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Experimental Studies in the Pathology of Allergy.

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Summary.

Numerous investigators have described morphologic changes attributed to experimentally induced hypersensitivity. Different observers have stressed different lesions, and the recent recognition of sulfonamide hypersensitivity has resulted in further additions to an already long list of tissue alterations. In the experiments forming the basis of this thesis, 77 rabbits were given large doses of horse serum intravenously, and their tissues searched for lesions. An acute arteritis, quite similar to the vascular lesions of periarteritis nodosa was found in 47 cases. Diffuse and focal inflammatory lesions were found in smooth, cardiac and skeletal muscle. The latter changes bore a striking resemblance to the muscle lesions of dermatomyositis, and less closely simulated the muscle changes associated with rheumatoid arthritis. Inflammatory cardiac lesions somewhat reminiscent of the cardiac changes in acute rheumatic fever were also found, but their occurrence in small numbers in control animals vitiated the significance of this experimental result. The joints of 6 animals showed subacute inflammatory changes considerably in excess of any changes seen in control rabbits.

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C.R.M.

EXPERIMENTAL STUDIES IN THE PATHOLOGY OF ALLERGY.

Review of the Literature.

Introduction.

Following Jenner's observations (1) on the "disposition to sudden cuticular inflammation" which those individuals previously infected with small pox or chicken pox exhibited when the skin was reinfected by direct inoculation, there was little awareness that bacterial infection, or parenteral contact with a foreign protein could alter the body's reaction in a subsequent infection or contact with that foreign protein until the experimental studies of Robert Koch (2) were published in 1891. In 1907, von Pirquet (3) published a monograph entitled "Clinical Studies in Vaccination and Vaccination-Allergie" in which he recorded in some detail the observations made by Jenner more than one hundred years previously. He utilized the term "allergy" to connote that changed state (altered reactivity) in the body which may result from infection or parenteral contact with a foreign protein. The early work directed toward recognizing and defining the several ways in which the allergic or hypersensitive state of an animal might become manifest, soon resulted in the recognition of three distinct entities: acute anaphylactic shock in experimental animals, serum sickness in man, and localized inflammatory-necrotic lesions in the tissues of man and animals, occurring at the sites of injection in suitably sensitized subjects.

Acute Anaphylactic Shock.

Portier and Richet (4) in 1902, and Richet (5) in 1907 were among the first to report on the clinical picture of acute anaphylactic shock induced in experimental animals. The protein extract used was somewhat toxic for the dogs tested, but those which survived a first injection, succumbed rapidly with a curious symptom complex if given a second injection of the same material after several days or weeks. In the usual course of events, increased resistance follows the injection of a

sublethal dose of a toxic material, and it was the reverse of this process, that is, loss of immunity, which led Richet to coin the term "anaphylaxis".

It was soon observed that whereas the picture of acute anaphylactic shock tended to be constant within a given species, it varied within rather wide limits from one species to another. A moment after injection, guinea-pigs were seen to develop marked restlessness with bristling of the hair over the head and neck. Loud spasmodic sneezes were accompanied by a brisk rubbing of the nose. A short series of jumps were followed by tonic and clonic convulsions, and increasingly slow but violent inspiratory efforts. Death usually ensued within a few minutes, and it was frequently observed that the heart continued to beat for a few moments after respiration had ceased. The autopsy findings in guinea-pigs dying acutely in anaphylaxis were reported by Gay and Southard (6) in 1907-08. The principal findings were intense intestinal congestion in the presence of active peristaltic movements, together with marked distension of the lungs and numerous small haemorrhages in the heart muscle, pleura, stomach wall and caecum. They called attention particularly to a fatty change in the capillary endothelium, which they believed to be the direct cause of the haemorrhages. Auer and Lewis (7) in 1910, confirmed these observations and showed that the distension of the lungs was not due to simple emphysema. A detailed examination showed that the distension was caused by bronchiolar spasm and the retention of trapped air in dilated alveoli. Section of the vagi and the administration of curare did not prevent the spasm, which was therefore assumed to be of muscular or neuromuscular origin. Death was attributed to asphyxia resulting from respiratory obstruction.

Acute anaphylactic shock in the rabbit was found to be not so readily induced as in the guinea-pig, but if a large enough shocking dose of antigen were given via the intravenous or intraperitoneal route to a sufficiently sensitive animal, a characteristic reaction could be produced. The preliminary signs of irritability and restlessness so prominent in the guinea-pig were absent. The animal quickly

lay flaccid with outstretched legs, gave a few terminal convulsive movements and died. Irregular respiratory movements sometimes continued for a short period after the heart had ceased to beat. At autopsy, the most striking feature was the extreme dilatation of the right auricle and ventricle. In 1919, Coca (8) showed that this dilatation was associated with a marked degree of obstruction in the pulmonary circulation. The evidence for this was found in the increased pressure required to drive fluid from the pulmonary artery to the right auricle. Coca concluded that acute anaphylactic death in the rabbit occurred as a consequence of spasmodic constriction of the branches of the pulmonary artery, which obstruction in the pulmonary circulation rapidly led to dilatation of the right heart and acute heart failure.

The pattern of behavior in a dog subjected to acute anaphylactic shock was found to be somewhat more complicated. Marked excitement and restlessness were rapidly followed by vomiting and the passage of urine and feces, the latter sometimes blood-stained. In rapidly fatal cases, muscular weakness, slow laboured respirations, and progressive vomiting and diarrhoea were terminated by convulsions and death. Biedl and Kraus (9) showed that anaphylactic shock in the dog is associated with a progressive drop in blood pressure. Pearce and Eisenbrey (10) demonstrated that it was not due to failure of cardiac output or influence from the central nervous system, but to collection and stagnation of blood somewhere in the tissues. Manwaring (11) in a series of ligation experiments showed that when the liver was excluded from the circulation, acute anaphylactic shock in dogs could be inhibited. These results dovetailed well with the autopsy findings, wherein it had been observed that congestion of the abdominal viscera was a prominent finding, and in the most acute cases, the congestion in the liver had been seen to reach an extreme degree (12).

Thus it soon became apparent that the organ systems and associated functions likely to be most affected in the generalized reactions of acute anaphylactic shock

were unpredictably different from species to species. There was found to be not only a difference qualitatively in the reactions of anaphylactic shock from species to species, but also a great quantitative difference in the relative ease or difficulty with which the various species could be induced to undergo the phenomenon. Birds were reported to be sensitized by the usual procedures (13). The anaphylactic reaction in the pigeon has been studied in some detail (14) (15). Demonstrable sensitization in the rat seems difficult to obtain (16) (17) (18), although Kellaway (19) has reported the production of a characteristic anaphylactic response. Zinsser (20) has reported that the monkey is also somewhat resistant to the development of sensitization--only mild anaphylactic symptoms could be produced after treatment with repeated doses of antigen.

The specificity of the reaction was recognized at an early date. In 1904, Theobald Smith, working in America, informed Ehrlich of his observations on the extreme sensitiveness of guinea-pigs to widely-spaced injections of toxin-anti-toxin mixtures. Otto (21) studied the phenomenon in considerable detail and showed that the horse serum in the mixtures, not the toxin, was the essential agent. He further noted that an interval of 10 days elapsed after the sensitizing dose before hypersensitiveness was established, and that no hypersensitiveness resulted when large injections of serum were given at short intervals. Rosenau and Anderson in 1906 (22) observed that when guinea-pigs were sensitized to horse serum, they showed virtually no reaction to sera from rabbits, cats, dogs, pigs, sheep, chickens or man. Widespread investigation soon established the fact that animals of a given species always presented the same symptoms and pathologic changes when subjected to anaphylactic shock, irrespective of the type of sensitizing antigen used. The histo-pathologic changes in any of the species submitted to fatal or near-fatal shocks were not marked, which is to be expected, considering the acute nature of the phenomenon.

Localized Inflammatory Reaction of Sensitized Tissues. Arthus Phenomenon

In 1903, shortly after the reports of Portier and Richet (4) on the phenomenon of acute anaphylactic shock, Arthus (23) and Arthus and Breton (24) described an unusual localized inflammatory reaction in the subcutaneous tissues of sensitized animals. When rabbits were inoculated subcutaneously with repeated small doses of horse serum at short intervals, local inflammatory reactions developed at the site of the later injections. The first reactions consisted of little more than transient swellings due to edema, but with successive inoculations, the reactions became progressively more intense. Finally the response took the form of a localized swelling which frequently developed a haemorrhagic and necrotic centre. This localized inflammatory necrotizing reaction (Arthus phenomenon) resulted when a suitable antigen was given to a sensitized animal in such a fashion as to remain localized at the site of injection. It always had the same general characters, although the severity of the reaction could be modified by the dose of antigen used and the degree of sensitivity in the animal employed.

It has been repeatedly observed that vascular damage is characteristic of the histo-pathologic changes in the Arthus phenomenon. Arthus, in his original paper (23) noted the haemorrhagic nature of the lesion. Opie (25), Laporte (26), Pagel (27) and others (28) have described and emphasized vascular damage and thrombosis at the site of inoculation. In an extensive report on the histologic changes in the Arthus phenomenon as seen in rabbits, rats and guinea-pigs, Gerlach (29) stated that vascular spasm may occur, that the haemorrhages may be the result of stasis, and that the pure Arthus phenomenon is expressed by haemorrhages and exudation of leucocytes, whereas the subsequent mobilization of histiocytes is presumably the result of reaction to antigen previously injected. Abell and Schenck (30) observed these effects directly in the living rabbit's ear. They studied the action of horse serum introduced into the moat of an ear-chamber in rabbits sensitized to horse serum, and

obtained direct evidence of the effect of an antigen-antibody reaction upon vascular permeability. Frequently there was arteriolar spasm and arrest of the circulation. Leucocytes adhered to the endothelium and passed through the capillaries in large numbers. At times the white blood cells formed clumps large enough to cause embolic blockage in capillaries and venules. With repeated introduction of horse serum into the moat, extravasation of erythrocytes and necrosis of the capillary endothelium occurred.

