.

The most important recent contribution to the pathology of rheumatic fever is that of Aschoff (1), who in 1904 described certain small collections of cells in the myocardium which he considered characteristic of rheumatic fever. Aschoff spoke of them as "submiliary nodules." They are usually found in the neighborhood of the smaller arterioles and consist of a collection of unusually large cells with one or more large irregularly shaped nuclei. These giant cells, according to Aschoff, are often arranged like a rosette about a center of necrotic confluent cellular protoplasm. Just outside the giant cells, which resemble somewhat those seen in Hodgkin's disease, there is a peripheral zone of leukocytes, in which lymphocytes predominate. The peripheral cells form streaks or out-shoots into the connective tissue septa. In these streaks are also found the adventitia or wandering cells which appear in all inflammatory processes. They arise from the vessels and it is from them, Aschoff thinks, that the giant cells develop.

In the following year Geipel (2) studied five cases of rheumatic myocarditis. His description of the submiliary nodule agrees with Aschoff's, but he disagrees with Aschoff as to the origin of the giant cells, for he believes that they arise from connective tissue. According to Geipel, the connective tissue cells swell

and in some instances fuse together to form the multinucleated giant cells. Both Aschoff and Geipel describe the gradual conversion of the submiliary nodule into a patch of fibrous tissue. Aschoff claims that these foci are specific for rheumatic fever, but Geipel will not admit this.

Fraenkel (3) confirmed Aschoff's observations as to the specificity of the nodules, but does not commit himself as to the origin of the giant cells. Coombs (4) has devoted considerable study to Aschoff's bodies, and, like Geipel, considers them "the product of a connective-tissue proliferation in response to an irritant."

Bracht and Wächter (5) found the Aschoff bodies in three out of four cases of rheumatic myocarditis. They also conclude that the giant cells are derived from the fixed connective tissue cells, basing their opinion on the fact that these cells stain with the Unna-Pappenheim stain like swollen fibroblasts. These investigators were the first to produce experimental focal lesions in the myocardium of rabbits by injecting them with streptococcus. Rabbits injected with *Streptococcus viridans* showed considerable necrosis of the cardiac muscle fibers. The older foci showed a considerable number of lymphocytes and fibroblasts and a few giant cells. In still older lesions, fibrous scars were forming. In animals, on the other hand, that were injected with *Streptococcus hemolyticus*, the heart muscle showed small abscesses and dense infiltration of polynuclear leukocytes.

Takayasa (6) describes the heart changes in one case of rheumatism in which he found Aschoff bodies, but he offers no theory as to the origin of the giant cells.

Thalhimer and Rothschild (7) have recently studied the cardiac changes in rheumatic fever and chorea and conclude that the presence of Aschoff nodules signifies a previous rheumatic infection.

Jackson (8) injected hemolytic streptococci and also a *Streptococcus viridans* into rabbits. With the latter she produced foci consisting of degenerated muscle fibers around which are large mononuclear cells, large multinucleated giant cells, and proliferating connective tissue. In other lesions "the cells occupying the center of the lesion are very large with round or oval lightly stained nuclei and a large amount of granular cytoplasm in which bacteria are often seen." About these cells are many smaller, more deeply stained cells which are irregularly shaped and definitely outlined, with round deeply stained eccentric nuclei (plasma cells). In the relative absence of necrosis and of polynuclear leukocytes and in the abundance of large endothelial cells, these lesions are peculiar, showing the same characteristics emphasized by Coombs and others as characteristic of the focal human lesions in rheumatism.

De Vecchi (9) in 1912 injected the blood from cases of rheumatic fever into rabbits and was able thereby to produce lesions in the heart muscle. The lesions consisted of diffuse infiltrations of cells usually about a small vessel. The muscle fibers are often devoid of contractile tissue and appear homogeneous or in amorphous blocks. The cellular foci are composed of (1) leukocytes, mostly lymphocytes, (2) cells with large vesicular nuclei containing a network of chromatin and

scanty protoplasts. De Vecchi remarks that these experimental lesions resemble the rheumatic nodules, but his description and drawings do not support this claim. They might more readily be explained as a result of the injection of a foreign protein, as shown by Longcope (10).

Coombs, Miller, and Kettle (11) inoculated rabbits with streptococcus and produced cardiac lesions which differed in no essential manner from the lesions of human rheumatism.

Schloss and Foster's (12) experiments on monkeys were performed with *Streptococcus hemolyticus*; hence they are hardly comparable with the experiments which have been described above. They found the myocardial vessels thickened and surrounded in places by groups of cells which took a deep red color with pyronin methyl green. These cells looked like plasma cells, but were a little larger. Their nuclei were eccentric and stained light blue or green. Thalhimer and Rothschild (7) injected rabbits with *Streptococcus mitis (viridans)* and produced focalized myocardial lesions which they describe as follows:

"The lesions are entirely different from the submiliary myocardial rheumatic nodules described by Aschoff. . . . The lesions in the myocardium of the rabbit are a combination of degenerative and productive processes. Degeneration of the muscle fibres occurs either accompanied by or soon followed by a proliferation of the interstitial tissue which is disproportionate to the extent of the myocardial change."

