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Experimental production of rheumatic carditis in rabbits by anaphylactic reaction to horse serum

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THE EXPERIMENTAL PRODUCTION OF RHEUMATIC CARDITS IN RABBITS BY ANAPHYLACTIC REACTION TO HORSE SERUM

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INTRODUCTION

Since the early descriptions of the gross and microscopic findings of rheumatic fever first appeared in the literature, several methods have been devised to experimemtally produce a similar disease syndrome in laboratory animals. Thus far none of these techniques have been entirely satisfactory in producing the disease syndrome, and the histologic lesions produced while similar to those of rheumatic fever are not accepted in all circles as those of the disease.

Following the association of streptococci with the etiology of rheumatic fever, numerous investigators have attempted to produce the disease by bacterial injections into sensitized animals. Some cardiac changes have been produced, but it is hard to accept the changes arising by this method as rheumatic lesions (Rich and Gregory, 1943). Gross and his co-workers (1929) carefully repeated much of this work using bacterial injections, and their results were unfavorable. Bauer (1947) modified the bacterial injections in that he gave anti-rat-heart rabbit serum injections intravenously concurrently with the streptococcal organisms. The changes produced chiefly affected the valves and the connective tissue structures of the heart, and he thought that these lesions had a striking resemblance to those seen in rheumatic fever.

Several years ago Vaubel (1932), Klinge (1933), Knepper and Waller (1934), Junghans (1934), and Knepper (1935) reported within

a short time that they had produced changes similar to those seen in rheumatic fever by repeated injections of horse serum into rabbits. Vaubel (1932) stated that in a study of the relation of hypersensitivity to the lesions of rheumatism, he noted vascular changes in rabbit hearts following repeated subcutaneous and intravenous injections of horse serum. He wrote tha the lesions seen consisted of a "hyperplasia of the intima and media with splitting of the elastic fibers and a proliferation of adventitial cells, histiocytes and connective tissue cells, mostly in nodular formations", which were similar to the Aschoff nodules of rheumatism. These changes were noted in five out of forty-five rabbits treated. The microscopic examination of tissues revealed these changes in the heart only, therefore, Vaubel stated that these alterations resembled those seen in rheumatic fever.

Brunn (1940) repeated much of the experimental work using horse serum and although he saw cardiac lesions in his animals, he came to the conclusion that he had not been able to produce an anlagan to Aschoff nodules. He believed that the lesions found only suggested that rheumatic lesions probably develop on a hypersensitive basis.

In 1942, while studying the basic characteristics of lesions similar to polyarteritis nodosa produced in rabbits by the intravenous injection of sterile horse serum, Rich and Gregory noted cardiac lesions morphologically similar to those of acute rheumatic

carditis. They undertook a further study of these lesions in order to confirm their original finding. The animals were killed between one and five weeks following treatment and similar cardiac findings as discovered in their previous rabbits were seen in eleven of the thirty-six rabbits in this series. Therefore, Rich and Gregory came to the conclusion that the experimental observations were highly suggestive that cardiac lesions of rheumatic fever may be the result of hypersensitive reactions of the anaphylactic type. Since they pointed out this similarity, Hopps and Wissler (1946), Fox and Jones (1946), Roberts and associates (1949), and others have confirmed the observations.

The characteristic changes seen in the rabbit heart following injections of sterile normal horse serum can be divided into four main groups: (1) Focal collagen degeneration, (2) Aschoff-like bodies, (3) Non-specific inflamatory lesions, and (4) Rheumatic-like valvular lesions according to Rich and Gregory (1943) and Roberts and associates (1949).

(1) Focal Collagen Alteration. This change is a focal degeneration of collagen consisting of a separation of the fibers by edema, swelling of the individual fibers, and degeneration of the fibers. It is seen focally in the cardiac connective tissue but is more prominent in the valves and in the mural endocardium at and near the valvular attachments.

(2) Aschoff-like Bodies. A focal accumulation of cells, closely

resembling the Aschoff body is found in serum injected animals. It contains cells similar to those seen in cases of rheumatic carditis. In several instances these cells are grouped about a focus of swollen and degenerated collagen fibrils, sometimes forming somewhat of a pallisade-like border as seen in the Aschoff body of human cases. This characteristic lesion is present in all coats of the heart, but it is more likely to be found in the myocardium (Roberts and associates, 1949). Rich and Gregory (1943) found the lesion to be more evident in the valve leaflets, near their bases, and in the endocardium near the valve attachments. Although the group of cells is usually perivascular in location, the focus of cells resembling the Aschoff body is seldomly present near the vessels. The Aschoff-like body is not present in the hearts of serum injected animals in a large enough number that can easily be seen with low power examination of the sections (Rich and Gregory, 1943).

(3) Non-Specific Inflamatory Lesions. This change consists of an ordinary edema with mononuclear cellular infiltration (lymphocytes, plasma cells, and macrophages) as seen in rheumatic fever. It is especially prominent in the pericardium, the base of the valves, and the mural endocardium at the valvular insertions. Roberts and associates (1949) in addition noted the presence of an occasional eosinophil in the lesion. They also described an atypical mononuclear cell that has an abundance of slightly acidophilic cytoplasm

and an oval vesicular nucleus, and in many instances it is identical to the Anitschow myocyte. In some cases the muscle cells in cardiac vessels of rabbits following multiple injections of horse serum have nuclei of a similar type.

(4) Rheumatic-like Valvular Lesions. The valvular change consists of edema, swelling, degeneration of collagen fibers, and a cellular infiltration projecting on the valve in some instances. Although the valvular endothelium on the surface of the infiltration may be damaged, no "eosinophilic thrombus material" is seen upon it.

In addition to the four major types of cardiac changes seen in their series, both Rich and Gregory (1943) and Roberts and associates (1949) describe a change consisting of simple necrosis of foci of cardiac muscles in some of their animals.

Although several individuals have reported findings of "rheumatic-like" cardiovascular lesions in rabbits subjected to injections of horse serum or other agents, it must be re-emphasised that as yet no one has reported a typical clinical case of rheumatic fever with marked gross cardiac lesions in animals. The reason for this failure may be due to the fact that in most instances the animals have been sacrificed shortly after serum treatment was carried out (one month or thereabout).

In this project we undertook to repeat much of Rich and Gregory's and Robert's work using a uniform method that would give a high incidence of cardiac lesions in rabbits. The experiment was

was carried for a period of six months and animals were sacrificed at monthly intervals so that we could study the time of occurrence of the primary lesions and the progress of these early changes to the more chronic lesions as reportedly seen in the human case of rheumatic fever.

PROCEDURE

The procedure used in this experiment can best be divided into two sections for clarity of presentation. They are first the preparation of horse serum and second the technique of the serial injection of horse serum into experimental animals.

(1) Preparation of Horse Serum. Fresh blood was drawn under aseptic conditions from the jugular vein of horses at the Corn State Serum Farm and placed in a sterile container without anticoagulant. This container of blood was stored over night at room temperature so that the blood could clot. The following morning the serum portion of the contents was aseptically pipetted into sterile centrifuge bottles and centrifuged to remove any cells that might have remained suspended in the serum. The serum was pipetted again and placed into sterile bottles without preservatives and refrigerated at four degrees Centigrage until used. A culture was inoculated from each container in order to insure sterility of the serum. This procedure of preparation of horse serum was repeated prior to each series of injections so that the serum would be fresh at each injection.

(2) The Technique of Horse Serum Injection into Experimental Animals. Sixty white albino rabbits with an average weight of from one to two kilograms were used in this experiment. The rabbits were housed in wire cages, segregated as to sex, fed commercial rabbit pellets (Nutrena), and given water at ad libitum. Each experimental animal was given an intravenous injection of 10 cc. of horse serum per kilogram of weight. The same dose of horse serum was given on the eleventh and on the twenty-first days of the experiment, making three injections in all. Fifteen to twenty-five minutes prior to the second and third injections of horse serum, an intraperitoneal injection of 5 mg. per kilogram of histadyl was given. This was done to prevent death from acute anaphylactic reaction.

During the second and third series of injections, the animals suffured a very high mortality rate due to acute anaphylactic shock (forty-five in all). The symptoms of shock were similar to those described by Coca (1919) as occurring in the rabbit. The animal just lay with outstretched legs or fell on its side, gave a series of convulsive movements often associated with a screem and the passage of urine and feces, and died. In many cases irregular respiratory movements continued for a brief period after the heart had stopped beating. In several instances an attempt was made to administer epinephrine intravenously, but once the rabbit had shown the signs of acute anaphylactic raction, it could not be

saved. It was noted in the later series of injections, that when the time of injection was increased to four or five minutes during the actual time of horse serum administration, all of the rabbits survived.

Eleven of the rabbits which died during the second series of injections were autopsied and all six of the rabbits that succumbed to the third injection were examined. Two months after the first injection, two rabbits were sacrificed and autopsied. Three animals were sacrificed and examined on the third, fourth, fifth, and sixth months of the experiment. Tissue sections were made from the heart to demonstrate the presence of any cardiovascular lesions. Sections taken included myocardium, endocardium, and cardiac valves, as shown in plates I, II, III, and IV. Sections of the kidneys, liver, adrenals, spleen, lungs, and thymus were also taken from each animal. The sections were preserved and fixed in ten percent formaldehyde prepared in the customary fashion, stained with hemotoxylin-eosin stain, and examined microscopically.

GROSS FINDINGS

Immediately following death or sacrifice, thirty-one experimental rabbits in this investigation were autopsied so as to allow maximum preservation of gross and microscopic alterations in the tissues. The primary intrest of this examination was focused on the heart, lungs, kidneys, adrenals, liver, and thymus gland.

In most instances, the heart did not demonstrate any distinct

gross changes; however, there were some suggestive findings. First, the right auricle and ventricle were markedly dilated, engorged with blood, and the walls of these chambers appeared paper thin in rabbits number 1 to 17. Possibly, this can be attributed to the fact that these rabbits had died in acute anaphylaxis. Coca (1919) showed that this dilatation was associated with marked obstruction to the pulmonary circulation, evidenced by the increased pressure required to drive fluid from the pulmonary artery to the left auricle. He concluded that acute anaphylactic death in the rabbit was due to a spasmodic constriction of the branches of the pulmonary artery, followed by rapid dilatation of the right side of the heart, and acute heart failure.

Second, the cardiac valves appeared to be normal; but on closer examination, a questionable valvular thickening was noticed. This consisted of very fine papillary-like varrucae along the line of valve closure and a deminished translucency of the valve. these questionable changes appeared most often in the mitral valve, but they were present in an occasional tricuspid valve as well. The aortic and pulmonic valves seemed to be normal.

In general the changes seen in the heart were very minimal. This is similar to the observations by Rich and Gregory (1943). The determination of specific cardiac findings was reserved to be made on microscopic examination.

The lungs appeared to be normal grossly in rabbits no. 18 to 31.