The picture of localized allergic inflammation has been studied in numerous tissues by many observers. As early as 1911, Friedberger (31) observed the development of pneumonitis in the lungs of guinea-pigs following the inhalation of a foreign serum to which they had been sensitized. In a report published the following year, Schlecht and Schwenker (32) described pulmonary haemorrhages and foci of acute alveolitis appearing as early as 6 hours after inhalation of the antigen. These observers emphasized the appearance of eosinophile leucocytes, both interstitially and as an exudate into the bronchi. Ishioka (33) emphasized the interstitial character of the inflammation. By the method of injecting horse serum directly into the trachea of sensitized rabbits, Fried (34) studied widespread intense inflammation of the lungs with edema, haemorrhage, leucocytic infiltration, deposition of fibrin, consolidation and necrosis. Prototypes of these procedures have been followed for most of the tissues of the body, including the brain (35) (36) (37), heart, pericardium and aorta (38), eye (39), liver (40), and kidney (41). A detailed account of the Arthus phenomenon in humans was first presented by Lucas and Gay (42) in 1909. Their report was concerned with the localized lesion which occurred frequently in the skin of children who had received two or more doses of antitoxin at frequent intervals. They felt that the local inflammatory reaction corresponded in all particulars to the Arthus phenomenon in rabbits, and the percentage of cases in which this reaction occurred increased directly with

the number of injections given at short intervals subsequent to a primary injection. The local reaction might or might not be associated with the immediate generalized reaction described by von Pirquet and Schick (43).

Serum Sickness

While the reactions of acute anaphylactic shock and the Arthus phenomenon are manifestations of the allergic state which have been studied almost exclusively in animals, our knowledge of serum disease has been derived almost exclusively from observations on man. Among the earliest descriptions of what we now recognize to be serum sickness was that of Dallera (44). In 1875 he transfused human subjects with lamb's blood; urticaria appeared a week to ten days later. However, our present conception of the disease is generally accepted to have been introduced by the monograph of von Pirquet and Schick, "Die Serumkrankheit", published in 1905 (43). These authors recognized three forms of the disease which they termed "normal reaction", "accelerated reaction" and "immediate reaction". The normal reaction follows the injection of serum into an individual who has not previously had an injection of serum, derived, at least, from the same species of animal, or who is not spontaneously sensitive to that particular protein. This injection is usually followed by an incubation period of 6 to 10 days before the appearance of clinical symptoms. Loncope (46) collected 1493 cases in which the incubation period was 8 to 9 days in approximately one-third, and 6 to 10 days in two-thirds. There are rarely prodromal symptoms, for the onset is usually abrupt. The most frequent symptoms are malaise, muscle pains, nausea, vomiting and arthralgia. The commonest signs are skin eruptions, fever, subcutaneous edema and lymphadenopathy. Urticaria is the commonest of the skin rashes. It is the first to appear and may be very trying to the patient. The arthralgia, which is exquisitely painful, is rarely associated with swelling, reddening, or increased heat about the joints. The joints most often involved are those of the hands. Occasionally an effusion of fluid can be demonstrated in the knee joints (45).

The lymphadenopathy may be localized, and those nodes nearest the point of injection are often the first to enlarge. Occasionally the spleen is palpable. The edema is particularly noticeable about the face and eyes; however, it may not be confined to the subcutaneous tissue. The lips, tongue, buccal mucosa and larynx may also be affected.

Disturbances in the conduction of cardiac impulses through the cardiac muscle often accompanies acute anaphylactic shock in cats (47) and rabbits and dogs (48). Cardiac arrhythmias have occasionally been observed in cases of human serum sickness (49). The former has been attributed by Andrus and Wilcox (50) to interference in the blood flow in the coronary circulation.

Headache, drowsiness and meningismus may occur in cases with severe reactions accompanied by considerable edema (45). In such cases, the pressure of the cerebro-spinal fluid is increased, the globulin content may be elevated and the number of lymphocytes increased. Numerous observers (51) (52) (53) have described symptoms and signs related to involvement of the peripheral nervous system following the use of various prophylactic sera, and this complication of serum disease is now generally accepted (45). The pathogenesis of the nerve lesions is not clear. While it is admitted that edema of the nerve sheaths or of tissues about the bony ostia might result in pressure on the nerves, similarity of the symptoms to those which sometimes accompany periarteritis nodosa, suggest that arterial changes, several times reported in serum disease (54) (55) (56) may well involve arterioles in the nerves or their sheaths and be responsible for the observed symptoms. The nervous symptoms and signs have been classified as spinal, radicular and neural, and the cervico-brachial plexus is alleged to be the most often affected (45). In 1922, Mason (57) reported the occurrence of optic neuritis in serum sickness, and other single nerves such as the long thoracic have been implicated.

Minor blood changes have long been recognized. Von Pirquet and Schick (43) wrote that a leucocytosis followed by leucopenia with increase in

the mononuclear cells may be associated with the disease. Eosinophilia in the peripheral blood stream is said not to be characteristic (45).

The "accelerated reactions" differ from ordinary serum disease in that the incubation period is reduced to one to three days, the onset of the clinical picture is more explosive, the symptoms are more violent and the course is frequently shorter.

"Immediate reactions" are the most serious. Alarmingly violent and even fatal reactions may be produced in persons, such asthmatics, who are naturally sensitive to the protein employed. Sudden dyspnoea, cyanosis, asphyxia, generalized edema, circulatory collapse, convulsions and profuse urticaria are among the common symptoms and signs. Kojis (58) has collected the accounts of 61 deaths following the injection of foreign serum. While not constituting an entity or symptom-complex that is too well defined, these cases may be considered in most respects as comparable to acute anaphylactic shock in susceptible laboratory animals.

Serological Relationships in Serum Sickness, Acute Anaphylactic Shock and the Arthus Phenomenon.

Hamburger and Moro (59) were the first to publish the results of serological studies performed during the course of serum disease. These observers studied the precipitin reaction in children following injections of diphtheria antitoxin. They found that the reaction appeared during serum disease, and as the precipitin titre in the circulating blood increased, the precipitinogen or horse serum decreased. They concluded that the interaction of precipitin and horse serum in the circulation produced a poisonous substance that was responsible for serum sickness. Not all studies of the serological background in serum disease which were performed at this time were in agreement. Von Pirquet and Schick (43) had not been able to find any constant relationship between the presence of precipitins in the circulation and serum disease. Lemaire (60) confirmed the results of Hamburger and Moro, and stated further

that precipitins were not demonstrable unless serum disease followed the injections of antitoxin. Wells (61) claimed, on occasion, to have found precipitin in the circulating blood even before the onset of serum disease. In his experience, the usual pattern followed was that of a low precipitin titre during the course of the illness which rose rapidly toward the end of the disease, falling gradually thereafter and eventually disappearing. Weil (62) confirmed these results. In 1918, Longcope and Rackemann (63) reported the results of a detailed study of 25 individuals who had received various quantities of antitoxic or antibacterial horse serum. Precipitin titres were followed in 14 cases. Of these, two excaped clinical serum sickness, and never showed a positive precipitin test. Of the twelve which developed serum disease, eleven had positive precipitin tests. In no instance were precipitins present in the circulation before or at the time of onset of serum disease. Usually, precipitins were not demonstrable in the patient's blood serum until 3 or 4 days after the onset of clinical symptoms. After this, they rose rapidly to reach their maximum at the time of clinical recovery, and diminished thereafter. Von Pirquet and Schick (43) had presented evidence to show that serum sickness was dependent upon a reaction between the residual precipitinogen and newly formed precipitins. The observations of Lemaire (60) Wells (61) and Mackenzie and Leake (64) were in accordance with those of Von Pirquet and Schick. Longcope and Rackemann (63) felt that the fixed tissue antibodies in contrast to the circulating antibodies were probably responsible for serum sickness. They suggested that the circulating antibodies neutralized or destroyed the antigen and initiated recovery from serum sickness. Coca (65) in 1922 submitted evidence that circulating antibodies may not be demonstrable in the presence of clinically typical serum sickness. He observed in his experiments on serum sickness in Indians that following the injection of normal horse serum, serum sickness might develop although precipitins to horse serum were absent. Similar observations were made by Tuft and Ramsdell (66). They pointed out that the reaction of human beings is somewhat different to normal

horse serum than to antitoxic and antibacterial sera. They found that the formation of precipitating antibodies to horse serum, which occurs regularly when antisera are used, is negligible or absent when normal sera is employed. Longcope and Rackemann (63) had further observed that when serum disease did not occur following the injection of horse antisera, the precipitins were not detectable in the patient's serum, and the antigen or horse serum persisted for long periods of time and could be demonstrated for weeks or even months. Mackenzie and Leake (64), Tuft and Ramsdell (67) and De Gara and Bullowa (68) have confirmed these observations.

Passive Anaphylaxis

In 1907 Otto (69) demonstrated that the anaphylactic state could be passively transferred by injecting the serum from an anaphylactic animal into a normal animal. An animal so treated will respond to a subsequent injection of the corresponding antigen by developing acute anaphylactic shock. An interval of several hours must elapse between the injection of the sensitizing serum and the injection of the corresponding antigen, if typical shock is to be regularly obtained. For maximal sensitization the interval is reported to be 4-6 days (70). Under suitable experimental conditions, an analogous reaction, "reversed passive anaphylaxis" may be induced in the guinea-pig by injecting antigen first and antibody later (74) (25) (71). Different antisera, produced by the sensitization of animals of different species against a single antigen, differ widely in their ability to induce passive sensitization in a given species of animal. Thus guinea pigs have been passively sensitized with antipneumococcal sera prepared in the rabbit, whereas, they were not rendered sensitive following the passive transfer of antipneumococcal sera prepared in the horse (72)(73). The passive sensitization of guinea-pigs with serum derived from patients with serum sickness has been reported by numerous observers (22a, 62, 63, 66, 67, 75).

Prausnitz - Küstner Reaction (76)

Küstner was quite sensitive to certain fish foods, but when extracts of the offending foods were tested with his serum, the presence of precipitins in his peripheral circulation could not be demonstrated. However, if a little of Küstner's serum was injected into the skin of a normal person, and the fish antigen injected into the same site 24 hours later, a marked local inflammatory reaction was produced. The reaction did not occur if the antigen and the serum were injected simultaneously. This localized inflammatory response in the skin following the subcutaneous injection of antiserum and the appropriate antigen has been obtained following the use of serum from patients with serum sickness by Tuft and Ramsdell (66) and others (77).