The following facts are evident from this review of the literature. (1) Opinion is almost unanimous in considering Aschoff bodies as specific for rheumatic fever. (2) There is considerable diversity of opinion as to the origin and character of the large cells which for the most part constitute the lesion. By Coombs they are considered endothelial; by Geipel, Bracht and Wächter, and others, fibroblasts; while Aschoff himself looks upon them as transformed macrophages or wandering cells. (3) Efforts to reproduce Aschoff bodies experimentally have met with varying results in the hands of different investigators. Coombs and de Vecchi and Jackson all claim to have produced lesions which they consider essentially identical with Aschoff bodies. Bracht and Wächter, and Thalhimer and Rothschild produced lesions which are not identical with Aschoff bodies and are easily distinguished from them.

It is obvious that once granted that Aschoff bodies are a specific manifestation of rheumatic fever, the experimental production of them by injection of streptococci would be strong evidence in favor of the streptococcal origin of rheumatism. The present study was undertaken to determine, if possible, the origin of the peculiar cells

in the myocardial lesion; as the work progressed it seemed desirable to include also a study of the other structures involved, especially the joints.

In view of the disagreement as to the origin of the cells which constitute the Aschoff body in the human heart and the lack of harmony in the results from experimental study of the lesion, it was thought that the employment of a vital stain might prove of value.

Evans, Bowman, and Winternitz (13) have recently used this method with considerable success for the histological study of the miliary tubercle, and by vital stains demonstrated that in the liver both the epithelioid and giant cells of the tubercle develop from the endothelial lining of the capillaries.

In using the vital stain I have followed the method of Goldmann (14) who first reported his experiments with trypan blue and isamin blue in 1912. Goldmann employed the dyes in 1 per cent aqueous solutions and administered them either subcutaneously, intraperitoneally, or intravenously. In any case, the animals rapidly took on a deep blue color and at autopsy the various organs and tissues showed more or less pigmentation with the dye. Microscopically it was at once obvious that certain cells had taken up the blue granules, while others had not. For instance, Goldmann noted that the white corpuscles of the blood and the plasma cells never took the blue; on the other hand, the so called wandering cells, which are present throughout all the tissues, invariably showed phagocytosis of the granules. These wandering cells have received a variety of names, being variously known as macrophages, polyblasts, adventitia cells, clasmatocytes, or endothelioid cells, and Goldmann lengthened the list by calling them pyrrol cells. He found them in the subcutaneous tissue and in all the internal organs and very abundant in the pregnant uterus and in healing wounds. The characteristic feature of these cells is their active phagocytosis of bacteria and foreign matter of any kind. Goldmann thinks that they eventually stretch to form the spindle cells of young connective tissue, thereby losing their affinity for vital and fat stains as well as their phagocytic power.

Evans (15) has recently described these vitally staining cells in detail. He speaks of them as macrophages and classifies them as follows: (1) endothelial macrophages which line the capillaries in the liver, spleen, bone marrow, and hemal glands, and the lymphatic sinuses of the lymph glands; (2) macrophages which are more or less fixed, such as the cells of the reticulum of lymph glands, spleen, and bone marrow; also resting wandering cells in the connective tissues, especially numerous in the omentum; (3) the free macrophages which comprise the large mononuclear cells of the serous cavities and those present in the lymphatic sinuses of lymph glands and sometimes seen in the hepatic and splenic capillaries. Evans has demonstrated that the intramuscular macrophages may develop from endothelium, but he is not willing to go so far as Mallory, who considers all macrophages of endothelial origin.

It will be seen from this review that the vital stain is of considerable value in the study of cellular foci as helping to distinguish the various types, leukocyte, endothelium, fibroblast, and macrophage from one another.

*Methods.*

In the present study, the technique employed was as follows: Freshly distilled water was heated to 56°C. and enough trypan blue added to make a 1 per cent solution. While still warm the dye was injected into the rabbits usually by the intravenous route, though occasionally subcutaneous or intraperitoneal injections were substituted. The dye has a tendency to produce thrombosis of the ear veins. The rabbits were usually injected first with streptococci, but in some cases the trypan blue was administered previously. Histologically it seemed to make no difference which preceded the other. The usual dose of trypan blue was 10 cc. of the 1 per cent solution every day for 1 to 2 weeks. The number of trypan blue injections varied from one to twenty-three, the average number being twelve. By this method it was found that the tissues of the rabbits acquired a deep blue color and at the same time the animals suffered little physical deterioration. Most of the rabbits were killed by intracardiac injection of formalin. The rabbit was anesthetized, and the jugular and femoral veins were exposed. The sternum was then rapidly removed and a cannula inserted into the left ventricle. A 4 per cent solution of formalin was then allowed to run in through the cannula and at the same time the jugular and femoral veins were severed. The animal was thus rapidly exsanguinated, and in a few minutes formalin could be detected in the venous outpourings.

The perfusion with formalin was continued for 10 to 15 minutes, usually until several hundred cc. of the solution had been injected. A complete autopsy was then performed and thin pieces of tissue were preserved in 4 per cent formalin for sections. Sections were also taken from the infected joints and decalcified in Müller's fluid. Films and sometimes cultures were made from the pus in the infected joints. The intracardiac injection of formalin did not interfere with the recovery of streptococci in culture from the joints.