(Those killed at monthly intervals). They were pink in color without any mottling, well expanded, and somewhat spongy or rubbery in consistency. The picture was the same on cross-section. There was not any evidence of pulmonary hemorrhage or congestion. The lungs of rabbits no. 1 to 17 (which died in acute anaphylaxis) differed from those previously described in that they had a hemorrhagic mottled appearance. On cross-section this mottling was accentuated and a sero-sanguinous frothy fluid exuded from the lung.

The liver seemed to be normal in all instances. The size did not vary from the average. It was dark red in color. On cut section, the lobular pattern did not show any gross and the consistency of the tissue was normal. Blunting of the edges and other signs of congestion and edema were absent. A number of the livers had an occasional subcapsular small hard white fibrous nodule, but no significance was attributed to this as fibrous nodules are known to appear in the livers of normal untreated rabbits.

The kidneys did not exhibit gross alterations from the normal. The size was similar in all cases. The capsules stripped easily, and the surfaces were smooth. The medullas and cortices appeared normal on cross-section. The pelves were normal.

The spleen appeared normal in all respects except for a marked prominence of the lymphoid follicles. These appeared light tan in color in contrast to the dark red background of the splenic pulp on cross-section.

The thymus and adrenals at the time of autopsy were without discernable gross alterations.

All tissues were fixed in ten percent formalin. Subsequently, blocks were cut for microscopic examination. At this time the fixed tissues were re-examined for any features that might have been overlooked at autopsy. Additional changes were not found, but it noted that the previously described cardiac valve changes were not as prominent, possibly, because of the change in color and shrinkage of tissues in the fixing process. Microscopic sections were prepared in the conventional way and stained with hematoxylin and eosin stain.

MICROSCOPIC FINDINGS

On microscopic examination of the experimental tissues, a large variety of specific and non-specific changes were present. In general, these were similar to those described by Rich and Gregory (1943) and those seen in the earlier stages of rheumatic fever in the human. These experimental rheumatic findings occur more prominently in the heart, liver, lungs, spleen, and kidneys in this series.

The histologic changes seen in the rheumatic fever damaged heart may be classified into six major categories: (1) Focal degeneration of collagen, (2) Non-specific inflamatory cellular infiltration, (3) Aschoff body formation, (4) Vascular changes, (5) Valvular changes, and (6) Fibrosis and scarring of valves

and muscle.

(1). Focal Degeneration of Collagen. A number of the rabbit hearts demonstrated a focal degeneration of collagen consisting of a separation of the fibers by edema, swelling of the individual fibers, and finally degeneration of the fibers. This change is seen in localized areas dispersed throughout the myocardium, but it tends to be located adjacent to or in vessels, valves, valve cushions, or other areas where collagen is normally seen in the heart. In our opinion this represents the earliest phase of the anaphylactic raction seen in the heart of the horse serum treated rabbit and tends to be more prominent in the animals sacrificed in the earliest part of the experiment.

(2) Non-Specific Inflamatory Cellular Infiltration. A large number of the hearts exhibit a diffuse and/or a focal accumulation of non-specific inflamatory cells (lymphocytes, plasma cells, and macrophages). These accumulations of cells seem to be more prominent at the base of the valves, in the endocardium, and about vessels, but many of them are dispersed throughout the myocardium in general and are associated with degeneration of muscle fibers in some areas. Those appearing in perivascular position are particularily prominent in the adventitial layers of small coronary arteries and arterioles. Many Anitschow myocytes are seen in close association with these cellular accumulations, especially in the adventitialof small coronary arteries and arterioles.

Most investigators now consider the Anitschow myocytes to be a cardiac histiocyte (Clawson, 1929).

(3) Aschoff Body Formation. Typical Aschoff nodules similar to those characteristically seen rheumatic fever are present. The nodule consists of an initial fibrinoid degeneration followed by an inflamatory cellular infiltration. These cells are mononuclear and slightly larger than lymphocytes, with a dense chromatic nucleus and scanty cytoplasm. Specialized mesenchymal cells, designated Anitschow myocytes, are present throughout the interstitial tissue in the nodule. The giant cells of Aschoff appear in a few cases, especially in the heart of rabbit no. 14. These cells are large, with a prominent vesicular nucleus containing a bar-like or round central nucleolus. The cytoplasm is abundant, basophilic, and irregularily outlined. Frequently, the cell is multinucleated. In a number of cases a network of reticulin fibrin is deposited between the cells. As a rule the Aschoff-like nodules are located in perivascular position. These lesions vary from small accumulations of cells to more extensive collections that tend to obliterate the lumina of the vessels (rabbit no. 14).

(4) Vascular Changes. A small number of the rabbit hearts demonstrated a polyarteritic lesion in coronary arteries and arterioles in many respects similar to that seen in human polyarteritis nodosa. It consists of a nodular and segmental inflamation of the walls of small cardiac vessels. The lesion

varies from an edema of the media to a more advanced lesion consisting of necrosis, fibrinoid alterations, and hyalinization of the media with infiltration of mononuclear and polymorphonuclear cells. Intimal proliferation and cellular infiltration of the intima are prominent findings in these arteries.

(5) Valvular Changes. A number of the rabbit hearts demonstrate microscopic changes in vavles. These changes are rather minimal and do not extend very far beyond focal inflamation and early scarring. In some cases valvular thickening consisting of edema only is present; in other cases, an occasional area of fibrinoid degeneration of collagen and proliferation is evident. These changes are supplemented by small nodular and focal collections of mononuclear cells and occasional polymorphonuclear leukocytes on the valve. One valve examined demonstrates early scar formation, consisting of fibrosis of the valve cushion, but this is minimal and does not extend far into the valve.

(6) Fibrosis and Scarring. Fibrosis and scarring is considered to appear in the healing process in human cases of rheumatic fever according to Klinge (1933). In as much as this investigation is of reasonably short duration and only three insults to collagenous tissues are assumed to have occurred, one would not expect extensive fibrosis and scarring. The scanty inflamatory and proliferative changes seen in heart valves can reasonably be expected to subsequently lead to fibrosis and scarring. In one valve cushion already

mentioned, fibrosis is evident. In a number of the animals sacrificed late in the experiment there seems to be an increased amount of dense fibrous tissues surrounding some of the smaller coronary arteries and arterioles. We assume that these irregular changes are the result of earlier allergic inflamatory changes in as much as this irregular perivascular fibrous tissue is not seen in the animals sacrificed early in the experiment. No conclusions concerning patch fibrosis and hyalinization within the myocardium can be drawn as this reaction was seen inconsistently and scattered throughout all ages of animals in the investigation. It should be emphasised that none of the reactions in this category were sufficient to produce lesions easily visible to the naked eye.

The lung demonstrates several of the features characteristically seen in rheumatic pneumonitis, as described by Rich and Arnold (1947). These findings are focal collections of mononuclear cells within the alveoli, about the vessels, and about the bronchi. A focal plugging of the capillaries with fibrinous or hyaline thrombi is prominent, and thrombosis of an occasional minute vein can be found. The lesions are usually focal and rather marked segmental intimal proliferation is present in a number of vessels (this is thought to be a prominent feature of rheumatic pneumonitis).

The alterations of the lung just described do not appear in very many of the lungs examined, but it is noted that the changes are more easily found in the lungs of rabbits which have a more extensive cardiac involvement.

A marked constriction of pulmonary vessels with transudation of erythrocytes and protein into the alveolar spaces is a prominent finding in the rabbits that died of acute anaphylaxis. There is an associated pulmonary edema and congestion in these cases. The se findings go hand in hand with the description of acute anaphylaxis in the rabbit by Coca (1919).

An incidental case of non-specific pneumonia is present in a few of the rabbit lungs, and a number of them show a focal atalectasis diffusely distributed throughout the lung sections examined.

Generally, the liver sections show reversible degenerative changes in the liver cord cells with an associated congestion of central veins. A small number of livers exhibit marked cloudy swelling with obliteration of the venous sinusoids.

A segmental polyarteritis is present in the livers of two rabbits examined (no. 14 and 15). This lesion varies from an edema of the media to an advanced lesion consisting of necrosis, fibrinoid alteration ah hyalinization of the media, and mononuclear and polymorphonuclear cellular infiltration of all layers of the vessel wall. In addition, a marked intimal proliferation is present. This lesion is more prominent in the moderate sized arteries and arterioles. Although many venous sinusoids are distended with erythrocytes, portions of the liver tissue are bloodless and are undergoing a degeneration similar to that seen in vascular insufficiency. This of course may be attributed to

thrombus formation onto an arteritic lesion of this type.

In most instances, the sections of kidney tissue do not show changes other than cloudy swelling or granular degeneration of the tubule lining cells. Occasionally, hyaline casts are present in a very small number of kidneys. One of the rabbits (no. 14) demonstrates a polyarteritic lesion in a moderate sized artery; this is similar to that described in the liver.

An incidental case of pyelonephritis is seen in rabbit no. 12(2). The kidneys of rabbit no. 30 show a rather marked fibrosis and segmentation of a moderate number of glomerular tufts, adhesions between visceral and parietal epithelium of the glomeruli, and increased cellularity of the tufts. These changes are consistent with those seen in experimental nephritis by Smadel (1936).

The thymus and adrenal glands do not show remarkable changes. The only alterations noted were cloudy swelling and granular degeneration in the adrenal cortex.

The spleen demonstrates a prominence of the lymphoid follicles and a marked congestion of the red pulp in practically every case. Peripheral palisading of lymphocytes and active germinal centers are present in the follicles.

A marked fibrosis of the spleen, consisting of a fibrous thickening of the capsule and trabeculi and fibrosis within the red pulp, is present in one of the rabbits (no. 17). The lymphoid elements are not indistinct in this case and numerous multi-

nucleated cells containing granular pigment appear in the venous sinusoids.

In summary the microscopic findings are very suggestive of and very similar to rheumatic carditis, rheumatic pneumonitis, and polyarteritis nodosa, and suggest somewhat of an interrelation of the three intities.

DISCUSSION

Through the years many people have attempted to establish the pathogenesis of rheumatic carditis and currently the following concepts have evolved from the work of several investigators.

The characteristic reaction of the tissue of the heart to the causual agent or agents of rheumatic fever is the formation of the Aschoff body. The initial change is a swelling, fusion and degeneration of the collagenous fibers so that they stain deeply with acidophilic dyes, and take specific stains for fibrin-- hence the designation "fibrinoid degeneration or <u>fibrinoid Verquellung</u> by Klinge (1933). At about the same time a few cells, slightly larger than lymphocytes, with a dense chromatic nucleus and scant cytoplasm, collect about the periphery of the swollen collagen. This was designated as the beginning of the inflamatory phase by Klinge (1933). Specialized mesenchymal cells (the Anitschow Myocytes) which are present only in the heart become conspicuous throughout the interstitial tissue in this inflamatory stage (Clawson, 1929). Other cells soon appear within the nodule,

known as "Aschoff cells"; they are seen at about the midphase of the development of the nodule (Gross and Ehrlich, 1934). These cells are large, with a prominent vesicular nucleus containing a central nucleolus. The cytoplasm is abundant, basophilic, and irregularily outlined. Multinucleated cells are frequently seen (Gross and Ehrlich, 1934).