It thus appears that the fundamental reactions of the tissues of the human being towards the artificial introduction of soluble foreign proteins is of the same nature as anaphylaxis in animals. The Arthus phenomenon, anaphylactic shock, passive sensitization and "reversed anaphylaxis" (78) can all occur or be induced in the human being. Antibodies occur in the circulation, during or after serum sickness, for precipitins; and anaphylactic antibodies are demonstrable, while the Prausnitz-Küstner reaction can frequently be obtained.

Typical serum sickness is, however, peculiar to the human being. Fleisher and Jones (79) have described a diffuse inflammatory reaction with reddening and edema in the ears of rabbits appearing several days after the injection of single large doses of horse serum. They felt that this sign, together with a rise in body temperature to be analogous to serum disease in the human. In repeating this work, Khorazo (80) was able to obtain ear flushing in 10 of 36 rabbits tested during the winter, and in none of 12 tested during the summer months. Attempts to produce similar ear reactions in rabbits with other foreign serums, such as normal human, sheep, guinea pig and dog serum were unsuccessful, irrespective of the time of year when the experiments were performed. There were no differences in precipitin production or antigen

elimination in rabbits which developed local serum reactions when compared with those which failed to show any symptoms at all. These observations led her to support the thesis that the occurrence of serum sickness in man may be due to a peculiarity of horse serum. Kraus (81) has frequently been quoted to the effect that bovine serum is less likely to be antigenic for man than horse serum. These results are countered by the reports of Wangensteen (82) and Taylor and Keys (83) who obtained urticarial reactions following the intravenous use of bovine serum. Carefully purified bovine serum used by Janeway and Beeson (84) caused typical serum sickness in 2 of 16 patients who received varying amounts in solution intravenously.

Histopathologic changes attributed to hypersensitivity

The earliest reports of generalized histopathologic changes attributed to hypersensitivity, other than the relatively slight changes associated with acute anaphylactic shock, were made by Longcope (85, 86, 87) and Boughton (88). In neither case was the effort made to similate conditions which parallel those of human serum sickness. In general, these observers sensitized their animals with repeated small subcutaneous injections of the antigen, allowed a rest interval to elapse, and then subjected them to as many severe, but non-fatal anaphylactic shocks as possible.

In 1913, Longcope (85) had observed that "man may develop a hypersensitiveness, or allergy, to some food stuffs --- and under these circumstances, be subjected from time to time to many non-fatal attacks of intoxication by the proteins of these substances". He therefore planned a series of experiments to determine if repeated anaphylactic shocks in animals would result in permanent damage to body cells. Longcope used guinea-pigs, rabbits, cats and dogs, which were sensitized to repeated small doses of whole horse serum, or egg albumen, or both. The animals were rested for 15 to 25 days, and then subjected to repeated shocking doses of the antigen. All guinea-pigs received their shocking doses intraperitoneally, while the majority of rabbits, cats and

dogs received serum or egg white intravenously. He reported on lesions in the kidneys (85), liver (86) and heart (87).

In general, the most advanced kidney lesions were found in those animals which had received the largest number of doses over the longest period of time. He felt that the lesions were very similar to those produced by the injection of uranium nitrate. "The changes in the epithelial structures in the kidney point to some direct toxic action upon the epithelium, particularly the cells lining the loops of Henle, and the collecting tubules. This is followed or perhaps accompanied by small round cell infiltration and later by connective tissue formation". There was frequently fatty change and cloudy swelling of the tubular epithelium, and necrosis of cells of the collecting tubules. Wide areas of small round cell infiltration; swelling of the capillary tufts and glomeruli; and periglomerular fibrosis and fibrosis about the arcuate vessels were also described and attributed to the repeated anaphylactic shocks. Frank glomerular sclerosis was not described, nor were any lesions of resembling periarteritis nodosa reported.

In the livers of these animals Longcope (86) observed small areas of necrosis, which might involve only 3 or 4 cells, or more massive lesions which might involve as much as one-third of the lobule. They occupied any part of the lobule and could heal with or without scarring, depending on their position within the lobule. Those near the central vein tended to heal completely, while those near the portal areas tended to heal with the formation of scar tissue which spread out from the portal tracts. In addition to the focal necrosis, he noted cellular infiltration of the portal spaces, mainly mononuclear in type. In animals which had been inoculated over very long periods, there was a dense fibrous tissue in the portal spaces. In his series, the guinea pigs and dogs failed to show significant lesions in the liver. Of 22 rabbits used, 20 developed

liver lesions, and of 9 cats used, 4 showed liver changes. It is noteworthy that in 15 control cats there were no similar spontaneous lesions. In the treated cats, the liver lesions were very similar to those seen in the rabbits, but in a less marked degree.

The smallest lesions in the heart (87) consisted of foci in which the muscle fibres were swollen but showed no other definite changes. Frequently, these fibres were surrounded by considerable numbers of small round cells. In the larger lesions there was definite evidence of muscle degeneration, and occasionally the muscle fibres had disappeared entirely. A late stage of this chronic focal inflammatory process could be seen in groups of irregularly vacuolated muscle fibres embedded in a mesh of connective tissue. The lesions were described by Longcope as occurring in all parts of the heart muscle. They were rarely, if ever, situated about the blood vessels. Longcope actually stated specifically that the smaller arteries and capillaries were normal. The final stage was that of a diffuse interstitial myocarditis. Apparently Longcope was not at all impressed with their possible similarity to rheumatic fever-like lesions.

In 1917, Boughton (88) reported his observations on the lesions produced in guinea-pigs which had been subjected to repeated anaphylactic shocks. Some of his animals received as many as 20 injections of foreign serum, and were under observation for periods up to 8 months. He examined sections from the heart, lungs, liver, kidneys, spleen and aorta, and reported the presence of arterial lesions in all organs except the lungs and aortas. The arterial changes occurred most frequently and in the most marked degree in the liver. He described changes which included swelling and vacuolation of the endothelial cells; swelling, splitting and fraying of the internal elastic lamina; and perivascular collections of inflammatory cells. In his experience, the most severe lesions were found in animals dying of shock, or killed within one week of the time of the last injection. The arterial lesions occurred in

100% of the livers examined, 66% of the kidneys and 66% of the hearts. The aortas were without pathological change. He concluded that the lesions were rarely found in the larger arteries and not at all in the aorta. Boughton did not describe or illustrate lesions of heart, liver, spleen or kidney other than those in the related vessels.

In 1929, Klinge (89) reported his observations on the changes produced in rabbits which had been sensitized by subcutaneous injections of foreign protein and then treated with numerous intraarticular injections of the same antigen. In preliminary procedures he noted that when a single intraarticular injection of horse serum was given to a normal rabbit, and the joint examined 24 hours later, there was little to be seen other than slight hyperemia and a few leucocytes in the villous processes. When rabbits sensitized with horse serum were given an intraarticular injection of guinea-pig serum 4 weeks later, the inflammatory reaction was still slight, although somewhat more marked than that seen in the first procedure.

In a third series, rabbits were sensitized with subcutaneous injections of horse serum, and 4 weeks later given an intraarticular injection of the same antigen. When the joints were examined 2 days later, they showed the characteristic picture of "hyperergic inflammation". The synovial membrane exhibited a diffuse, phlegmonous, inflammatory reaction, with an exudate of leucocytes, swelling of the connective tissue fibres, and proliferation of the fixed tissue elements.

Rabbits in a fourth series were sensitized with subcutaneous injections of horse serum, rested for a month, and then given several intraarticular injections of the same antigen over a 2-3 month period. Histological alterations were seen in the synovial membranes, joint cartilage and capsule, periarticular tissue, skeletal muscle, blood vessels, heart valves and myocardium.

The lesions seen in the synovia and subsynovial tissues were described as severe inflammatory changes. The lining cells of the synovial membrane

were swollen and frequently several layers deep. The underlying connective tissue was heavily infiltrated with leucocytes, many of which were eosinophilic granulocytes. Proliferative changes were seen particularly well in the fatty tissues, which resembled "young scar tissue". The collagenous connective tissue showed foci of hyaline swelling. From the angle formed between the fusion of the joint cartilage and capsule, pannus tended to grow inward over the free surface of the cartilage. The contiguous hyaline cartilage showed degenerative changes characterised by a homogeneous eosinophilic appearance and loss of chondrocytes. Fibrillar changes in the hyaline matrix and the development of cartilage cells into fibroblasts were also seen. The red marrow of the epiphysis showed a tendency to proliferate and invade the joint cartilage from its under-surface.

The skeletal muscle lesions consisted of foci of degenerating muscle fibres in association with a variable degree of inflammatory reaction. The affected fibres stained a dark blue colour in haematoxylin and eosin preparations and showed loss of striations and fragmentation. Large mononuclear phagocytes surrounded the muscle fragments, and occasionally multinucleated giant cells were seen. The latter only superficially resembled tubercle giant cells. Polymorphonuclear leucocytes were present but not numerous. As many as 10-15 such lesions could often be seen in a low-power field. Individual fibres were occasionally affected.

Vessels at varying distances from the joints were examined. In arteries and veins of all sizes there were encountered localized hyaline swellings of the subendothelial tissue which extended a short distance to involve the underlying media. The endothelium appeared to be intact and thrombosis was observed but rarely. The subendothelial hyaline swelling resembled the subsynovial changes, not only in the character of the inflammatory exudate,

but also in the degeneration of the connective tissue fibres. The outer layers of the vessel walls showed little change beyond occasional large mononuclear cells in the perivascular tissues.

A regular finding on the free edges and along the points of the tendinous insertions on the heart valves were characteristic nodose swellings. In the fresh specimen, these presented a clear gelatinous appearance. Microscopically, these areas showed swelling of the connective-tissue fibres, which was almost mucoid-like. The cell content varied a good deal, and large mononuclear forms were frequently seen arranged in a radiate fashion. The circumscribed swellings contained no polymorphonuclear leucocytes and resembled similar nodes in the joints and walls of blood vessels.

Somewhat analogous changes were observed in the heart muscle. Numerous focal collections of mononuclear cells of varying sizes were seen scattered throughout the muscle together with perivascular collections of round and spindle-shaped cells. Fragments of hyaline connective tissue and giant cells were occasionally seen among the cell collections. Sometimes the hyaline swelling and proliferative reaction in the perivascular connective tissue assumed the form of a small granuloma. Proliferative inflammatory reactions replacing muscle fibres were frequently seen beneath the endocardium.