Thirty-eight rabbits in all were used in the experiments (Table I). In addition, three controls were injected with trypan blue but received

TABLE I.

No. of rabbit	Weight. gms.	No. of trypan blue injections.	No. of streptococcus injections.	Duration of life after 1st injection of streptococcl. days	Arthritis.	Lesions in heart.	Lesions in liver.	Lesions in kidneys.	Remarks.
1	1,820	2	1 (59-F)	1	0	Focal necrosis.	Focal necroses.		
2	1,670	2	1 (59-F)	2	0	"	"	Infiltration of leukocytes. Parenchymatous changes.	
3	1,900	2	1 (59-F)	7	0	Endocarditis, acute. Pericarditis, acute.	0		
4	2,230	3	1 (59-F)	7	0	Focal necrosis and infiltration.	0	Infiltration of small round cells.	Streptococcus recovered from heart's blood at autopsy.
5	1,850	3	1 (59-F)	7	0	Focal necrosis.	0	Severe parenchymatous nephritis, focal necroses.	"
6	1,715	9	4 (A-30)	26	0	0	Infiltration of leukocytes in lobules.		
7	1,620	0	3 (A-30)	18	Acute. Left wrist.	Acute pericarditis.	Infiltration of lymphoid cells.	Infiltration of lymphoid cells. Collection of lymphoid cells in cortex.	Died of pneumonia.
8	1,720	7	4 (59-F)	26	Subacute. Left shoulder.	0	0		

9	1,800	0	3 (59-F)	12	Acute. Both ankles, left wrist, right shoulder.	0	Diffuse infiltration of round cells.	Collection of lymphoid cells in cortex.	Died of pneumonia. Streptococcus from joints.
10	670	6	1 (A-30)	8	Acute. Right knee.	0	Marked cloudy swelling.	Collection of lymphoid cells.	Culture from heart's blood sterile.
11	554	0	1 (A-30)	1	0	0	0	0	0
12	692	18	4 (59-F)	70	Subacute. Right elbow, left shoulder.	Focal necrosis and infiltration. Re-pair.	Focal necroses, early cirrhosis, endocarditis.	Collection of lymphoid cells.	Died.
13	490	1	1 (59-F)	1	0	0	0	0	0
14	634	9	2 (A-49)	19	Acute. Both knees and shoulders.	0	0	Collection of lymphoid cells.	Died.
15	624	6	2 (A-49)	17	Acute. Both shoulders, left wrist.	A few foci of round cell infiltration.	Focal necroses.	"	0
16	635	1	1 (59-F)	1	0	Foci of round cell infiltration.	0	0	Died.
17	600	3	1 (59-F)	8	0	0	0	0	Incomplete autopsy.
18	425	5	1 (A-32)	6	0	Focal necrosis and round cell infiltration.	Small adenomata of ducts.	0	Incomplete autopsy.
19	779	6	1 (59-F)	19	0	0	0	0	Incomplete autopsy.
20	500	6	1 (A-32)	7	0	0	Coccidia.	0	Incomplete autopsy.

TABLE I—Continued.

No. of rabbit.	Weight.		No. of trypan blue injections.	No. of streptococcus injections.	Duration of life after 1st injection of streptococci.	Arthritis.	Lesions in heart.	Lesions in liver.	Lesions in kidneys.	Remarks.
	gm.	days								
21	682	1	2	1 (59-F)	1					Incomplete autopsy.
22	1,610	225	14	20 (59-F)	225	Chronic. Both shoulders, left wrist.	Foci of round cell infiltration.		Infiltration of lymphoid cells.	
23	1,480	180	14	13 (A-26)	180	Chronic. Both shoulders.	0		0	
24	1,800	180	14	17 (A-32)	180	Chronic. Right shoulder.	0		0	
25	1,895	40	4	8 (59-F)	40	Subacute. Both shoulders.	Acute endocarditis and pericarditis. Foci of round cell infiltration. Thrombosis of vessels.		0	
26	1,820	50	14	8 (59-F)	50	Subacute. Both shoulders.	A few foci of cellular infiltration.		Parenchymatous changes.	
27	1,880	140	14	8 (59-F)	140	0	0	Moderate cirrhosis.	0	
28	1,760	50	14	8 (59-F)	50	Subacute. Both shoulders.	Foci of necrosis and cellular infiltration.		0	
29	1,830	50	14	5 (59-F)	50	0	0		0	
30	2,050	14	9	2 (A-49)	14	0	0	Central necroses. Coccidia.	0	
31	2,490	28	18	3 (A-49)	28	Acute. Right elbow.	Foci of cellular infiltration. Repair.		Collection of lymphoid cells.	Streptococcus re-covered from joints.