As healing proceeds, the cells become elongate and appear as fibroblasts (Gross and Ehrlich, 1934). Between the cells a reticulin fibrin is deposited (Gross and Ehrlich, 1934). Finally, all evidence of previous change disappears, and a small focus of scar tissue remains (Gross and Ehrlich, 1934, Clawson, 1929, and Klinge, 1933). This was designated as the third stage of rheumatic carditis or the stage of fibrosis and scarring by Klinge (1933). Klinge (1933) states that a similar process may occur in the valves and other structures of the heart without the actual formation of the Aschoff body proper.

The primary interest of this investigation as previously stated rests upon our desire to confirm the work done by others, to demonstrate to ourselves whether this sequence of events in pathogenesis is accurate, and (to be reported elsewhere) whether cortisone would modify the course of development of these lesions. Let us see if these goals have been reached in this investigation.

The stages of rheumatic carditis, as described by Klinge (1933), consist of three consecutive processes: the degeneration of

collagen, the inflamatory cellular infiltration, and the healing process consisting of fibrosis and scarring of the primary lesions.

In this experiment, we were able to reproduce all three of these stages to some extent.

Fibrinoid degeneration appeared in the hearts of a sizeable number of experimental rabbits, but it was an especially prominent feature in eight of the thirty-one rabbits. These eight rabbits died of acute anaphylactic shock during the earlier part of the investigation. Either at the time of the second or third innoculation of horse serum. These innoculations were given on the eleventh and twenty-first days respectfully. We, therefore, came to the conclusion that fibrinoid degeneration is a change occurring early in the active stage of experimental rheumatic carditis, as was previously stated by Klinge (1933) and others.

Inflamatory cellular infiltration, similar to that seen in human cases of rheumatic carditis, was seen in almost all of the rabbit hearts examined. It consisted of a non-specific cellular infiltration (diffuse or focal) and also in the form of Aschoff nodules, which are characteristic of rheumatic fever. In some instances, there were numerous Anitschow myocytes indicating a marked reaction. A few multinucleated giant cells of Aschoff type were also present in the Aschoff nodules. According to Gross and Ehrlich (1934) this type of cell is seen only in the midphase of development in the Aschoff nodule; therefore, this finding would tend to place the development of the nodule in that stage of

development. This inflamatory cellular infiltration seemed to be in the most active phase in several of the experimental animals, and it appeared at all stages of the experiment. As a rule the Aschoff-like nodules were located in a perivascular position. These lesions varied from small accumulations of cells to more extensive collections that tended to obliterate the lumina of the vessels.

Fibrosis and scarring in its earliest stages appeared in several of the rabbits, occurring in the valves, Aschoff bodies, and other places where collagen is normally seen in the heart. In these cases, the fibrosis was very minimal and no gross scarring or deformity was present. It appeared as a minimal but definitely prominent feature in nine of the thirty-one rabbits. In these cases it was still in an early phase; but it was marked enough to be distinguishable under the microscope. No gross scarring was present, such as occurs throughout the myocardium in the human case of rheumatic fever.

Valvular changes consisting of active and healing phases of rheumatic fever appeared in a number of rabbit hearts examined. They were more prominent in eleven of the thirty-one rabbits. The changes varied from a mere initial swelling and degeneration of the collagen fibers to a cellular infiltration followed by an early fibrosis. The fibrotic and scarring processes when present were very minimal in the valve and could be distinguished accurately

only by microscopic examination. The occurence of the valvular changes was seen inconsistently-scattered throughout the series of animals; therfore, no conclusion can be made as to the time that these changes begin to occur.

A small number of the test animals demonstrated a definite vascular change in the coronary arteries and arterioles, this seemed to be a prominent feature in four of the rabbits. The changes varied from a mere intimal proliferation to an inflamatiory lesion involving all coats of the vessel wall with a mononuclear cellular infiltration and fibrosis similar to that seen in the Aschoff nodule. The lesion is segmental in character and is very similar to polyarteritis nodosa, but it is designated as rheumatic arteritis by Vaubel (1932). He stated it is characteristically seen in the heart in rheumatic fever. The development of this lesion seemed to closely parallel that of the Aschoff nodule in our series. At times the only distinguishing feature was the absence of intimal involvement in the Aschoff nodule.

In summary, twenty-four rabbits of the thirty-one examined demonstrated positive signs of carditis. We were convinced that the lesions seen were similar to those appearing in early cases of human rheumatic carditis. However, we were not able to produce gross chronic rheumatic cardiac changes. In as much as this investigation was of reasonably short duration and only three insults to the heart tissues are assumed to have occurred, one

should not expect these chronic changes to occur.

We come to the conclusion that we were able to reproduce the work of Rich and Gregory (1943) and others in this field.

In as much as the lesions appear inconsistently in the series of animals and are not clear cut in all cases, it would be quite difficult to definitely state the time of occurence and duration of each stage of the active and healing processes of experimental rheumatic carditis.

CONCLUSION

An attempt was made to repeat the work of Rich and Gregory (1943) and others in producing a rheumatic-like carditis in rabbits by three massive intravenous injections of sterile normal horse serum. The animals were sacrificed at monthly intervals and the heart, liver, lungs, kidneys, spleen, and thymus were examined for gross and microscopic changes.

Gross findings were insignificant, but definite microscopic findings were present in twenty-four of the thirty-one rabbits examined consisting of fibrinoid degeneration, inflamatory cellular infiltration, and very early fibrosis as seen in rheumatic fever. Marked scarring of the valves and other gross features of chronic rheumatic carditis were absent. We concluded that we were able to reproduce the work of rich and Gregory (1943) in producing rheumatic caridits in rabbits and that the lesions produced were very similar to or the same as those seen in the human heart

during the active phase and early healing processes in rheumatic fever.

In addition, the microscopic findings demonstrate lesions very similar to those seen in rheumatic pneumonitis and polyarteritis nodosa. This tends to suggest an interrelation of the three entities, namely, rheumatic fever, rheumatic pneumonitis, and polyarteritis nodosa.

ACKNOWLEDGEMENT

In the preparation of this thesis, the advice and assistance of many friends has been sought and frequently accepted, and to all who contributed suggestions I am sincerely grateful. While it is not possible to list all who were helpful, I would like to express my gratitude to Hans Rath who aided in the treatment of the animals, the sacrifice and autopsy of the animals, and the cutting of blocks for microscopic sections from the fixed tissues. Dr. H. W. Mc Fadden undertook the laborious task of reading the several microscopic sections and gave many suggestions and timely bits of advice in the compiling of the data for this paper. The fund with which the rabbits for this investigation were purchased, was donated by the Phi Chi Medical Fraternity Research Fund.

PLATE I

This illustration demonstrates the incisions made in opening the left ventricle of the heart. They are indicated by the dark heavy lines. A transverse block was taken through both ventricles (shown in plate II) and a longitudinal section was taken from the left ventricle (shown in plate II).

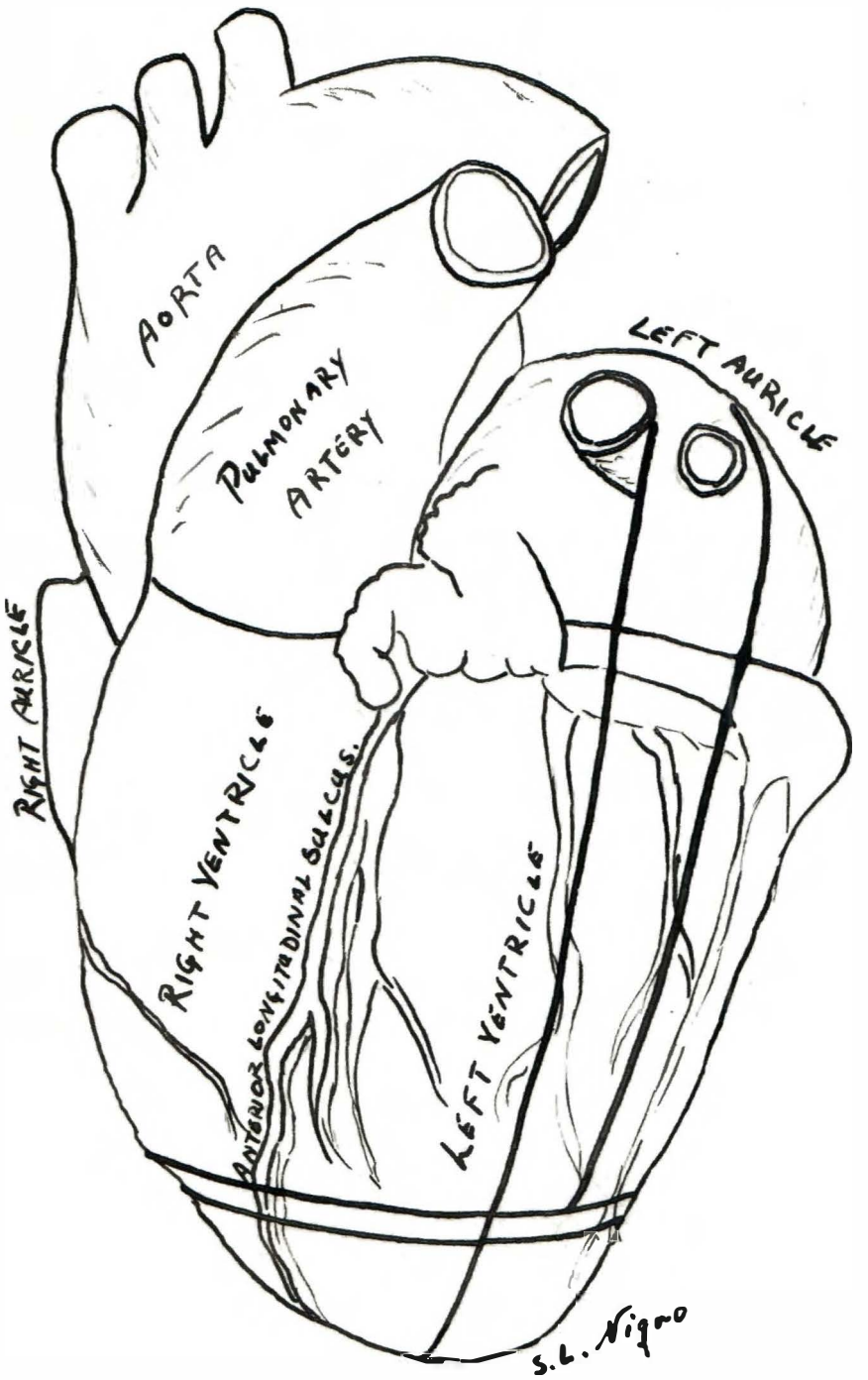

AORTA
PULMONARY ARTERY
LEFT AURICLE
RIGHT AURICLE
RIGHT VENTRICLE
ANTERIOR LONGITUDINAL SULCUS
LEFT VENTRICLE
S.L. Nigro

PLATE II

Plate II demonstrates the transverse block taken through the distal part of both ventricles in order to get a portion of myocardium of the wall of the right ventricle, left ventricle and transverse interventricular septum. This block was taken routinely from each heart examined.