In assessing the value of these alterations, Klinge concluded that the "morphologic changes in the vessels, connective tissues and musculature were exceedingly similar to those in cases of human rheumatism".

Vaubel (90) in 1932 reported the results of a series of experiments somewhat similar to those of Klinge (89). He treated rabbits with subcutaneous, subcutaneous and intravenous, and intravenous injections of horse serum. In a second series, particular attention was paid to the articular tissues. Following sensitization with repeated subcutaneous injections, the joint cavities and periarticular tissues were injected with serum. In other cases, the joints were subjected to cooling and physical trauma in association with intravenous

injections of serum. With small doses of serum (up to 2 cc.) given subcutaneously, the lesions produced in heart, lungs, liver, spleen and kidney were trivial and distinguished with difficulty from the minor tissue alterations seen in normal rabbits. When the intensity of the treatment was increased by raising the quantity in single doses from 2-5 cc., injecting the animals over longer periods of time, and supplementing the subcutaneous with intravenous injections, morphologic changes were induced whose severity grew with the intensity of the treatment. The cardiac lesions which Vaubel emphasized consisted of foci of destruction of the heart muscle, the formation of inflammatory "nodes" about vessels, and damage to the vessel walls themselves, with swelling and fibrinoid change in the media, and proliferation of the intimal endothelium. This latter he termed "anaphylactic arteritis". In the lungs he found "no changes in the treated animals which could be attributed to the effects of foreign protein", although he does record the presence of capillary thromboses in 3 animals. These were included in a group of 8 rabbits which received large doses of serum intravenously (10-30 cc.) and of which 4 had died in anaphylactic shock during the course of treatment. He could not convince himself that repeated injections of serum had caused a vigorous proliferation of the lymphoid tissue of the lung as described by Pentimalli (91) in somewhat similar experiments. In the liver, Vaubel noted that even in normal animals the periportal connective tissue is rich in fibres which lie circularily around the vessels and bile ducts. In treated animals he felt that the lobular structure was accentuated by increased cellularity, strong basophilia of the cells, and an increase in the number of bile ducts, large lymphocytes and histiocytes with much protoplasm and vesicular nuclei.

In the joints he concluded that the intraarticular injection of small doses of serum produced a "hyperergic" inflammation, and that periarticular injections produced an extensive inflammation of all parts of the

knee joint. The intraarticular injection of antologous blood in sensitized animals caused only slight changes with hyperplasia of the synovial lining cells and an increase in the advential tissue. Repeated coolings of the joints of sensitized animals, he felt, could lead to a severe anaphylactic arteritis as could repeated contusion, the latter with a slight degree of intraarticular participation. Focal damage to skeletal muscle with the formation of interstitial granulomas was frequently observed in the region of these affected joints.

In considering the arterial lesions which he observed only in the coronary arteries, in 5 of 46 treated rabbits, Vaubel remarked that the "lesions bore a resemblance to periarteritis nodosa, thrombo-angütis obliterans, and above all to rheumatic arteritis".

Klinge and Vaubel (92) in a discussion of the fundamental tissue reactions characteristic of rheumatic fever, particularly in the vascular system, emphasized the swelling and fibrinoid change in the collagenous fibres and smooth muscle of these vessels. This they took to be an expression of hypersensitivity. They also considered the similarities and differences between the arterial lesions of rheumatic fever and periarteritis nodosa. While many authors had speculated on the etiologic agents responsible for the clinicopathologic syndrome of periarteritis nodosa, Gruber (93) in 1925 was probably the first to suggest that the lesions had their origin in some form of allergic or hypersensitivity reaction. In 1920, while trying to isolate an infectious etiologic agent from a human case of periarteritis nodosa, von Haun (94) unknowingly set up experimental conditions which roughly parallel the injection of a large quantity of foreign protein into an animal in which hypersensitivity is readily induced. The clinical diagnosis of periarteritis nodosa had been established in his patient through examination of a subcutaneous nodule. Von Haun injected two guinea-pigs intraperitoneally with blood removed from the human case. The animals died in 8 weeks, but showed no microscopic evidence of disease. Blood was drawn from the hearts of the injected animals, and

emulsions prepared from the organs for subsequent guinea-pig inoculations. In both instances in the animals of the second generation, microscopic arterial lesions were found. Macroscopic lesions were not seen. Von Haun had not filtered the blood to see if the responsible agent was filterable, and control animals treated with normal human blood were not prepared, however, he concluded that periarteritis nodosa was a specific infectious disease due to an unknown etiological factor present in the circulating blood, and capable of inducing inflammatory changes in the walls of blood vessels of guinea-pigs.

In 1922 Harris and Friedrichs (95) reported the results of a similar series of transmission experiments. Using autopsy material from a case of human periarteritis nodosa, these observers prepared an emulsion of several typical nodules from the kidneys and injected it into two rabbits. These animals showed ill-defined changes in the arteries of the lungs and liver. Portions of kidney, liver and heart from one of the first generation rabbits were utilized to prepare an emulsion, part of which was injected directly into two further rabbits, and part of which was passed through a Berkefeld filter before inoculation into a third animal. The most marked vascular lesions seen in these transmission experiments were observed in this last animal. The arteries of the heart and kidneys showed occasional periarterial adventitial nodules, while those of the lungs and liver showed inflammatory changes with polymorphonuclear leukocytes in the adventitia and necrosis of the media. Harris and Friedrichs concluded that periarteritis nodosa was a specific infectious disease capable of being transmitted to the rabbit, and that the microorganism inducing the disease was capable of going through a Berkefeld N filter, and therefore to be classed with so-called "filter passers" or viruses.

Following the reports of Klings (96, 97, 98, 89), Vaubel (90) and Klinge and Vaubel (92) numerous investigators reexamined the whole problem of hypersensitivity to foreign protein, and the histologic changes which might be attributed to such reactions. Metz (99) in 1932 compared the vascular

lesions of periarteritis nodosa and those he claimed to have produced in rats by experimental means. He used two groups of white rats; one injected with increasing quantities of haemolytic streptococci, and the other with cattle serum. He concluded that both methods were effective in producing lesions in vessels comparable to those of periarteritis nodosa. The illustrations shown by Metz do not entirely support his contention that the experimental lesions closely resemble those of periarteritis nodosa. The most extensive changes appear to have occurred in those animals treated with streptococci, and the lesions pictured show little more than proliferation of the intimal endothelium and organization of adherent thrombus material. The damage done to the blood vessels appears to have been relatively superficial, and frequently associated with the deposition of thrombus, neither of which are particularly characteristic of periarteritis nodosa.

Apitz (100) in 1932 studied the effects of large doses (10-30 cc) of horse serum given intravenously to sensitized rabbits in such a manner as to cause severe anaphylactic shock. He was interested in the changes which might occur when the animals died in acute anaphylactic shock, in what he called "protracted" or delayed shock, and in those which might result when the animals survived such large doses and severe reactions. His series was composed of 24 animals. Seven died in acute shock, five in "protracted" shock and twelve withstood the large shocking dose of serum. The changes seen in the first group were relatively slight. Subendothelial proliferative changes were seen in the pulmonary arteries, and in one instance subendothelial proliferation with granuloma formation was observed in the heart. The coronary arteries in one case of the group of five which died in "protracted" shock after 24 hours, showed acute inflammatory changes with necrosis of the media. Similar acute changes were found in one of the twelve which survived the shocking dose of serum. Apitz also described and illustrated widespread, diffuse inflammatory lesions in the myocardium characterised by loss of muscle

fibres, with infiltrating mononuclear histiocytes and proliferating fibroblasts. The spleen showed hyperemia of the pulp and large hyperplastic follicles. In the kidneys he described a parenchymatous degeneration of the tubule cells and endothelial proliferation in some of the larger vessels.

Junghans (101) studied the changes induced in a series of ten sensitized rabbits following the intravenous injection of swine serum in doses that ranged from 1 to 10 cc. In the heart valves he noted hyaline swelling of the collagenous fibres, foci of fibrinoid degeneration, and a diffuse proliferative reaction of the endothelial lining cells. The coronary arteries showed an acute arteritis with fibrinoid changes in the media and the development of paravascular granulomata. In addition to these lesions, he observed a mild inflammatory and proliferative reaction in the intimal layer of the aorta and pulmonary arteries. The inflammatory cells were small round cells for the most part, and occasionally extended a short distance into the media. Granulocytes were not seen, and there were no acute necrotizing changes in the underlying vessel wall. These changes were seen in rabbits that had received as little as three subcutaneous sensitizing doses of serum followed by two 1 cc. doses given intravenously and separated by an interval of 6 days.

In a general way, the observations of Klinge (89), Vaubel (90) and Apitz (100) tended to show that the generalized tissue reactions which followed repeated subcutaneous injections of foreign protein in sensitized animals could be elicited more readily and in a much severer form if the antigen were given intravenously, and in large doses. The factors which would limit foreign protein to the circulation or permit it to pass from the blood stream and produce local lesions in internal organs were the subject of much speculation. Knepper (102) in 1935 suggested that the endothelium provides an effective barrier against the penetration of foreign protein into sensitized tissue. Auer (103) showed that the ear of a rabbit sensitized with horse serum undergoes necrosis if preceding the injection of serum the ear has been gently rubbed with xylol.

He assumed that the inflammation caused by the xylol resulted in an unusual accumulation of the antigen within the tissues of the ear, which then reacted with the sensitized tissue. Knepper (102) caused similarly localized allergic inflammation by heating a rabbit's ear in warm water for a half hour, and by applying cold to the thigh of a calf for an hour. In 1934 Knepper (104) claimed to have reproduced the hepatic and renal lesions of eclampsia by repeated simultaneous injections of horse serum and hormone of the posterior lobe of the pituitary. Injurious action of the hormone in large amount was held to cause injury to the liver which in turn brought the antigen in contact with sensitized tissue in unusual quantities. In an effort to relate the localization of lesions attributed to allergy to increased functional activity of a less artificial character, Knepper and Waaler (105) subjected sensitized rabbits to a short period of running on a treadmill and then injected them intravenously with shocking doses of horse serum. When the shocking dose was large (20-30 cc.) andgiven in the absence of exercise, an acute necrotizing arteritis was seen in the coronary arteries. When the animals were exercised and given the shocking dose immediately thereafter, a small (2 cc.) injection was found to produce a necrotizing arteritis in both the pulmonary and coronary arteries.