32	2,200	8	1 (A-49)	10	Acute. Right elbow, left knee.	0	0	Slight sclerosis, infiltration of lymphoid cells. Collection of lymphoid cells.	Streptococcus re-covered from joints.
33	2,295	18	3 (A-49)	28	Acute. Both elbows and knees, both wrists and left ankle.	0	0		"
34	2,640	14	2 (A-49)	21	Acute. Right knee. Synovitis, right leg.	Foci of necrosis and cellular infiltration.	0	0	"
35	1,890	19	3 (A-49)	26	Acute. Left shoulder.	Foci of cellular infiltration.	0	0	Rabbit received very large doses of streptococci (100-200 cc.) (broth culture).
36	1,700	17	2 (38-D)	12	Acute. Synovitis, both heels.	Large foci of necrosis, cellular infiltration, and repair.	0	0	Rabbit received very large doses of streptococci.
37	1,760	6	1 (38-D)	1		0	Central necroses.	0	"
38	1,810	6	1 (38-D)	1		None. Many macrophages.	Many macrophages.	0	Control.
39	1,580	16	0	0		"	"	0	"
40	1,680	23	0	0		"	"	0	"
41	1,770	23	0	0		"	"	0	"

no streptococci. The rabbits were usually of medium size, varying in weight from 425 to 2,500 gm. For one series, an attempt was made to use five young rabbits, but their veins were not well adapted to the trypan blue injections.

Six different strains of streptococci were employed for the experiments. They were obtained from blood culture from the following sources:

1. Rheumatic fever (59-F).
2. Rheumatic fever (A-49).
3. Rheumatic fever (38-D).
4. Infectious endocarditis (subacute) (A-30).
5. Infectious endocarditis (subacute) (A-32).
6. Infectious endocarditis (subacute) (A-26).

The six strains all belonged to the non-hemolytic group of streptococci. Four were green producers, while two, A-26 and A-32, produced no change whatever in blood.

The number of streptococcus injections varied from one to four and were separated by 5 to 10 day intervals. The average number of injections was two. This excludes the eight immunized animals (Nos. 22-29) which first received several injections of killed streptococci and then a series of injections (five to twenty) of living streptococci.<sup>1</sup> The dosage of streptococcus culture used in my experiments has varied considerably. In the earlier work I used 5 to 20 cc. of a 24 hour broth culture. In the later experiments much larger doses were given (three to five rabbit blood agar slants), and in the last series 100 to 150 cc. of a broth culture were injected at one time.

#### RESULTS.

##### *Joint Lesions.*

In this investigation I have given considerable study to the pathology of the joints, for in reviewing the literature it was noticeable that very little attention has been paid to the pathological anatomy of arthritis, either in the human being or experimental animal. Poy-

<sup>1</sup> The privilege of studying the lesions in the immunized rabbits was granted to me by Drs. Swift and Kinsella who were employing them for the study of another problem.

ton and Paine (16), Cole (17), and Jackson (8) have described the gross, and to some extent the microscopic appearance, but the cellular changes have not been carefully studied. In every case of arthritis in the present series, sections have been taken through the joints involved and studied microscopically. The sections were all decalcified in Müller's fluid, which did not injure the vital stain to any great extent. The sections were cut by the paraffin method and stained (1) with hematoxylin and eosin, (2) with aqueous cochineal (to bring out vitally stained cells), and (3) by the Gram-Weigert method for bacteria. The Unna-Pappenheim stain could not be used on account of the formalin fixation. By referring to the table it will be seen that of the thirty-eight rabbits injected with streptococci, nineteen, or 50 per cent developed arthritis; in seven the disease was monoarticular, and in twelve polyarticular. The shoulder was the joint most frequently involved; the right nine times, the left ten. The other large joints were affected with about equal frequency. In many cases it was difficult to determine the exact duration of the arthritis, for the reason that the rabbit showed no symptoms. Many infected joints were not discovered until the autopsy was performed.

The rabbits with arthritis lived from 8 to 225 days after receiving their first injection of living streptococci. Four animals lived less than 2 weeks; eight of the nineteen rabbits lived from 2 to 4 weeks after their first injection. The remaining seven lived from 6 to 32 weeks.

The four rabbits that lived or were killed in less than 2 weeks after receiving streptococci (Nos. 9, 10, 32, and 36) had arthritis in a very acute form. Two of these rabbits received only one injection each of streptococci; another received two, and the last three injections. Faber (18) found that it usually took more than one injection of streptococci to produce arthritis. As a rule, this is true, but not invariably, as our results show. In the gross these joints were moderately swollen and contained bluish viscid mucoid material, films of which showed many pus cells filled with coarse blue granules of trypan blue. In two of the cases streptococci were recovered in cultures from the joint exudate. Microscopical sections through the infected joints show extensive infiltration of the synovial membrane with leukocytes, chiefly of the polynuclear type. In addition there

are present a few large endothelioid cells (macrophages) which contain blue granules and are similar in every respect to the large cells found in the joint exudate. The membrane is thickened from edema and cellular infiltration. The synovial membrane is covered with an exudate of leukocytes and a few endothelioid cells. The cartilage is intact and shows no infiltration. The marrow shows a slight hyperplasia. The capsule is little if any changed.

In the second group of cases that lived for 2 to 4 weeks after the first injection of streptococci (Nos. 7, 8, 14, 15, 31, 33, 34, and 35), the gross appearance of the infected joints differed in no way from those of the first group.