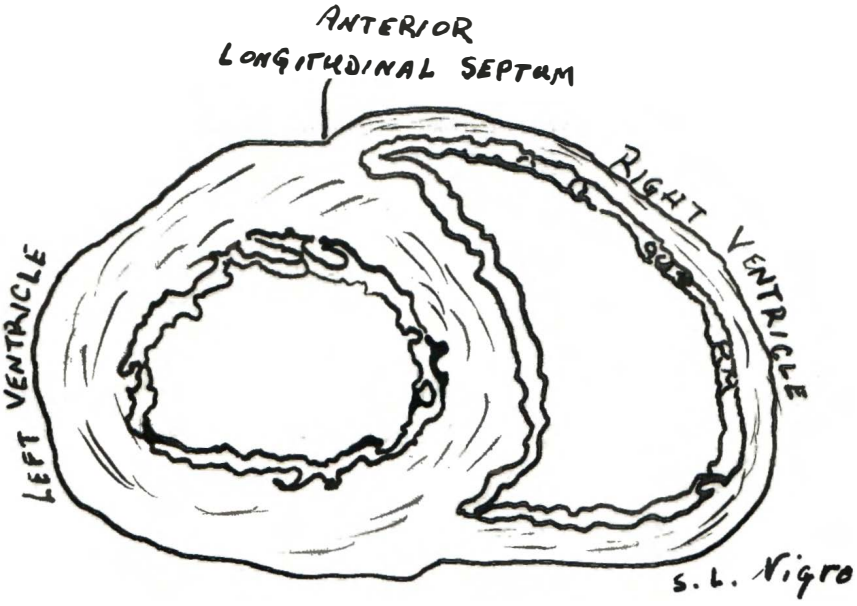

ANTERIOR
LONGITUDINAL SEPTUM
RIGHT VENTRICLE
LEFT VENTRICLE
S.L. Nigro

PLATE III

This drawing demonstrates the block taken from the left ventricle with a portion of mitral valve intact with the papillary muscle. This section was taken routinely from every heart examined.

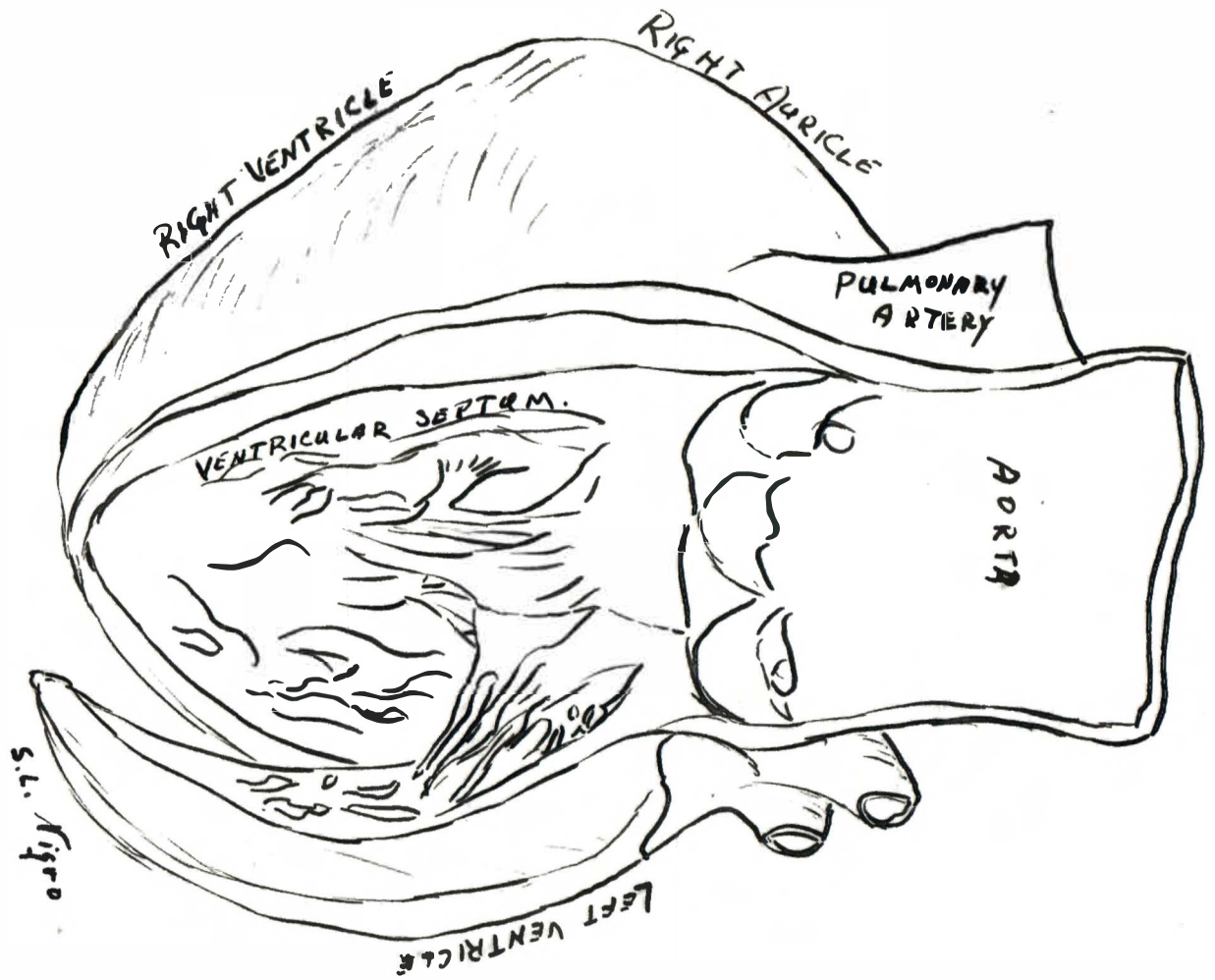
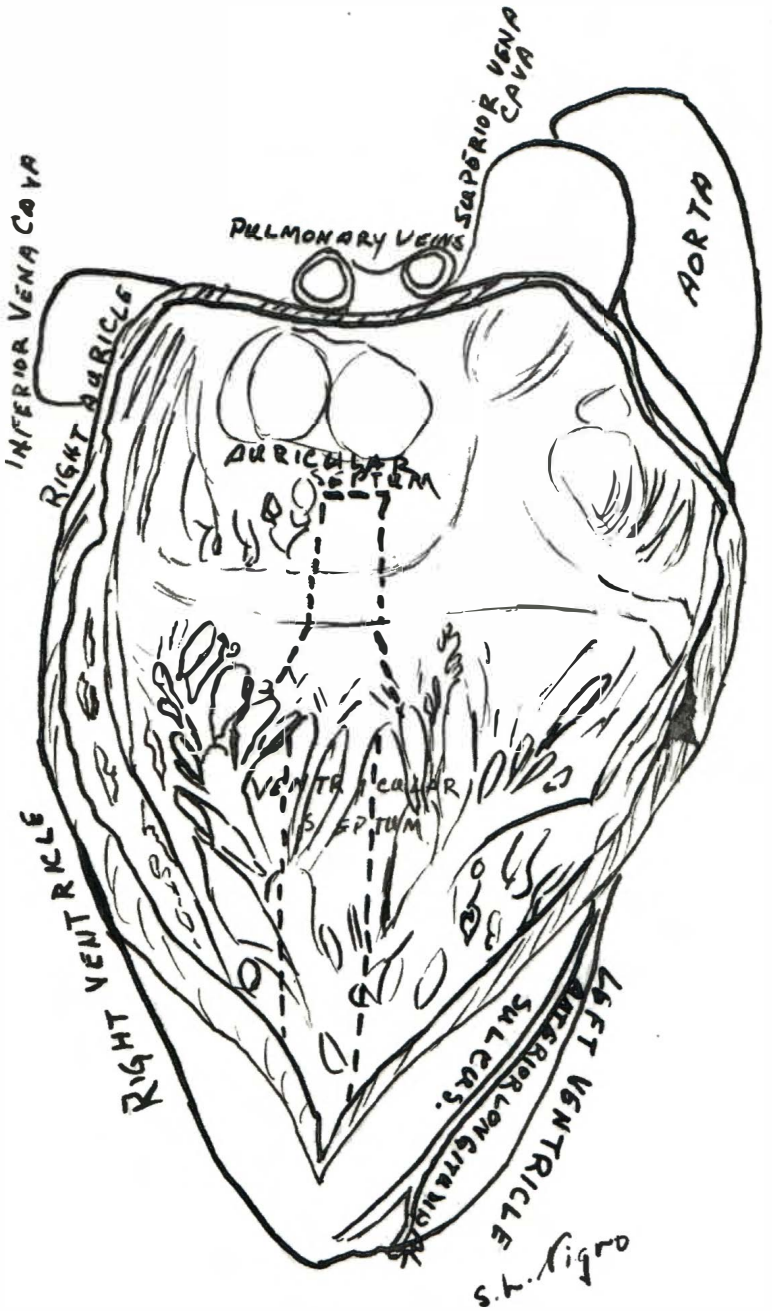

RIGHT VENTRICLE
RIGHT AURICLE
PULMONARY ARTERY
VENTRICULAR SEPTUM.
AORTA
LEFT VENTRICLE

PLATE IV

This drawing illustrates the block taken from the ventricular septum including a portion of the tricuspid valve intact with the papillary muscle (indicated by the dotted line). This was taken only when there was a questionable thickening of the tricuspid valve.

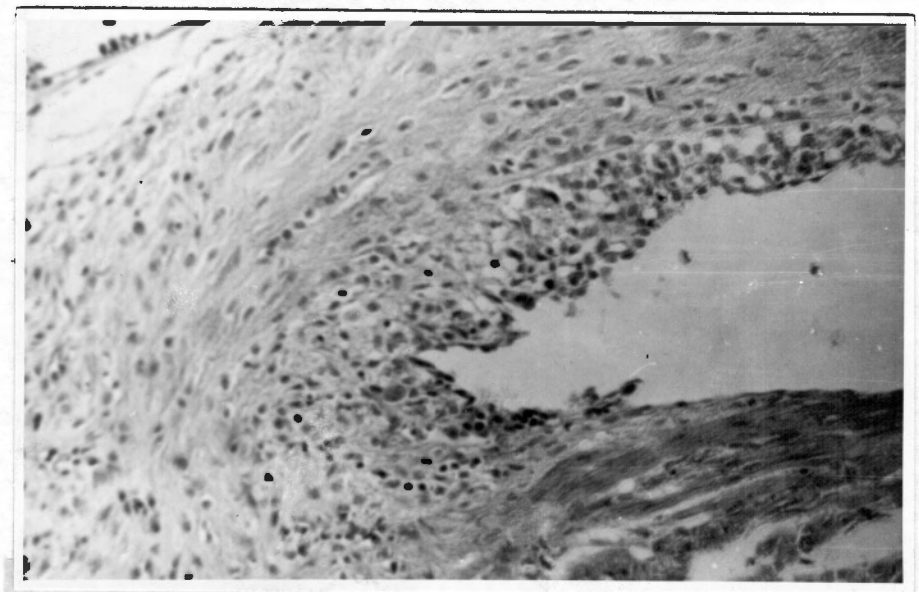

INFERIOR VENA CAVA
RIGHT AURICLE
PULMONARY VEINS
SUPERIOR VENA CAVA
AORTA
AURICULAR SEPTUM
VENTRICULAR SEPTUM
RIGHT VENTRICLE
ANTERIOR LONGITUDINAL SULCUS
LEFT VENTRICLE

PHOTOMICROGRAPHS

(1) Low power view of the base of the mitral valve of hear no. 14. This shows a non-specific inflamatory cellular infiltration associated with a degeneration of collagen.