Masugi and Sato (106) achieved a high local concentration of antigen in sensitized tissues by injecting serum into the renal artery and then temporarily compressing both renal artery and vein. Under these conditions, acute inflammatory changes were seen in the kidney, with vigorous proliferation of the capillary endothelium of the glomerular tufts.

In 1933 Masugi (107) described a method of producing specific organ changes in rabbits following a single injection of antiserum. He used rabbit kidneys to prepare extractswhich were injected into ducks over long periods of time. Finally the ducks were bled, and duck serum, presumably rich in antibodies to rabbit kidney, was injected into previously untreated rabbits. In this instance, the supposed union of antigen (rabbit kidney) and antibody

(duck serum) was invoked to explain the rather extraordinary glomerulonephritis which resulted. Kay (108) has questioned the validity of this thesis and the efficacy of sera supposedly rich in antibodies to organ extracts in producing allergic inflammatory responses in particular organs. He found that the onset of nephritis in the rabbit occured at about the same time that precipitins for duck serum were demonstrable in the peripheral blood of the test rabbits; and further, if the formation of precipitins was suppressed by the previous treatment of the rabbits with x-rays, duck serum remained for long periods of time in the blood of the rabbit; precipitins did not appear and nephritis did not occur. If, however, during this period, the rabbit was given serum from rabbits that had been treated with duck serum and had a high titre of precipitin for duck serum, an attack of acute glomerulonephritis was precipitated in a large proportion of the x-rayed rabbits. Nettleship (109) has reported the production of small areas of focal necrosis in bone marrow in rabbits following the intravenous injection of antisera prepared against extracts of bone marrow.

The literature contains very few case reports with autopsy protocols in which serum sickness has been present at, or before the time of death. The reports of Clarke and Kaplan (54) Easson and Carpenter (115) and Rich (55) are among the few available.

The report of Clarke and Kaplan (54) details the findings in two patients who died during serum sickness following treatment with large doses of anti-pneumococcus horse serum. There was present a mild, diffuse, inflammatory reaction in the intimal layer of the aorta and pulmonary arteries, characterised by an infiltrate of mononuclear leucocytes, and proliferation of histiocytes. A somewhat similar reaction was seen in the endocardial layer of the heart, which showed foci of proliferating cells and a similar infiltrate of large and small mononuclear leucocytes. There was, in addition, a necrotizing arteritis and periarteritis of the smaller coronary arteries in one case. These authors felt that the complete pathological picture was not compatible with any disease

previously described and concluded that it represented an expression of hypersensitivity and was related to the administration of foreign serum.

In the case reported by Eason and Carpenter (115), the relationship between therapy and lesions was not at all clear cut. These observers treated a series of cases of rheumatic polyarthritis with concentrated antiscarlatinal serum. One of the patients developed serum sickness and died 32 days later. At autopsy, widespread lesions typical of periarteritis nodosa were found. Eason and Carpenter felt that the vascular lesions were more probably related to the rheumatic injection and were only coincidentally associated with serum therapy and the ensuing serum sickness. "We suggest that the serum was in no way responsible for the death."

In 1942, Rich (55) published the case reports of seven patients all of whom had as a common denominator, serotherapy or chemotherapy with sulphathiazole (or both), and a generalized necrotizing arteritis which was indistinguishable from periarteritis nodosa. The vascular lesions included necrosis and fibrinoid alteration, hyalinization of the media and perivascular infiltration, or infiltration of the entire wall with mono- and polymorphonuclear leucocytes. Eosinophilic leucocytes were prominent in 4 cases. While the presence of the lesions could not be proven to have not been a coincidence, none of the seven patients had symptoms suggestive of periarteritis nodosa before entering hospital for their terminal illness. Further strong evidence that hypersensitivity to sulphathiazole could result in the development of a necrotizing arteritis was found in an additional case report by Rich (56) in the same year. In this instance, a 66-year old negro underwent biopsy because of an ulcer on his scrotum. Five months later a radical removal of the carcinomatous ulcer and the inguinal lymph nodes was performed. Immediately following operation, the patient was given sulphathiazole as a prophylactic measure in anticipation of the development of aspiration pneumonia. The sulphathiazole therapy was discontinued on the fourth post-operative day, and restored on the eleventh post-operative day. At this time

his temperature rose to 102°F. and a conjunctivitis developed. The patient continued to receive 406 gms. of sulphathiazole daily until the eighteenth post-operative day, when he died. At autopsy, there was considerable haemorrhage in the intestinal lumen. The testes were atrophic and the lungs edematous. No other gross lesions were seen. Microscopically, widespread lesions of periarteritis nodosa were found. They occurred in the tissue at the operative site, in the heart, liver, spleen, kidneys, testis, bladder and prostate. In addition to the vascular lesions, there was a diffuse inflammatory infiltration in the myocardium, composed of mononuclear, and neutrophilic and eosinophilic leucocytes. Also present in the lungs, spleen, kidneys and bladder were very minute focal necroses densely infiltrated and surrounded by mononuclear and polymorphonuclear leucocytes, including eosinophiles. The significant finding in this case, of course, was the normalcy of the tissues (with respect to vascular lesions) which were obtained at the time of biopsy and radical operation, and the presence of very marked vascular changes in these sites 18 days after operation and sulphathiazole therapy.

Rich followed these observations on clinical material with a series of experiments in which he treated rabbits with large doses of horse serum intravenously and observed the histologic changes induced. Longcope (85) (86) (87) Boughton (88) and Apitz (100) had deliberately treated their animals in such a way that acute anaphylactic shock was a likely result. Many of the animals of Klinge (89) and Vaubel (90) had been treated with subcutaneous injections of the antigen. Rich treated his animals with single, or infrequently repeated, large doses of serum given via the intravenous route only, and in so doing attempted to emulate somewhat the conditions associated with serum sickness in man. When the injections were repeated, he attempted to partially desensitize his animals with a small intravenous injection of the antigen 24 hours prior to the injection of the large dose. In 1943, Rich and Gregory (110) reported

on the arterial changes induced in a series of 14 rabbits, 9 of which received horse serum and 5 of which received horse serum and 0.5 gms. sulphadiazine daily. The serum was given in quantities of 10 cc. per kilo. of body weight of rabbit. Arterial changes were found in 12 of the 14 treated animals. All stages of lesions typical of periarteritis nodosa were encountered. The simplest lesion consisted of edema of the media, which spread the muscle fibres widely apart. More advanced lesions showed necrosis, fibrinoid alteration, and hyalinization of the media, infiltration of inflammatory cells which were predominantly mononuclear, but included neutrophilic and eosinophilic granulocytes. Marked perivascular infiltration and occasionally necrosis were encountered. Some vessels showed necrosis of the entire circumference, some showed involvement of a segment only. Aneurysmal dilatation was not seen, but these authors felt it would have developed if hypertension had been present. Infarction from vascular occlusion occurred only once, and that in the liver. A noteworthy finding in this experiment was the presence of acute arteritis following the administration of a single large dose of the antigen. Serum plus sulphadiazine was no more effective in producing the lesions than was serum alone.

In addition to acute arteritis, Rich and Gregory observed what they felt was an acute diffuse glomerulonephritis in 10 of the 14 treated rabbits. They described condensation of the glomerular tufts, proliferation of the glomerular epithelium and haemorrhage into Bowman's capsule. The tubules contained albumin and casts, and showed fat droplets in the lining cells. They stressed the point that these glomerular lesions were entirely independent of the focal inflammatory lesions familiar in the kidneys of many untreated rabbits.

Rich and Gregory followed their paper on the experimental production of necrotizing arterial lesions with a report (111) on experimentally induced lesions in rabbit hearts which they felt had the basic characteristics of rheumatic carditis. Thirty-six rabbits were injected with large doses of horse serum under conditions similar to those of their first procedure. Lesions in the heart were

produced in 11 animals. Rich and Gregory make clear that the experimental lesions were not identical with those seen in acute rheumatic fever, but the changes were closely allied to those seen in the human disease.

Focal alterations in the collagen of the myocardial connective tissue were present. They were most prominent in the valves and in the mural endocardium at and near the valvular attachment. There was separation of the fibres by edema, swelling of the individual fibres, and finally, degeneration of the fibres.

Also observed were focal collections of cells which in many ways resembled Aschoff bodies. They were present in the valve leaflets near their base, and in the endocardium near the valvular attachments. They included not only mononuclear inflammatory cells, but also the large pale basophilic cells that characterize the Aschoff body. Cells of the "Anitschkow myocyte" type were also present. In "numerous" instances the cells were grouped about a focus of degenerated and swollen collagen fibrils, sometimes forming a rather palisade-like border closely resembling that familiar in rheumatic lesions. Non-specific inflammatory lesions in the pericardium, the base of the valve leaflets and in the mural endocardium at the insertion of the valves were also noted.

Cellular infiltrations in the valve cusps which produced projecting "vegetations" on the valve cusps were described. The valvular endothelium over these infiltrations appeared swollen and damaged, but no eosinophilic thrombus material was found on them. Small foci of necrosis of cardiac muscle were observed in 4 of the 36 animals injected.