Microscopically, however, there are some differences. In these rabbits the infection is obviously of longer duration. In some places the cartilage is superficially necrotic and the destroyed portion is being replaced by connective tissue. The bone marrow shows considerable hyperplasia. The synovial membrane is thickened and densely infiltrated with leukocytes and the same large endothelioid cells filled with blue granules (Fig. 1). Lymphoid and plasma cells are more numerous in the infiltration and exudate than in the first group.

There are signs of beginning organization in the exudate and it is interesting in this connection to study the part of the large endothelioid or macrophage cells. On the surface they appear as large round, or oval cells packed with coarse blue granules. Deeper down in the exudate near the synovial membrane these cells have apparently become pulled out into fusiform and stellate shapes, appear smaller, and contain fewer and finer granules. The impression is obtained that the macrophages, having accomplished their first duty as phagocytic cells, have become gradually transformed after this function is completed into fibroblasts to assist in the process of repair. Eventually they become adult connective tissue cells and lose their phagocytic power entirely.

In the third group of cases (Nos. 12, 22, 23, 24, 25, 26, and 28) many of the infected joints began to show chronic changes, such as great thickening of the capsule with considerable limitation of motion; the bone was very friable and crumbled easily when sections were sawed for microscopical examination. There was no bony ankylosis, however, in any of the cases.

Microscopical sections through these joints show extensive necrosis of the cartilage which sometimes extends down into the bone. There is marked hyperplasia of the marrow which has replaced the cancellous bone to a great extent. The synovial membrane is thickened and the deeper part composed of newly formed connective tissue; the superficial part is very cellular. The cells are leukocytes, mostly lymphoid and plasma cells, though there are a moderate number of polynuclear cells also present and large numbers of macrophages which appear to be undergoing the transition into fibroblasts, as just described. No bony changes were observed, even in the more chronic cases, but there were hyperplasia of the bone marrow and occasional thrombi or foci of granulation tissue in the marrow.

It will be seen from these descriptions that the changes which take place in experimental arthritis represent a gradual transition from a stage of acute inflammation in which there is little more than an infiltration of leukocytes into the synovial membrane and an outpouring of mucoid material, leukocytes, and macrophages into the synovial fluids to a stage of subacute or chronic inflammation in which the following changes occur. The synovial membrane and the cartilage undergo more or less necrosis. The cellular exudate in the synovial membrane consists chiefly of lymphoid cells and macrophages. There is marked hyperplasia of the bone marrow with more or less absorption of the cancellous bone. It is possible that if some of these rabbits had lived long enough, new formation of bone and possibly ankylosis would have occurred, as described by Poynton and Paine. The capsule of the joint thickens as a result of the long continued inflammation. The changes resemble those observed in cases of acute infectious arthritis in man which become subacute and finally pass into the chronic stage of arthritis. From the standpoint of the pathology and histology of experimental arthritis, there is little to differentiate it from human arthritis of the infectious or metastatic type. It has been observed by previous investigators of experimental streptococcus arthritis that complete recovery of the joint may take place as in human rheumatism. Unfortunately, I have not had an opportunity to study any joints of this type.

*Cardiac Lesions.*

The hearts were studied in considerable detail and it was chiefly in the hope of throwing some light on the foci found in the heart in experimental streptococcus arthritis that the rabbits were vitally stained. Almost all the sections studied were from the left ventricle, and particular care was taken to include the papillary muscles in the sections.

Of the thirty-eight rabbits injected with the streptococci, eighteen showed pathological changes in the heart or pericardium; eleven of the eighteen belonged also to the arthritis series. In three rabbits complete autopsies were not performed. Rabbit 3 showed an endocarditis and pericarditis. In Rabbit 25 a vegetative endocarditis was found associated with pleurisy and pneumonia. Rabbit 7 had a pericarditis. In the remaining fifteen the lesions were in the myocardium. These lesions were usually not visible with the naked eye on account of the trypan blue stain. The earliest focal lesions observed have been small areas of necrosis in the muscle fibrillæ (Fig. 2). At first these consist only of necrotic fragments of muscle with here and there a surviving nucleus. At this stage no infiltration of leukocytes or wandering cells has occurred. These foci are usually small, but occasionally one sees necrotic areas of considerable extent situated in the muscle, never in the supporting stroma, and sharply defined from the surrounding healthy parenchyma. The line of demarcation from healthy tissue is emphasized by the vital staining of the necrotic foci, which take a light blue color due to the deposition in them of very fine blue granules of trypan blue. A later stage of such focal necroses is characterized by the presence of leukocytes, usually of the lymphoid type, and macrophages filled with blue granules (Fig. 3). Focal necroses were observed in nine cases. In three of these (Nos. 1, 2, and 5) the focal necroses were the only lesions present. The other six (Nos. 4, 12, 18, 28, 34, and 36) showed in addition to necrosis more or less extensive cellular infiltration. In seven other cases, foci of infiltration, chiefly by lymphoid cells, were present with little or no necrosis (Fig. 4).