(2) High power view of the same area at the base of the mitral valve in heart no. 14. This shows a mononuclear cellular infiltration. The nuclei of these cells are somewhat larger than those of the lymphocytic luekocytes. Anitsohow myocytes are present (one is indicated by and arrow).

1



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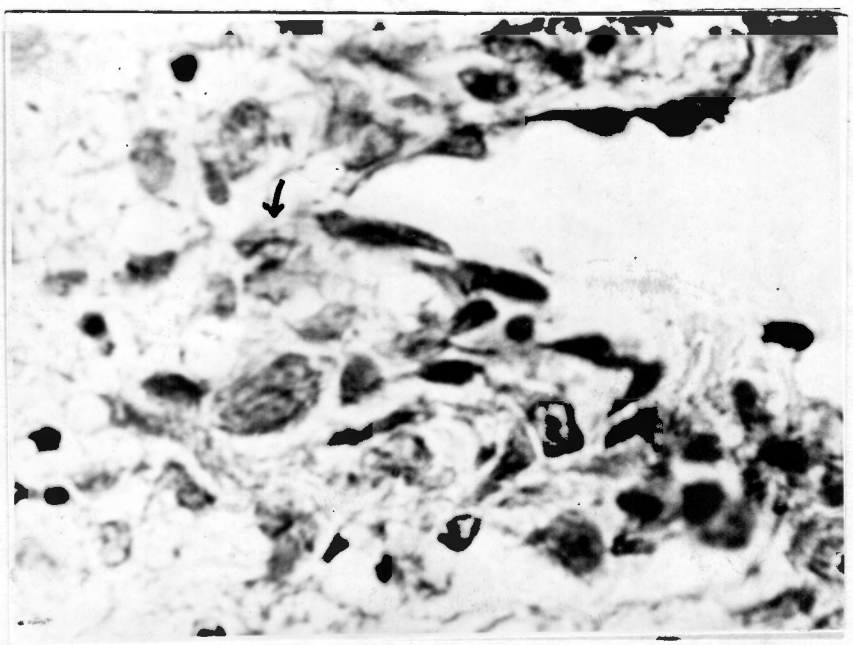
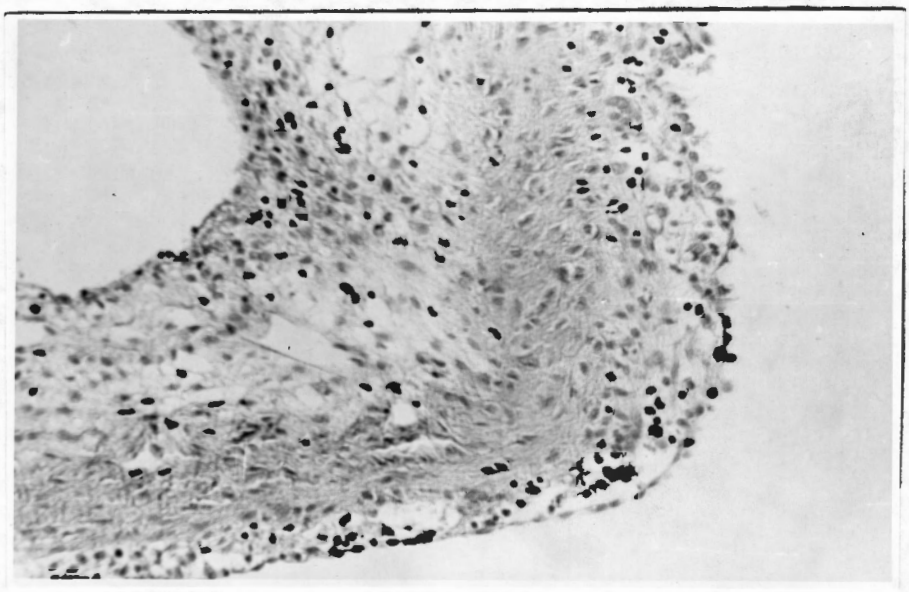


(3) Low power vie of a nodule on the endocardial surface of this same valve in heart no. 14. Note the area of fibrinoid degeneration and the cellular infiltration present.

(4) High power view of the nodule shown in photomicrograph no. 3. This shows the mononuclear cellular infiltration and the Anitschow myocytes present.

3



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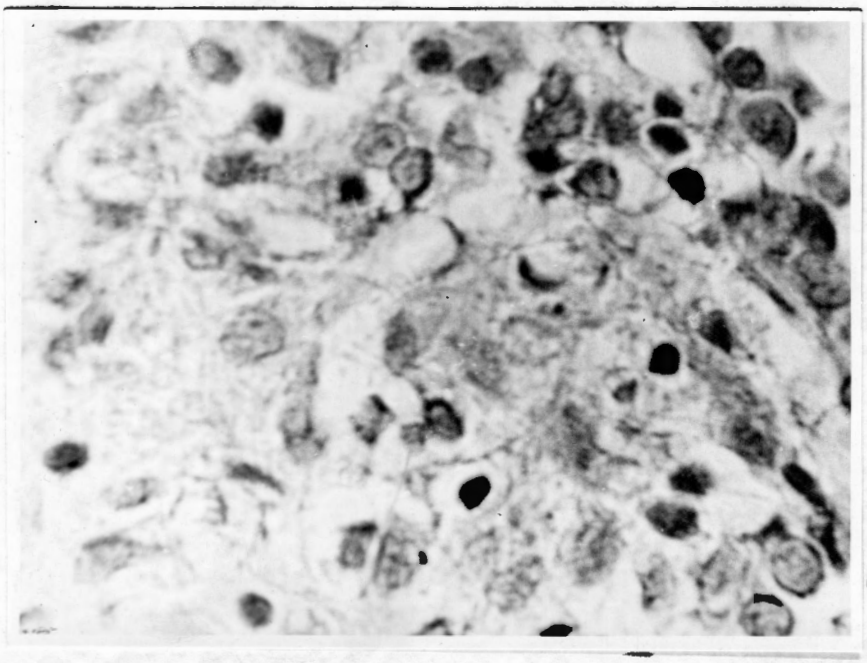
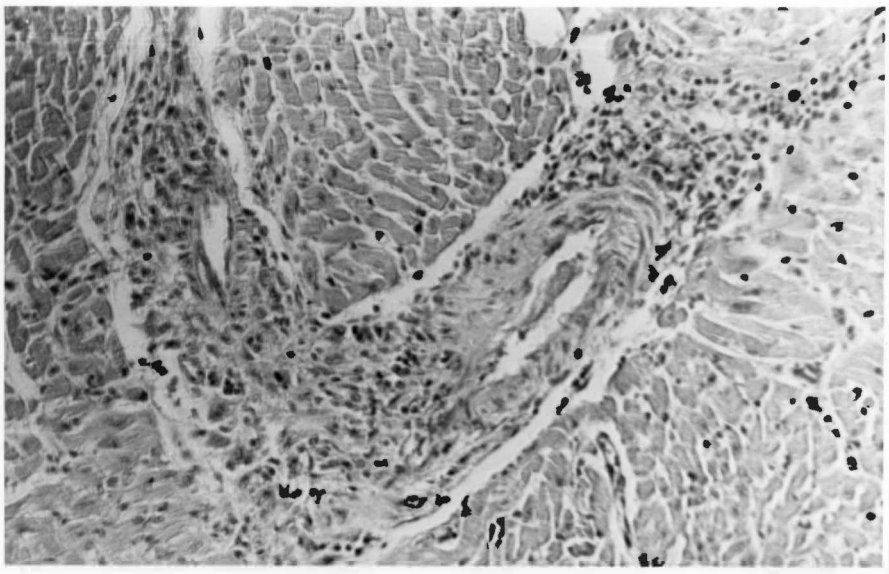


(5) Low power view showing an Aschoff nodule in perivascular position in the myocardium of heart no. 14. There is fibrinoid degeneration, inflamatory cellular infiltration, and an early fibrosis present throughout the adventitia of the vessel and surrounding supporting tissue. This tends to obliterate the lumen of the vessel.

(6) High power view of the Aschoff nodule shown in photomicrograph no. 5. Note the multinucleated cells present. These cells are large with prominent vesicular nuclei containing a bar-like central nucleolus. The cytoplasm is abundant and irregular in outline. A few Anitschow myocytes are also present.

5



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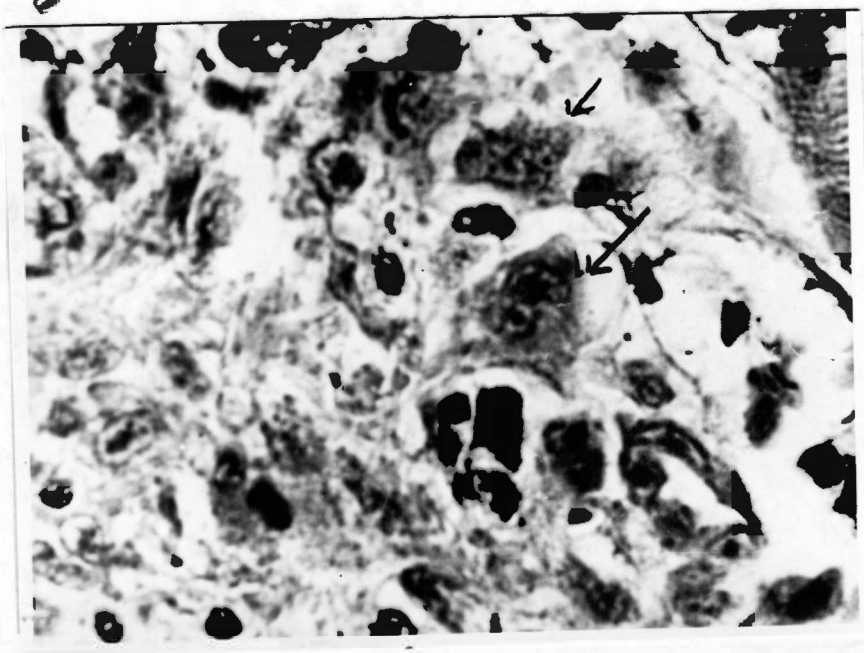
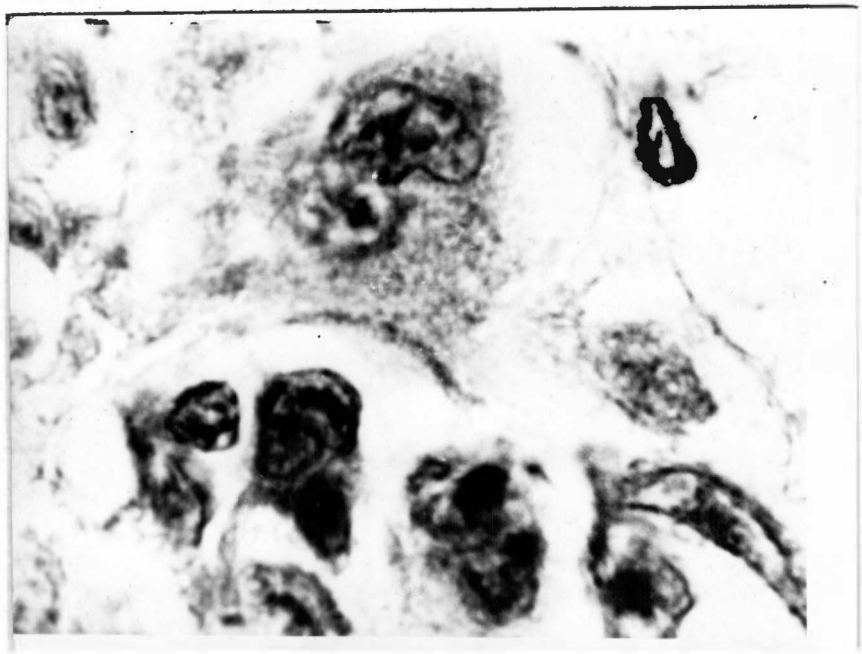