In 1943, Rich and Gregory (112) compared the pneumonic lesions common to seven cases of rheumatic fever with acute cardiac lesions, and five cases of pneumonitis attributed to hyper sensitivity to sulfonamides. The purpose of the paper was to draw attention to what they considered to be the basic identity of the pulmonary lesions in rheumatic fever, and to relate them to those allegedly resulting from sulfonamide hypersensitivity. They felt that the primary tissue alteration was a focal damage to the alveolar capillaries. In its mildest form, the ~~capillary~~ damage was expressed by an exudation of fluid into the related

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alveolar spaces, but leucocytes often infiltrated the alveolar wall about the affected capillary, and also escaped into the alveoli in numbers varying from a very few to a great many. Focal necrosis of the capillary endothelium sometimes occurred, and the lumen became plugged with a fibrinous or a hyaline thrombus. Sometimes the damage permitted the occurrence of intra-alveolar haemorrhages. Often the respiratory bronchioles and their adjacent alveoli were lined with an eosinophilic hyaline membrane. Focal necrosis of the entire alveolar wall occurred when the damage was severe. The lesions were decidely focal, sometimes affecting only one segment of a capillary in an entire low power field, and on the other hand they often involved the vessels of a sufficient number of adjacent alveoli to produce confluent areas of consolidation. The affected portions of lung were sterile on culture and contained no micro-organisms stainable in the sections. Rich and Gregory noted that the "large deeply stained cells, with vesicular nuclei, often multinuclear and markedly basophilic" seen by Gouley and Eiman (144) in the lungs of cases of rheumatic pneumonitis were only occasionally present in their rheumatic material. However, they were reported to be present by Rich and Gregory in the pneumonitis attributed to sulfonamide hypersensitivity.

In a sequel to their observations in pneumonitis in acute rheumatic fever and sulfonamide hypersensitivity, Gregory and Rich (113) in 1946 published the results of an experimental study in which they described "pulmonary lesions with the basic characteristics of rheumatic pneumonitis" in rabbits which had been injected with one or two large doses of horse serum or egg albumen. Thirty-eight of the 56 had been injected with serum, 12 with egg albumen and 6 with a mixture of equal parts of serum and egg albumen. The sections of the lungs of 10 rabbits showed definite lesions. Three of the 10 had been sensitized with serum, one with a mixture of serum and egg albumen, and 6 with egg albumen alone. The pulmonary lesions exhibited, in varying degree, focal capillary damage of the type that was previously reported by these authors as characteristic of the pneumonitis of acute rheumatic fever and of sulfonamide sensitivity in man. Capillary thrombosis, focal edema and haemorrhage were the most frequent lesions.

Using rabbits as test objects, and horse serum as antigen, Fox and Jones (114) studied the histologic changes in sensitized animals given several injections of serum. They employed 31 rabbits, some of which were kept under observation for periods up to nine months. Their technique varied from that employed by Rich and Gregory (110) (111) (113) in that each animal received one, two or three large doses of serum (10 cc./kilo. of body weight) intravenously, intraperitoneally or subcutaneously, as sensitizing injections. The subsequent shocking injections which were given intravenously were relatively small (1 cc. or less) in comparison to the initial injections. The total number of doses, the time interval between them, and the time of skin testing were arbitrarily chosen, varied considerably, and in their experience seemed to bear no significant relationship to the observed results. Of the 31 rabbits treated, 20 exhibited pathologic changes of sufficient intensity to be considered significant. Seven showed alterations which were considered to be only minimal in degree and the remaining 4 were negative. The significant histopathological changes, which were limited almost exclusively to the heart, consisted primarily of mild vascular and perivascular alterations of the smaller branches of the coronary arteries. A few animals showed a mild infiltration of leucocytes (mainly eosinophile granulocytes) in the myocardium. Vascular and perivascular lesions were occasionally noted in the liver, lung, testis, kidney and mesentery.

The vascular changes noted in the heart were characterised by intimal hyperplasia, and proliferation of the adventitial cells. The latter were frequently interspersed with, or surrounded by lymphocytes and large mononuclear cells together with an occasional granulocyte. Fibrinoid change in the intima and media was noted infrequently, and necrosis was not observed. The mononuclear cells varied from medium to large. They frequently occupied a paravascular position, and, intermingled with lymphocytes, adventitial cells and a few granulocytes, vaguely resembled Aschaff bodies. The vascular lesions

in which the cells were more circumferentially placed, resembled those often described as "rheumatic arteritis". The arterial lesions described by Fox and Jones differed from those reported by Rich and Gregory (110) in the relative mildness of the inflammatory reaction and their rather conspicuous tendency to be restricted to the heart. Fox and Jones also failed to report the presence of lesions in the heart valves, myocardium or lungs. They did note, however, that arterial lesions in the heart could be produced in some instances following a single intravenous injection of serum.

Hopps and Wissler (116) treated rabbits in a manner somewhat similar to the procedure adopted by Rich and Gregory. Large doses of horse serum were given intravenously to a series of 10 rabbits; 6 additional animals were given sulphadiazine together with serum. Acute and subacute periarteritis, characterised by polymorphonuclear and/or mononuclear leucocytic infiltration of the adventitia, was seen in 11 of the 16 animals. Proliferative lesions of the endothelium and chronic inflammatory changes of the media and adventitia were observed in 5 of these, and a marked, generalized necrotizing arteritis in a single case. The majority of the vascular lesions were seen in the heart. The heart valves were not examined, but paravascular collections of cells in the heart were likened to Aschaff bodies. Diffuse collections of small round cells in the myocardium were labelled focal interstitial myocarditis and attributed to the effects of the treatment. Also described were sulfadiazine crystals in the collecting tubules of the kidney, hypertrophy of the juxta-glomerular apparatus, hypertrophy of the spleen with follicular hyperplasia and an increase in fibrous connective tissue in the portal triads with small areas of focal necrosis of liver cells. These authors had been concerned over the vascular localization of the lesions, and speculated on the possibility that the sudden infusion of antigen in a large amount might be responsible for the localization of the necrotizing antigen-antibody reaction in a manner somewhat comparable to that illustrated by the Auer phenomenon (103). Accordingly, they varied the periods required to infuse

from a few minutes to over 8 hours. In their short series they found that the rate of infusion bore no relation to the severity of the arterial lesions that resulted.

On the whole, they felt the vascular lesions were qualitatively similar to those described by Rich and Gregory, but quantitatively less marked and less generalized. As a group, those animals which received sulphadiazine in addition to horse serum exhibited less arteritis histologically. They searched for, but failed to find lesions of acute diffuse glomerulonephritis which Rich and Gregory (110) had observed in 10 of their 14 animals.

In a procedure incidental to an experimental attempt to produce necrotizing arteritis and hypertension in rats, Smith and Zeek (117) injected rabbits with horse serum and searched for histologic changes in the vessels. Their conclusions were rather sweeping considering the number of animals examined, and the way in which they were treated. The series was composed of 8 albino male rabbits. Five of these received an initial injection of 10 cc. of serum per kilo. of body weight. Twenty-nine days later, these five animals, and the three "controls" were given 3 cc. of serum intravenously. All animals survived this injection,and all were sacrificed two hours later. None of the 8 rabbits had "lesions remotely suggestive of periarteritis nodosa and no evidence of glomerulonephritis was found". In summing up, these authors concluded that their observations tended to refute the theory that periarteritis nodosa is the result of hypersensitivity to known or unknown antigens.

Effect of different serum protein fractions

Normal serum is composed of several different protein fractions which are separable, not only by their chemical constitution and physical properties, but also by their ability to act as specific antigens. Dale and Hartley (118) have demonstrated that anaphylaxis will discriminate between three purified proteins separable from normal horse serum by chemical methods. A "guinea-pig which had received a preparatory injection of the pure euglobulin became sensitive to this, but remained indifferent to the pure albumin and vice versa".

There was also a difference in the time of appearance of sensitivity to the proteins employed. Sensitiveness to euglobulin appeared within 8 to 10 days after injection, while sensitiveness to albumen was not demonstrable until after an interval of 16 to 20 days. Hooker (119) found that the skin of patients who had been sensitized to horse serum by a small prophylactic dose of diphtheria antitoxin, reacted differently to the intracutaneous injection of three protein fractions of horse serum. The wheal produced by pseudoglobulin appeared in 30 minutes, that caused by euglobulin in 5 hours and that elicited by albumin in 7 hours.

There has recently appeared a report by Hawn and Janeway (120) which emphasizes the specificity of the histologic change which may occur when the antigen utilized is composed of a purified fraction of plasma protein. These observers used crystallized bovine serum albumin, bovine serum gamma globulin and whole bovine serum. Each animal received a single intravenous injection of the foreign protein, approximately 1 gm. of protein per kilo. of body weight. The principal pathological lesions in rabbits given whole bovine serum were similar to those reported by Rich and Gregory (110), and were characterised by widely dispersed but segmental acute inflammatory lesions of the arteries. There was no evidence of acute glomerulonephritis, and no focal hepatic lesions or changes in knee joints were noted. Focal collections of cells in the heart muscle and valves similar to those described by "Longecope (87), Klinge (89) and Rich and Gregory (111)", were said to be present. These lesions were at their height two weeks after injection and showed marked repair at 4 weeks.

Crystallized bovine serum albumin produced lesions almost exclusively confined to the arteries. The lesions were less numerous, less intense, and occurred in fewer animals than the series which received whole bovine serum. They were at their height in two weeks, were healing at 3, and healed by 4 weeks.

Bovine serum gamma globulin elicited quite different histologic sequences. The most striking lesions involved the glomeruli of the kidneys,

and to a lesser degree, the heart. Focal necroses in the liver, and inflammatory lesions in the joints were present but less conspicuous, and arterial lesions were rare and slight in degree. The lesions differed from those in rabbits given albumin not only in distribution, but in timing. They were most widespread and acute at one week and were healing at 2 weeks after injection. Moreover, lesions were observed in almost every animal.

Lesions attributed to sensitivity to sulfonamides.

Recently there have appeared in the literature numerous reports of tissue alterations attributed to sulfonamide sensitivity which embrace a much wider variety of lesions than the vascular changes described by Rich (55, 56). Thus, focal interstitial myocardial lesions, rich in eosinophiles were reported by French and Weller (121) in 1942. Similar focal lesions were also observed by them in the lung, liver, and kidney, and comparable lesions were reproduced in mice and rats. In the same year, Lederer and Rosenblatt (122) reported on their findings in 4 cases treated with sulfathiazole, in which numerous areas of focal necrosis were noted microscopically in practically all viscera. Merkel and Crawford (123) described similar lesions in 4 cases, and Lichenstein and Fox (124) reported visceral focal necroses in association with necrotizing arterial lesions resembling those of periarteritis nodosa. In a series of 22 cases with widespread lesions, More, McMillan and Duff (125) found that the commonest lesion in their material was a granulomatous inflammatory reaction, seen in the heart, liver, kidney, lung and bronchus, peritoneum and bone cellus. Focal visceral necroses and a necrotizing polyvasculitis were also described.