The foci of cellular infiltration were of particular interest, especially as regards their resemblance to Aschoff's bodies. The foci

with necrotic centers surrounded by leukocytes and macrophages (Fig. 3) bore the nearest resemblance to the submiliary nodules of rheumatic fever, but even in these the resemblance was not close. In the first place, the location of the lesions in the rabbit heart is different from that in the human heart. In the rabbit I have always found the necrotic foci in the heart muscle proper and not related in any way to the blood vessels. Sometimes smaller collections of lymphoid and macrophage cells are found in the stroma, but this is the exception rather than the rule. The submiliary nodule is always found in the supporting connective tissue, usually about a blood vessel. In the rabbit's heart the necrosis is definite; in the human heart it is slight and of the hyaline type. There is a difference, too, in the cellular exudate about the necrotic foci. In the rabbit the cells are chiefly lymphoid and plasma cells and large macrophages. In the human lesion there are, in addition to these types, a considerable number of giant cells which resemble those seen in Hodgkin's disease. Furthermore, the radial arrangement of the cells in a submiliary nodule is characteristic, whereas in the experimental nodule the cells are arranged in a purely haphazard manner.

In Rabbit 36 I succeeded in demonstrating streptococci in one of the myocardial lesions. This rabbit, however, had received such large doses of streptococci that perhaps too much significance should not be attached to this point. Thalhimer and Rothschild were unable to demonstrate bacteria in the myocardial foci which they studied, and I have not found them in any other of my own cases.

In a number of cases, especially Nos. 12, 31, and 36, there were signs of repair (Fig. 5), and here, as in the joints, the macrophages seemed to play an important part. They were present in large numbers and were apparently being converted into fibroblasts.

It may be said, then, that while the myocardial lesions in experimental streptococcus arthritis follow in a general way the course of those occurring in the rheumatic human heart,—that is, necrosis and infiltration and finally proliferation with the formation of scars,—the histological appearance of the two lesions when carefully studied, is different.

*Endocarditis and Pericarditis.*

Sections through the vegetations on the heart valves in Rabbits 3 and 25 showed a fibrinous network, partially organized, and infiltrated with leukocytes and a moderate number of macrophages. Sections through the pericardial exudates presented similar pictures (Rabbits 3, 7, and 25).

*Lesions in the Kidneys.*

In the study of the rabbit's kidneys the investigator is always handicapped by the frequency of pathological changes in supposedly normal kidneys. Christian, Smith, and Walker (19), Ophüls (20), Longcope (21) and others have found round cell infiltration and patches of sclerosis in 22 to 33 per cent of kidneys of normal rabbits.

In the present series of thirty-seven cases in which the kidneys were examined microscopically, the kidneys of the three controls, from rabbits that received trypan blue but no streptococci, were normal; and of the remaining thirty-four from rabbits that had received injections of streptococci, eighteen, or 52 per cent, showed definite pathological changes. Of these, fourteen were associated with arthritis. As in all there were nineteen cases of arthritis, it follows that fourteen out of the nineteen, or 74 per cent, were associated with renal changes. In nine cases the lesion consisted of collections of lymphoid cells in the cortex with more or less patchy sclerosis. In four cases there was a well developed parenchymatous nephritis, with extensive destruction of the tubular epithelium of the convoluted tubules. In one case both parenchymatous and interstitial changes were noted (No. 36).

The glomerular changes in streptococcus infections have been studied by Baehr (22), Löhlein (23), and others. Baehr has described characteristic glomerular lesions in infectious endocarditis due to the lodgment of bacterial emboli in the glomerular capillaries.

*Lesions in the Liver.*

In six cases the liver showed focal necroses, which were usually about the central veins and were themselves in most cases surrounded by a zone of leukocytes. In only two of the six cases was



there an associated arthritis. In two other cases there was a well marked cirrhosis (Nos. 12 and 27). A slight degree of round cell infiltration and cirrhosis was present in some of the other cases, but the change was not sufficiently marked to be significant.

*Lesions in Other Organs.*

The spleen and lymph glands were enlarged in all the rabbits. Microscopical sections showed hyperplasia of the lymphoid tissue and large numbers of macrophages. These changes were apparently due to the vital stain as they were also observed in the control rabbits. In Rabbit 9 there were infarcts in the spleen.

The lungs showed nothing of interest except in two cases. Rabbits 7 and 9 both died of pneumonia. Microscopical sections showed the usual exudate in the pulmonary alveoli.

DISCUSSION.

The injection of one or more doses of *Streptococcus viridans* into the vein of a rabbit produces in many cases an acute arthritis which bears some resemblance to the arthritis of rheumatic fever, but is perhaps more nearly analogous to the so called acute or subacute infectious arthritis so frequently associated with focal infections in the teeth, tonsils, or other parts of the body. Microscopically, joint sections from vitally stained animals show in addition to numerous leukocytes, a considerable number of large endothelioid cells or macrophages, which take the vital stain. These cells migrate from the tissue into the joint exudate and could not be seen to develop from the endothelial lining of the joint as commonly supposed. As the infection becomes older these macrophages become more and more numerous and, gradually losing their phagocytic power, appear to develop into fibroblastic cells. In the chronic stage the infected joints show ulceration of the cartilage and synovial membrane, with hyperplasia of the bone marrow and fibrous thickening of the capsule.