(7) Oil Immersion view of an Aschoff cell in photomicrograph no. 6. This shows the features previously described. It accentuates the granularity of the cytoplasm and the irregular indistinct outlie of the cell. The nuclei are large and vesicular containing a bar-like central nucleolus in each of the two nuclei. Gross and Ehrlich (1934) referred to this as the "fibrocytoid type nucleus".

(8) High power (oil immersion) of the same field as in photomicrograph no. 6. Note the typical Anitschow myocyte present in the lower right corner of this photomicrograph. The distinguishing feature of this cell is stringy distribution of the chromatin through the center of the nucleus and the relative indistinctly delineated nuclear membrane.

A



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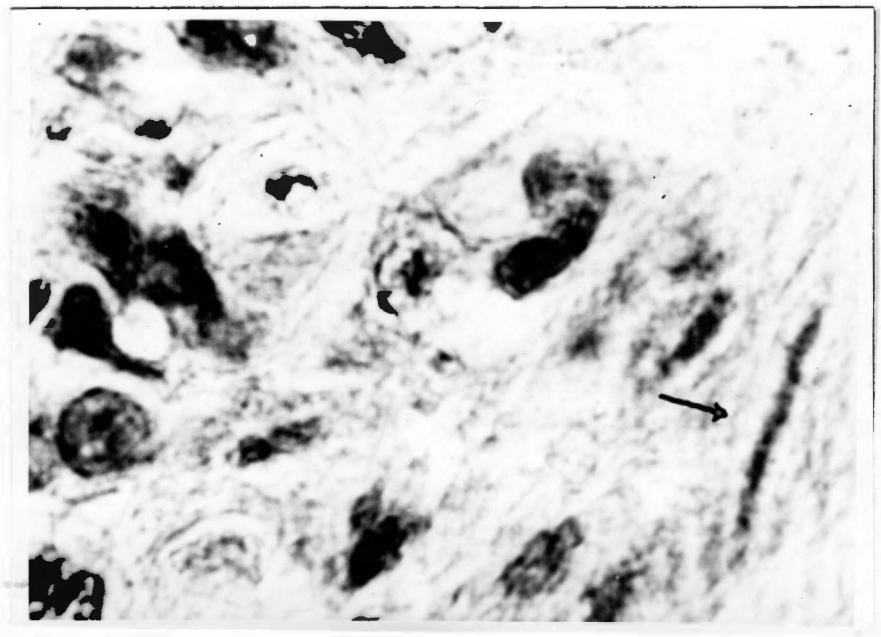
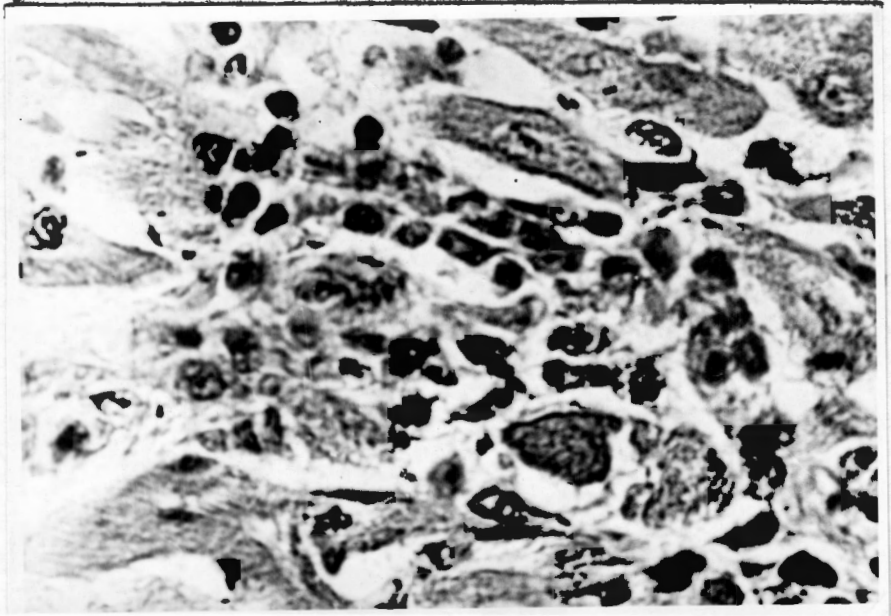


(9) High power view of another Aschoff body in the myocardium of heart no. 14. There is a fibrinoid degeneration gpresent associated with myocardial degeneration. The inflamatory cells appear to be mononuclear in type.

(10) Oil immersion view of the same lesion depicted in photomicrograph no. 9. The cells appear to be mononuclear in type.

9



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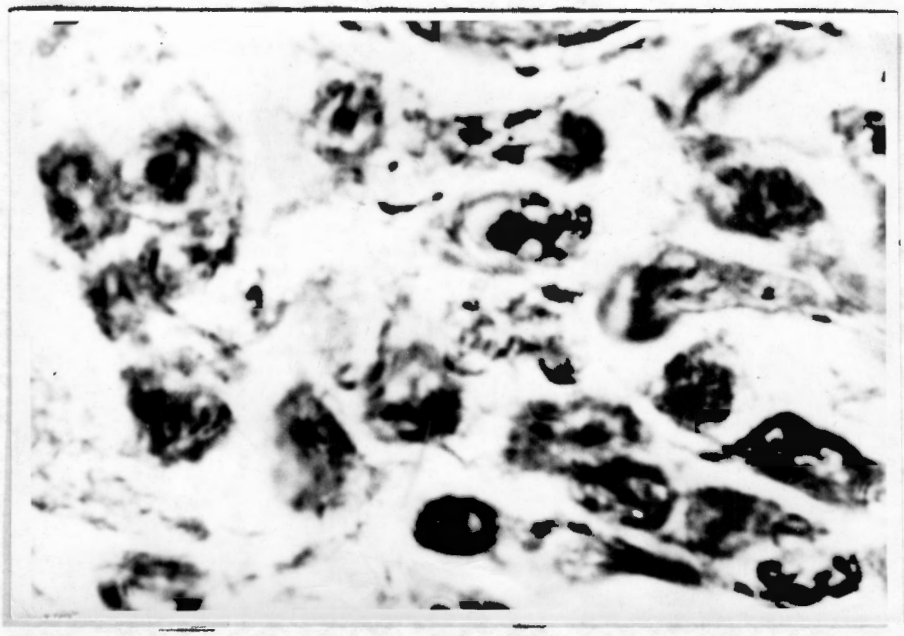
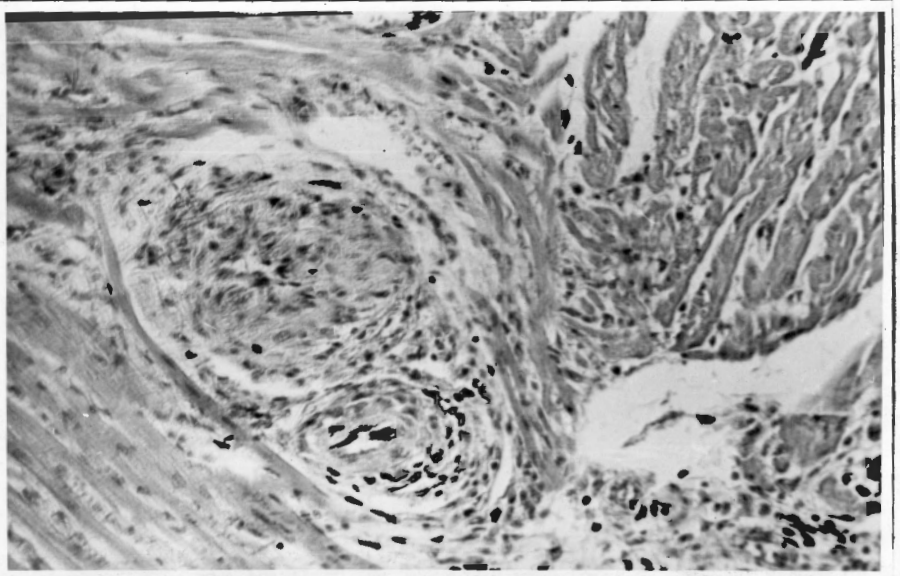


(11) Low power view of periarteritis lesion very similar to polyarteritis nodosa in two coronary arterioles of the heart in no. 14. It shows an involvement of all layers of the arteriolar wall with fibrinoid degeneration, inflamatory cellular infiltration, and hyalinization of the media. The intima has proliferated and is encroaching upon the lumina of the two vessels.

(12) Low power view of periarteritis in the liver of rabbit no. 14. The picture here is of a similar type as seen in the heart (of this rabbit) but more severe in character. It shows a marked necrosis, fibrinoid alteration, and hyalinization of the media with infiltration of mononuclear and polymorphonuclear cells. An intimal proliferation and a cellular infiltration of the intima is prominent with a complete obliteration of the lumen of the vessel.