Summary of Work Proposed in Experimental Procedure

Even a casual survey of the literature shows that much disagreement exists on the nature of the lesions, if any, which may be produced as a result of allergy. Slight variations in fundamentally similar experimental procedures have netted very different results in the hands of different investigators. The lesions described by Longcope (85) (86) (87) had little in common with those of

Boughton (88), Klinge (89) and Vaubel (90). Klinge and Vaubel felt that the experimentally induced focal necroses of collagen and perivascular granulomata sufficiently resembled those of rheumatic fever to warrant the assumption that rheumatic fever was a manifestation of hypersensitivity. Aschoff (126) refuted the identity of the two lesions. Vaubel (90) failed to produce an acute arteritis following a single injection of serum, but in larger series this was accomplished by Rich (110), Fox and Jones (114) and Hawn and Janeway (120). Diffuse inflammatory reactions in the intima of the aorta and pulmonary arteries were reported in experimental animals by Junghans (101) and in two cases of human serum sickness by Clarke and Kaplan (54). Subsequent investigators have failed to confirm these observations.

Focal inflammatory reactions in skeletal muscle in cases of rheumatoid arthritis, a disease of suspect allergic origin, have recently been described by Steiner, Freund, Leichentritt and Maun (127). Alterations in muscle somewhat reminiscent of these lesions were found by Klinge (89) and Vaubel (90) in relation to the joints which they injected, but have not been reported since.

Some doubt has been cast on the actual specificity of lesions hitherto attributed to hypersensitivity, in man and in experimental animals, by the report of Selye and Pentz (128) that lesions similar to Aschoff nodules and those of periarteritis nodosa occur in rats following the administration of massive doses of desoxycorticosterone acetate.

The issue has been further confused by conclusions drawn from experiments in which the total number of animals in a given series are unreasonably small (117) and also by the failure on the part of some to differentiate between spontaneous and experimentally induced lesions, through inadequate examination of untreated control animals. The renal, myocardial and hepatic lesions of Longcope (85) (86) (87) and some of the myocardial lesions of Hopps and Wissler (116) are cases in point.

As an approach to the solution of some of these problems, a series of experiments were carried out, with a view to determining how great a variety of lesions can be produced in different tissues as a result of hypersensitization induced by massive injections of horse serum. These procedures follow in natural succession those of Longcope, Klinge, Vaubel and Rich and Gregory, differing from these previous experiments only in that a standard technique of hypersensitization was employed in a much larger series than most of the above authors. It is planned that the present studies which employ whole horse serum as antigen will serve as a base for comparison with successive procedures utilizing purified protein as antigen.

MATERIALS AND METHODS

The rabbits used in this experiment were derived from several sources. The majority of the animals were albinos, though small numbers of several other species were included. The proportion of males to females was roughly equal. At the beginning of the experiment, their average weight was 1800-1900 gms. although animals as small as 1400-1500 gms. were used in small numbers.

Normal whole horse serum was used as an antigen. When it had separated from the clot, it was freed from bacterial contaminants by passage through a Seitz filter, and was subsequently stored in rubber-capped bottles at 4°C. until ready for use. Preservative agents were not added to the serum, but test cultures of numerous lots taken immediately before use failed to show the presence of any bacterial contaminants.

EXPERIMENTS: Each animal was given an initial dose of serum into the lateral ear vein of 10 cc. per kilo. of body weight. When successive injections were given, they were repeated every 17 to 18 days. Since the animals gained weight, the absolute dose tended to increase, but was arbitrarily limited to 20 cc. per injection. In order to partially desensitize the animals, and somewhat reduce the mortality rate from anaphylactic shock, the repeat injections were preceded by a 1 cc. intravenous injection of serum 24 hours prior to the

administration of the large dose.

The total duration of the experiments varied from 8 to 146 days. Seventy-seven different animals received increasing numbers of injections. While approximately one half the series were treated with 2 massive doses, seven animals were given but a single injection, and the remainder received increasing numbers of treatments up to a group of seven which received 8 injections (see Table I). Eight animals died as a result of anaphylactic shock immediately, or within a few hours after their last injection. The remaining animals were sacrificed by air embolism, usually 6-10 days after the final injection of serum. Complete autopsies were performed and blocks of tissue were fixed in 10% formolsaline. Histological sections of tissues were prepared from brain, spinal cord, bone marrow, skeletal muscle, larynx, thyroid, lung, heart and aorta, stomach, intestine, adrenal, pancreas, liver, spleen, testis or ovary and knee joint, and stained with Haemotoxylin and Eosin. Where indicated, Glynn's stain for bacteria, Weigert's elastic stain, Masson's trichrome stain and stains for fibrin were also employed.

CONTROLS: Particular attention was directed toward securing adequate control material. Twenty-five animals were utilized for this purpose. They were derived from the same sources at the same time as the treated subjects, and lived in the animal house during the period of treatment. They were not treated in any way. They too were killed by air embolism and the same tissues were examined.

RESULTS

Spontaneous lesions were found in many organs, in a large percentage of animals. Those most frequently encountered were seen in the brain, liver, stomach and intesting, kidney, lungs and testis. Vascular and focal inflammatory lesions of the brain of both treated and control animals were found in approximately the same incidence as that reported by Bender (129). The frequent occurrence of coccidiosis in some degree in the gastrointestinal tract and liver of both treated and control animals was noted, as was the frequent occurrence of

focal and pyelonephritic inflammatory lesions in the kidney. An unidentified parasite was found in the testis in one instance. Approximately 1/5 of the lungs of both treated and control animals showed foci of what appeared to be a very mild, chronic, interstitial inflammation with thickened alveolar walls and mononuclear exudate medial calcification of the aorta was also noted.

Significant lesions were not seen in the spinal cord, bone marrow, thymus, adrenal, pancreatic islet and acinar tissue and ovaries in either treated or control animals.

Lesions occurring in the treated animals, but not in the controls, were found in skeletal, smooth and cardiac muscle; in the arteries of the heart, lungs, mesentery, stomach, pancreas, liver and fallopian tubes; and in the aorta.

The heart valves and valve rings, and joints required special consideration. Non-specific inflammatory lesions were found in the joints of both treated and control animals, but on the whole, appeared more marked in the treated group. Focal inflammatory lesions were found in the valve cusps of both treated and control animals, but similar lesions were seen in the valve rings of the treated group only.

TABLE I

NUMBER OF MASSIVE INJECTIONS	1	2	3	4	6	7	8	
NUMBER OF ANIMALS IN GROUP	6	38	7	4	9	6	7	77
NUMBER OF ANIMALS IN GROUP WITH ARTERITIS	1	22	3	4	7	5	5	47

Table II

	Animal Number	93	3	11	53	55	60	19	36	37	58	59	32	44	44	64	89	41	14	72	82	86	100	102	108	110	112	113	51	1	6	9	15	28	54	56	45	7	34	35	41	26	29	33	38	40	18	43	31	47	49	27	50	52	17	20	22	23	24		
	Treatment time in days	15	22	18	19	19	19	21	21	21	21	21	22	23	23	23	25	25	26	27	27	27	27	27	27	27	27	27	31	34	37	37	33	34	36	36	37	67	69	69	69	99	99	99	101	101	101	102	102	127	127	129	136	134	134	137	146	146	146	146		
	Number of large injections	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	4	4	4	4	6	6	6	6	6	6	6	7	7	7	7	7	7	8	8	8	8	8		
	Death as a result of anaphylactic shock			+																													+			+							+		+	+			+									8	10.4%
Heart	Massive necrosis of myocardium				+			+	+				+	+																													+					+									7	9.09
Heart	Lesion in valve ring					+						+									+				+	+	+					+		+			+				+															10	13.0%
Heart	Lesion in valve cusp						+															+										+			+										+			+								+	6	7.8%
Lungs	Proliferative lesion in vessels				+	+				+																							+																+									5	6.5%
Lungs	Capillary thrombosis																																																		+									1	1.3%
Skeletal Muscle	Acute Myositis	+							+	+			+	+		+						+	+									+	+	+		+	+					+																	15	19.5%
Smooth Muscle	Acute Myositis												+																								+																							2	2.6%
Joints	Arthritis												+									+																+	+																					6	7.8%
Arteries	Coronary Arteritis	+	+	+	+		+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47	61.1%
Arteries	Aortitis				+							+										+	+		+	+								+																											11	14.3%
Arteries	Pulmonary Arteritis				+				+	+			+			+																																													5	6.5%
Arteries	Mesenteric Arteritis								+					+			+								+																																			4	5.2%
Arteries	Gastric Arteritis															+								+																																			3	3.9%
Arteries	Pancreatic Arteritis															+	+									+																																		4	5.2%
Arteries	Fallopian tube Arteritis					+																																																							2	2.6%
Arteries	Hepatic Arteritis																																																											1	1.3%

Table I shows the relationship of the incidence of arterial lesions to the number of massive injections given. While lesions attributable to hypersensitivity did occur following a single injection, and occurred frequently with 2 injections, their percentage incidence became very high when more than 3 injections were given.

The distribution of lesions in all animals which showed changes attributed to hypersensitivity is shown in Table II, together with the duration of treatment and number of injections which they received.

ARTERIES: Seventy-seven animals received one or more large injections of serum, and of these, 47 showed arterial lesions. Among the 47 animals which showed an arteritis, 16 showed inflammatory changes which were classified as acute, 35 as subacute, and 15 as healing or chronic. In every animal that an acute, subacute or chronic arteritis was found, the coronary arteries were involved. In addition, the pulmonary arteries in 5 cases, the mesenteric arteries in one case and the arteries of the fallopian tubes in 2 cases were so involved. In no instance did the renal or splenic arteries show a similar inflammatory change. In those animals which had received two or more injections, there was no significant correlation between the duration of treatment and the severity of arteritis which resulted.