The most interesting changes in the heart are the myocardial lesions, which may be briefly described as small foci of necrosis infiltrated and surrounded in most cases by lymphoid cells and macrophages. The location of these lesions in the heart muscle proper, the

arrangement of the cells, and the absence of giant cells distinguish them sharply from Aschoff bodies. In a small percentage of cases bacterial endocarditis or pericarditis occurs.

The lesions produced in other organs are not especially characteristic. Focal necroses are frequently found in the liver, and the kidneys often show foci of round cell infiltration. In the more chronic cases there may be some production of fibrous tissue.

The large endothelioid cells in the heart muscle appear to develop from the vascular endothelium. In this respect my observations agree with those of Evans. In many sections the endothelial sprouts can be seen already developing phagocytic power, as shown by the presence in them of the trypan blue granules.

In the joint exudates the origin of the macrophages is not so clear. One thing is obvious in all the organs; namely, that the injection of trypan blue stimulates an increased production of macrophages.

In the three control rabbits that received no streptococci but were injected with trypan blue alone, the increased number of macrophages was noticeable in all the organs, but strikingly so in the liver and heart muscle.

#### CONCLUSIONS.

1. Repeated injections into rabbits of non-hemolytic streptococci isolated from human cases of infectious endocarditis or rheumatic fever will produce an acute arthritis in the rabbit similar in most respects to the arthritis of acute rheumatism.

2. Microscopical sections of the joints show a gradual transition from an acute exudative inflammation to advanced organization.

3. Endocarditis and pericarditis occur in a small percentage of cases, and focal lesions in the myocardium consisting of necrosis and the infiltration of cells are frequent. These focal lesions differ considerably from Aschoff's submiliary nodules.

4. Lesions in the kidneys and liver occur but are not characteristic.

5. By means of the vital stain it has been shown that the large endothelioid cells which play a prominent part in the joint and myocardial lesions belong to the group of so called macrophages or wandering cells and probably develop from the vascular endothelium.

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## EXPLANATION OF PLATES.

## PLATE 49.

FIG. 1. Rabbit 8. Section through capsule of left shoulder. Subacute arthritis. The membrane, which is considerably thickened, shows internally a layer of necrotic material beneath which the tissue is densely infiltrated with macrophages and leukocytes. Leitz obj. 3, oc. 4.

FIG. 2. Rabbit 5. Focal necrosis in heart muscle. No cellular infiltration has occurred. Leitz obj. 5, oc. 4.

## PLATE 50.

FIG. 3. Rabbit 36. Focus in heart muscle, showing necrotic center, surrounded by macrophages and lymphocytes. Vially stained cells contain blue granules. Counter-stained with aqueous cochineal. Zeiss obj. 5, oc. 4.

## PLATE 51.

FIG. 4. Rabbit 12. Focus in heart muscle, showing infiltration of lymphoid cells. Slight necrosis. Leitz obj. 5, oc. 3.

FIG. 5. Rabbit 31. Focus in myocardium showing repair. A few macrophages and lymphoid cells, but connective tissue cells predominate. Leitz obj. 7, oc. 3.

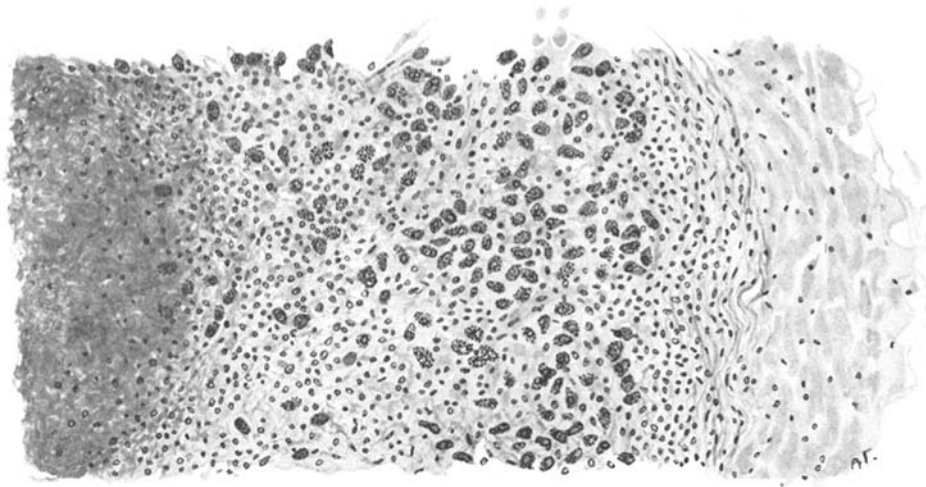


FIG. 1.