11



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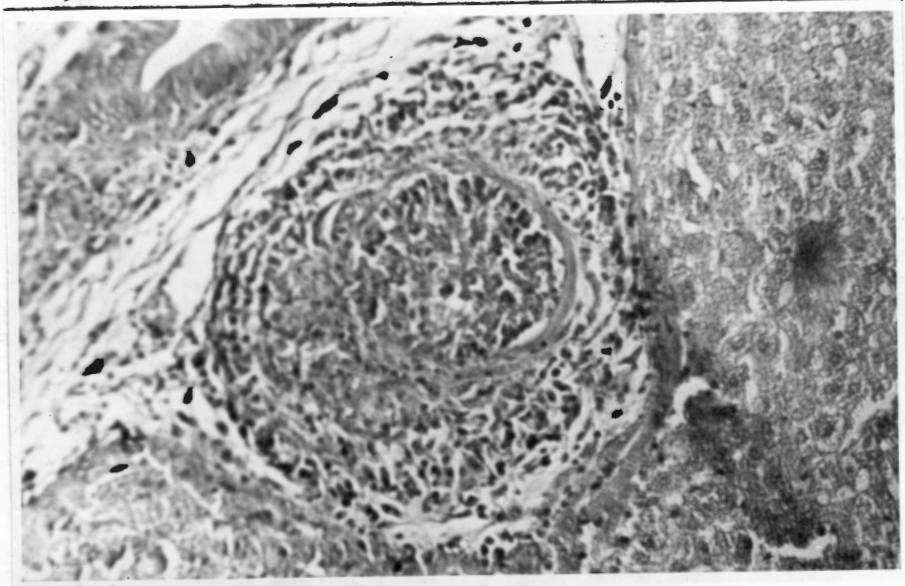
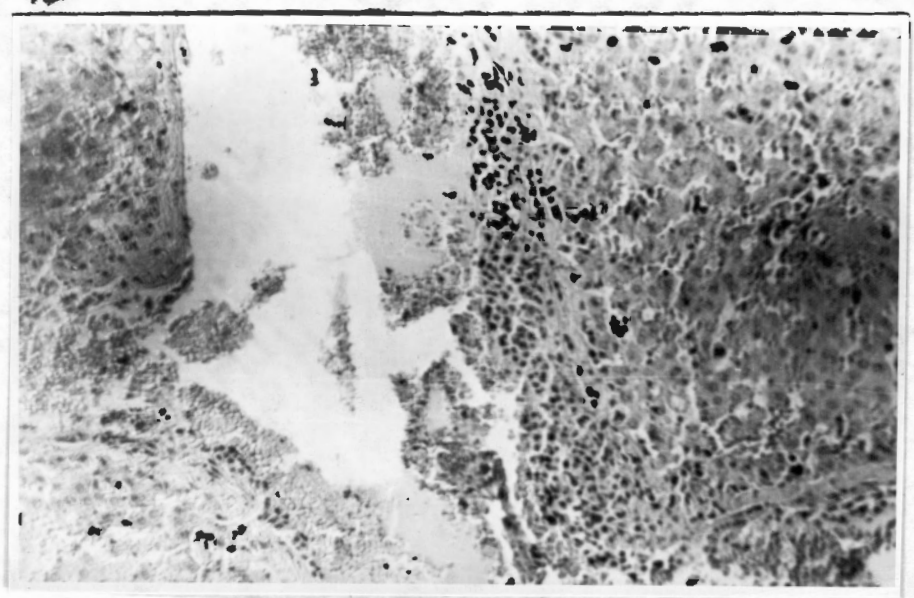


(13) Low power view of a vascular inflamatory lesion in the liver of rabbit no. 15. This lesion does not demonstrate the profound changes seen in photomicrograph no. 12, but a fibrinoid degeneration and cellular infiltration of the vessel wall is present. The intima does not show the very marked changes as demonstrated in photomicrograph no. 12, but it does appear to be proliferating.

(14) High power view of the same vascular inflamatory lesion depicted in photomicrograph no. 13. It demonstrates the presence of mononuclear and polymorphonuclear inflamatory cells. In addition a number of fibroblasts are present.

13



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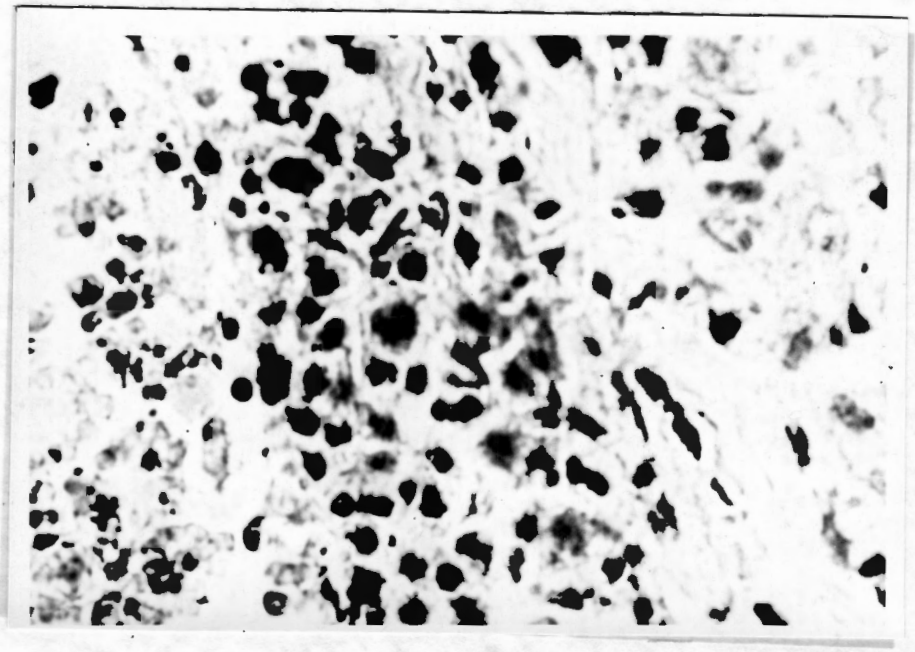
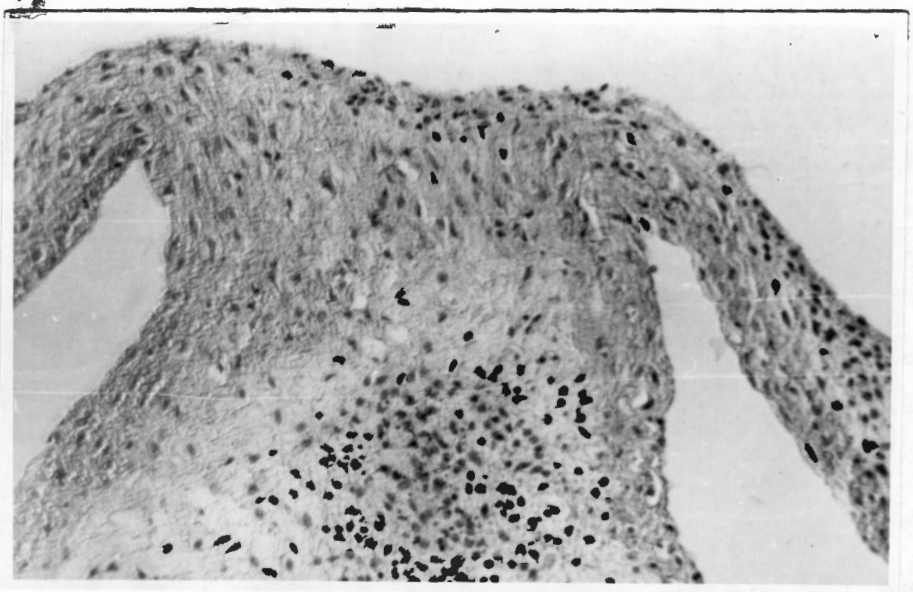


(15) Low power view of a nodule in the base of the mitral valve in the heart of rabbit no. 13. It shows a prominent fibrinoid degeneration and inflamatory cellular infiltration of mononuclear and polymorphonuclear cells.

(16) High power view of the same nodule in photomicrograph no. 15. This shows the mononuclear and polymorphonuclear cells more prominently. There si a fibrinoid change present, but it does not appear distinct in this photograph.

15



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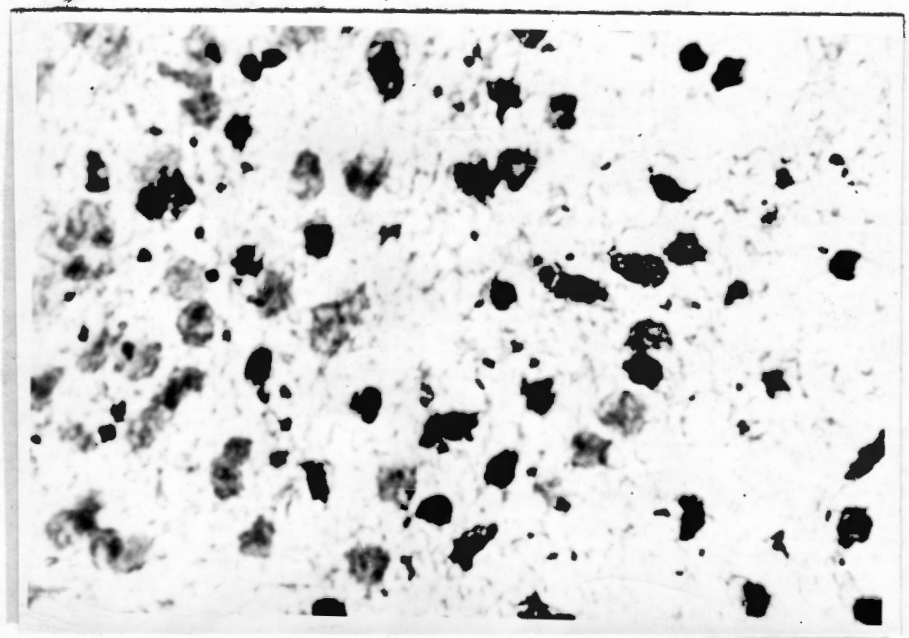
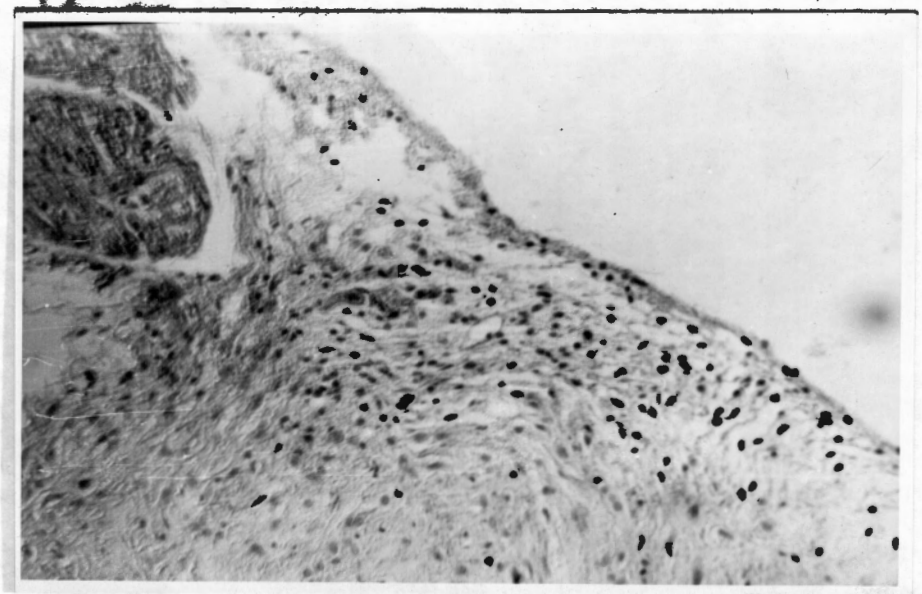


(17) Low power view of fibrinoid degenration at the mitral valve seat in the heart of rabbit no. 13. It consists of a degeneration of collagen consisting of a separation of the fibers by edema, swelling of the individual fibers, and finally degeneration of the collagen fibers. A degeneration of muscle fibers is also present. An associated diffuse non-specific inflamatory cellular infiltration is apparent in the immediate area.