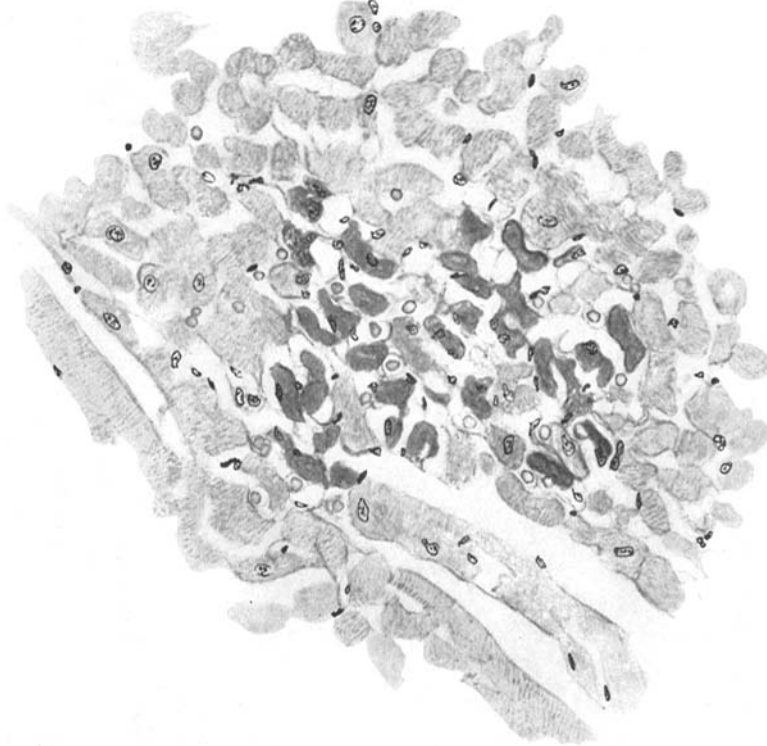


FIG. 2.

(Cecil: Non-Hemolytic Streptococcus Lesions.)



FIG. 3.

(Cecil: Non-Hemolytic Streptococcus Lesions.)

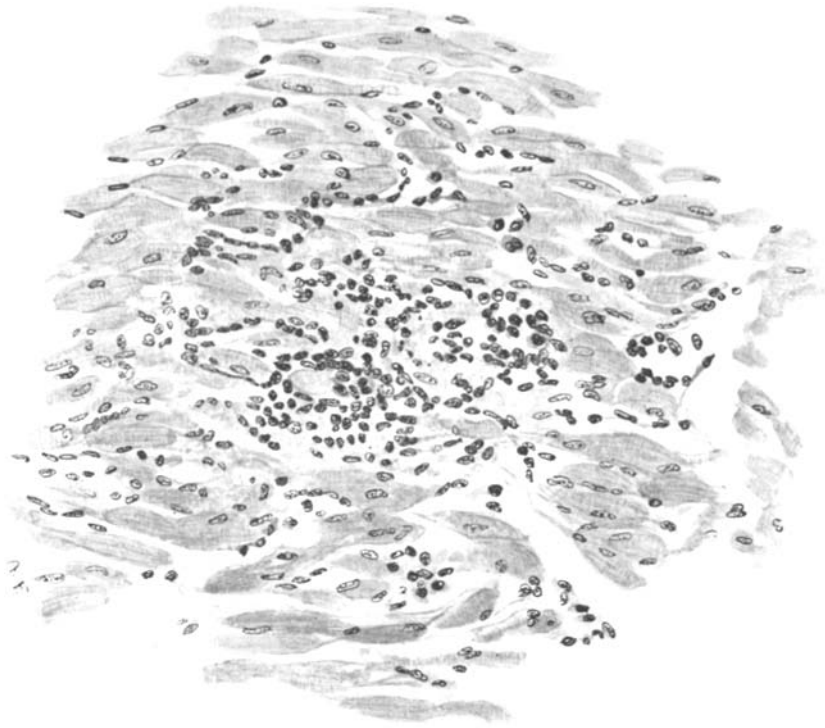


FIG. 4.

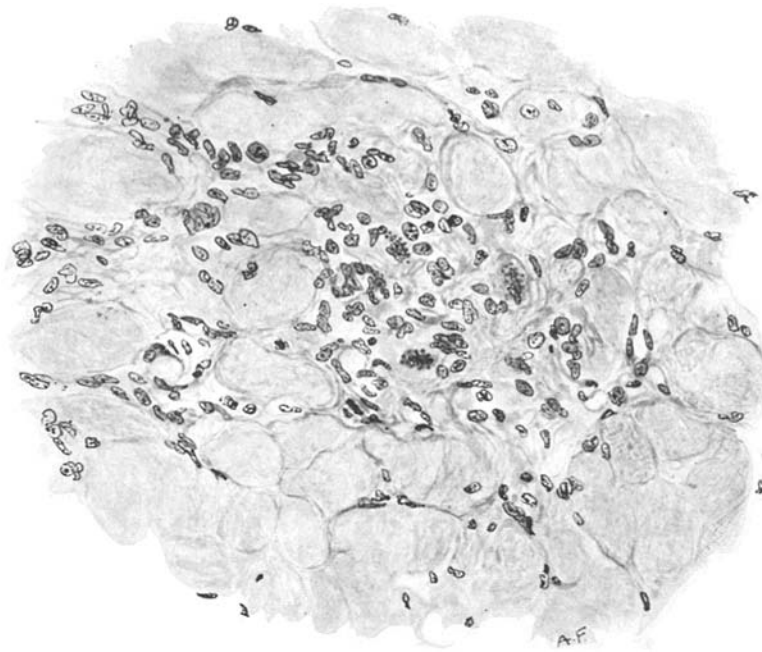


FIG. 5.

(Cecil: Non-Hemolytic Streptococcus Lesions.)