(18) High power view of a focal non-specific inflamatory cellular infiltration in the myocardium of rabbit no. 24. It specifically demonstrates the presence of polymorphonuclear cells and the Anitschow myocytes in addition to mononuclear cells.

17



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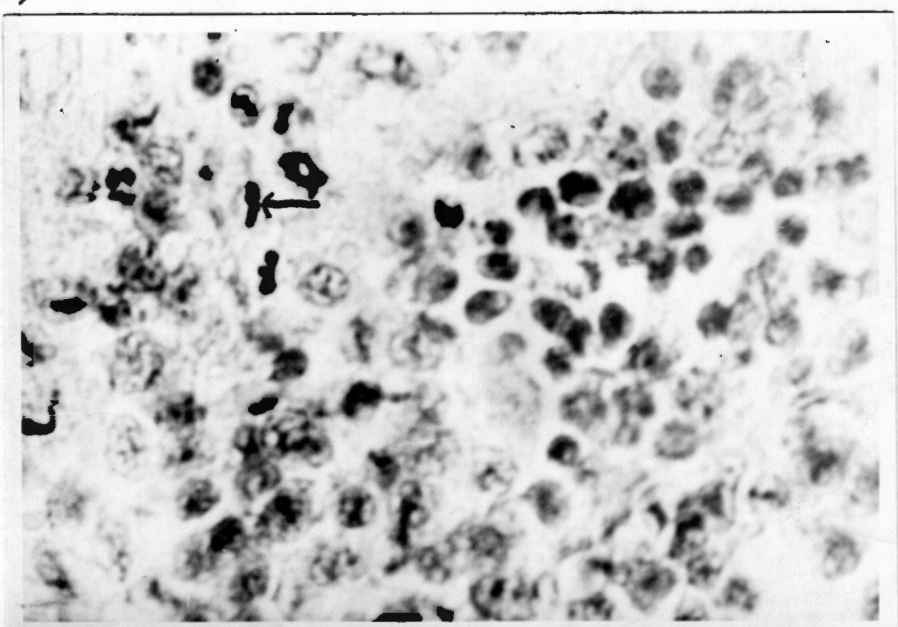
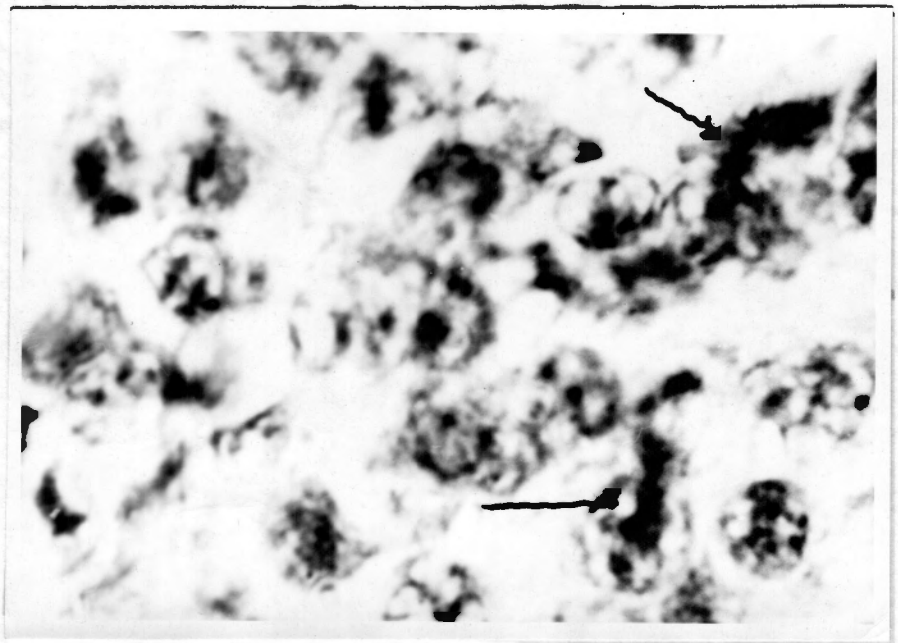


(19) Higher power (oil immersion) of the same area to show the Anitschow myocyte.

(20) High power (oil immersion) view demonstrationg Anitschow myocytes in a papillary muscle of the left ventricle in the heart of rabbit no. 5. The chromatin appears in a stringy strand through the center of the nucleus; this is a characteristic feature of this cell.

19



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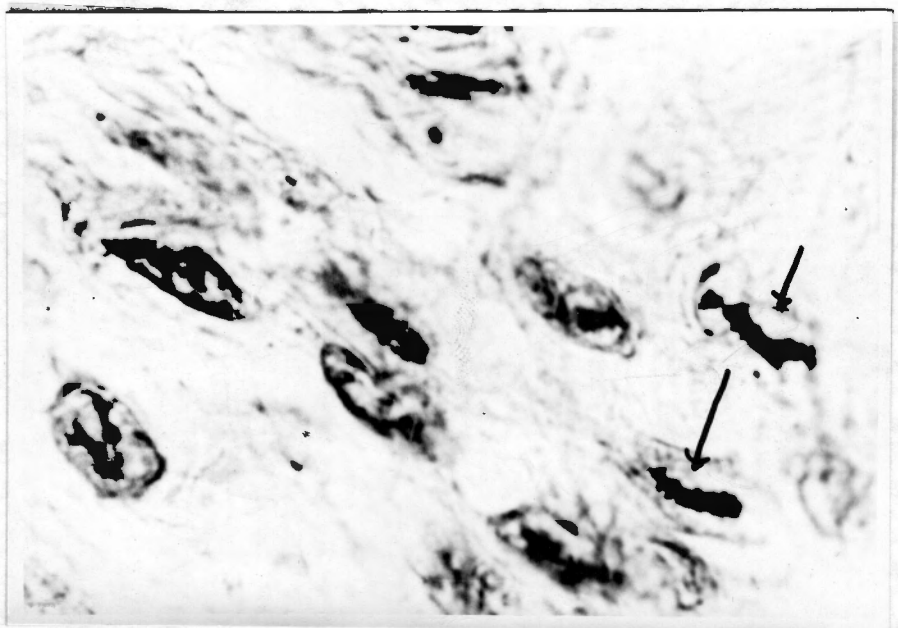
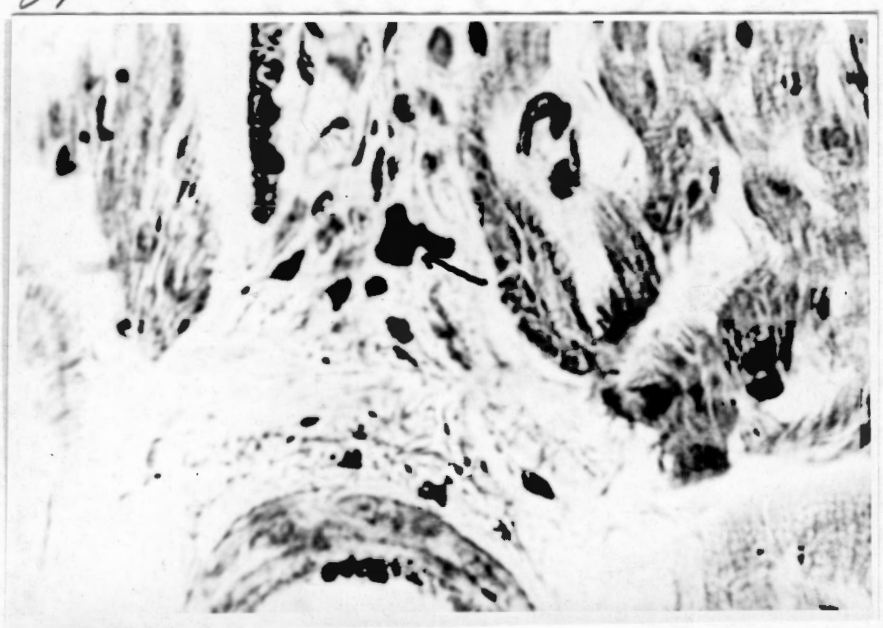


(21) High power view of a small Aschoff nodule in perivascular position in the myocardium of heart no. 5. It consists of a fibrinoid degeneration with an early inflamatory cellular infiltation. A few Anitschow myocytes are evident in this early infiltration. A giant cell is present in the nodule.

(22) Low power view of rheumatic pneumonitis in the lung of rabbit no. 13. The findings appear indistinct in the photomicrograph, but they are present as focal collections of mononuclear cells within the alveoli, a focal plugging of the capillaries with fibrinous or hyaline thrombi and thrombosis of an occasional minute vein.

21



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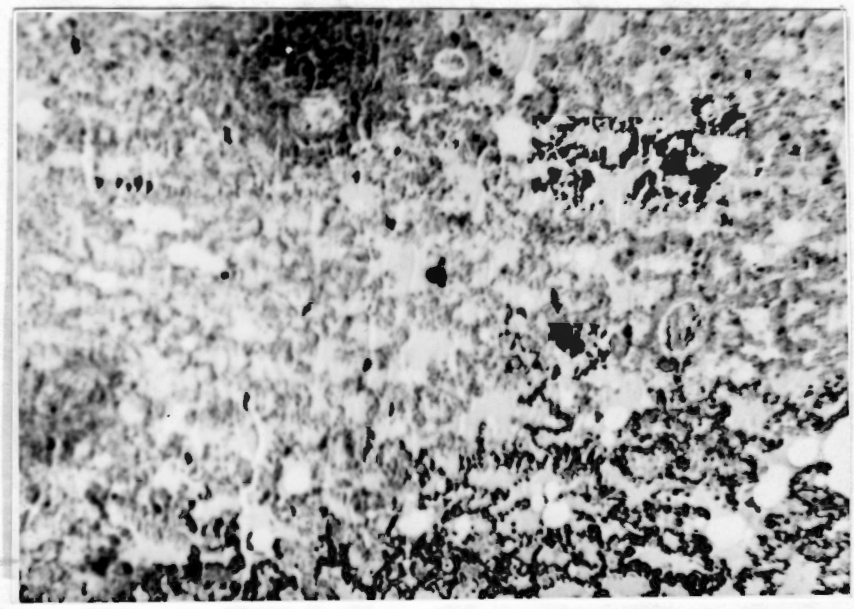

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