The earliest acute changes which were noted occurred in small coronary arteries, and consisted of an eosinophilic homogeneous swelling of a segment of the media, in which there was a loss of nuclei and cell detail, and slight swelling of the overlying endothelium (Fig. 1). With progressively severe involvement, fibrinoid necrosis of the media was present in association with a variable amount of mixed cellular exudate (Fig. 2). In larger vessels such as the main branches of the coronary and mesenteric arteries (Figs. 1 and 2), the nuclei of the muscle of the media assumed a swollen hydropic appearance, tiny clefts containing fluid appeared between the muscle fibres, the intimal endothelial cells became enlarged and swollen and small vesicles of fluid

appeared in the subendothelial tissues, which tended to raise the intimal endothelium from the underlying media. An interrupted layer of fibrin was deposited in the subendothelial layer and the intimal endothelium tended to proliferate until it often presented a solid layer, 5-8 cells in thickness, diffusely sprinkled with leukocytes, chiefly of the mononuclear type. In the severest lesions, segmental or complete necrosis of the media occurred with fragmentation of nuclei and cells and loss of the normal architectural pattern. The degree of inflammatory reaction in the adventia was quite variable. A mixed leucocytic exudate was seen most commonly, with large mononuclear cells predominating. Fragmentation of the elastic laminae was present in the severe acute cases.

In subacute lesions, the inflammatory reactions in vessels were characterised particularly by proliferative changes in the intimal and advential cells, together with a variable amount of perivascular exudate of mononuclear leucocytes (Fig. 10). Slight swelling of the connective tissue fibres, and separation of the various cellular elements by edema fluid also led to undue thickening and prominence of the intimal layer. Occasionally focal collections of mononuclear leucocytes in a paravascular distribution presented appearances which superficially resembled Aschoff nodules (Fig. 13 and 14).

In the stage which was regarded as chronic or healing, the subintimal layer was irregularly thickened with cellular connective tissue, the cells of the intimal endothelium were somewhat swollen and hyperchromatic, and the media was slightly distorted due to fibrosis. The adventitia was rendered irregularly thick and dense by the presence of collagenous scar tissue, and in this coat variable numbers of lymphocytes and large mononuclear cells were seen (Fig. 3).

Arterial changes comparable to any of the above were not seen in any of the 25 control animals.

AORTA: The first portion only of the aortic arch was examined. In 11 animals, an inflammatory reaction in association with proliferative changes of the intimal endothelium was observed (Fig. 6). The change was a diffuse one involving wide areas of the aortic lining and occasionally extending into the coronary ostia, there to become continuous with an acute necrotizing arteritis of the coronary arteries (Fig. 9). The subintimal tissues were frequently edematous and sparsely sprinkled with a mixed mononuclear exudate including lymphocytes, plasma cells and large mononuclear cells. In only one case did the media appear to be involved. In this instance, small masses of fibrinoid material, pyknotic nuclei and nuclear fragments, together with occasionaly pseudo-eosinophilic polymorphonuclear leukocytes were observed in a few areas of the inner one-third of the media. These lesions bore no resemblance to the familiar spontaneous calcification of the media of the aorta of rabbits which was encountered not infrequently amongst the animals of the experimental group.

Proliferative changes in the intima, or an inflammatory reaction of the media was not seen in any of the control material, although they, too, showed calcification of the media in about the same incidence as the experimental group.

HEART: Inflammatory and degenerative lesions of the myocardium and valve cusps were found in both treated and control animals, and of valve rings and coronary arteries in treated animals. The inflammatory reactions involving the coronary arteries have already been discussed.

The majority of hearts in both treated and control animals showed nodular or fusiform swellings of the valve cusps. These lay usually on the under surface of the valve. The swellings were poor in cells and presented a clear, almost mucoid appearance, with slender tenuous fibres widely separated by clear staining intercellular material (Figs. 14 and 15).

Inflammatory lesions of the valve cusps were seen in 2 control rabbits, and in 6 of the treated group. In some instances the changes consisted of a localized swelling of the valve cusp largely due to a dense collection of lymphocytes and larger mononuclear cells. In some cases, the inflammatory cells rested close to the free surface of the cusp and were associated with relatively slight changes in the deeper portions of the cusps (Fig. 19). In other instances, the inflammatory cells rested among and partly replaced the connective tissue fibres (Fig. 18). Lesions of a more diffuse character, in which there was considerable thickening of the greater portion of the valve cusp as a result of fibroblastic activity and collagen deposition, were also seen (Fig. 20). In our material, the histopathologic change which most resembled an Aschoff nodule was found in a control animal (Fig. 17). There was nothing in the histologic appearances of the inflammatory foci in the valve cusps which would serve to differentiate the treated from the untreated animals.

In the valve rings of 10 of the treated animals, exudative and proliferative focal inflammatory reactions were seen. Some showed little more than a small irregular rather dense collection of large and small mononuclear leucocytes (Figs. 28 and 29). In others, the collagenous fibres of the involved areas exhibited edematous swelling, separation, and hyaline change. Fibrocytes in these areas showed swelling and proliferative activity, and variable numbers of mononuclear leucocytes were scattered throughout the area. (Figs. 21 22 and 26). In addition to lymphocytes and large mononuclear cells, large cells with 2-5 nuclei and an abundant, granular, basophilic cytoplasm were occasionally seen in these foci (Fig. 25). Clustering of nuclei in a palisade fashion about a focus of swollen, hyaline collagen was also seen (Figs. 23 and 24).

Comparable areas of focal inflammatory reaction and collagen damage were not seen in the valve rings of any of the control animals.

In the myocardium, diffuse and focal collections of small round cells together with occasional areas of proliferation of connective tissue elements

were seen in some degree, even if slight, in virtually all of the animals of both treated and control groups. These changes were quite comparable to the spontaneous myocardial lesions of rabbits so well illustrated by Miller (130). Typically, they consisted of small collections of large and small mononuclear cells, scattered among myocardial fibres which themselves showed no obvious changes (Fig. 33). Occasionally, a few fibres appeared to have dropped out, to be replaced by inflammatory cells. Usually the inflammatory foci lay in the myocardium in no particular relationship to any other structure, but occasionally they occupied a paravascular distribution (Figs. 34 and 32). Rarely, the change was widespread enough to occupy several low power fields (Fig. 35).

Over and above these lesions, which were considered as "spontaneous", there was observed in the hearts of 7 treated animals a massive necrosis of large areas of heart muscle for which there was no counterpart seen in the control material. The involved muscle fibres exhibited what appeared to be an acute degenerative change characterised by swelling of the cell body, with loss of striations and fragmentation; pyknosis and loss of nuclei; and the separation of adjacent fibres by edema fliud. Perhaps the most striking feature in the histologic picture was the intense reddish-purple staining reaction of the affected fibres, attributable to their very pronounced tendency to take up calcium salts. With Haematoxylin and Eosin stains, very large areas were seen in which virtually all of the fibres and cell fragments were converted into dense, opague, reddish-blue masses as a result of the necrotic change and calcium deposition (Figs. 36 and 37). The inflammatory reaction in these lesions varied within rather wide limits. Proliferation of connective tissue elements and a moderate infiltrate of lymphocytes and mononuclear leucocytes was the usual finding (Fig. 38). Polymorphonuclear leucocytes were extremely few in number.

SKELETAL AND SMOOTH MUSCLE: Samples of skeletal muscle from the paravertebral region, the laryngeal region and thigh were sectioned. In the treated group 15 animals showed varying degrees of necrosis of skeletal muscle. In 10 instances, numerous small clusters or even isolated fibres were altered (Fig.43); in 4 additional cases moderately widespread areas of degeneration with a vigorous inflammatory reaction were seen (Fig. 40) and in one case the foci of destruction became confluent and a massive necrosis of large areas of muscle occurred (Figs. 41 and 42). The earliest changes seen in the affected fibres appeared to be swelling, with loss of striations and the assumption of a deep basophilic staining reaction due to excessive deposits of calcium salts (Fig. 39). Such swollen homogeneous fibres sometimes appeared to evoke a vigorous but rigidly localized inflammatory reaction with an abundant polymorphonuclear exudate which rapidly brought about solution of the necrotic cells (Fig. 41, 42 and 43). In other instances the fibres appeared to become extremely dense with calcific deposits, and often in relation to these changes the inflammatory reaction was of somewhat granulomatous character, with proliferation of sarcolemmal nuclei, an abundance of large mononuclear cells and occasional multinucleated giant cells (Fig. 44).

In the wall of the stomach in two cases, and in the smooth muscle of the small intestine in one case, foci of acute necrosis of muscle fibres were observed. The inflammatory exudate was not as pronounced as that seen in association with the striated muscle lesions, nor was there any "foreign-body" reaction with the formation of multinucleated giant cells.

Allowing for the histological differences in tissues, it was felt that the focal necroses of striated muscle, cardiac muscle necrosis and foci of necrosis in the smooth muscle of the gastrointestinal tract were quite comparable lesions.

LUNGS: The pulmonary arteries of five animals showed an acute or subacute arteritis which was identical with that seen in the vessels of other viscera (Figs. 2 and 10). In addition, the main branches of the pulmonary arteries

in 3 of the above cases showed inflammatory and proliferative changes in the intimal and subintimal tissue similar to that seen in the aorta (Figs. 7 and 8).

In addition to the above changes, there was observed in 5 instances, a lesion which involved vessels, but took the form of a vigorous proliferation of round and spindle-shaped mononuclear cells in the adventitial and subintimal layers of the vessel wall (Figs. 45, 46 and 47). This proliferative activity involved small and medium sized arteries and veins. While small numbers of lymphocytes were included in the dense mantles of cells about these vessels, the reaction was distinctly proliferative rather than exudative in its nature. The layer of cells present on the inner lining of the vessel wall was occasionally of sufficient thickness to completely occlude the vessel lumen. Fibrinoid necrosis of the vessel wall was not seen.

One rabbit received 6 massive injections of serum, and died of anaphylactic shock immediately following the injection of the seventh large dose. Most of the pulmonary capillaries in this case contained a dense, somewhat refractile, eosinophilic, homogeneous substance not unlike thrombus material. Free haemorrhage into the alveolar spaces was not seen, nor was there any evidence of leukocytic collections in relation to the capillaries which appeared to be so occluded (Figs. 48 and 49). Seven other animals died in anaphylactic shock, but this change was not identified in these or any other of the experimental animals.

Lesions comparable to the above were not seen in the lungs of any of the control animals.

Small foci of interstitial pneumonitis, characterised by thickened alveolar walls with variable numbers of mononuclear leucocytes were found in approximately 1/5 of both treated and control animals. These alterations were regarded as due to spontaneous disease in the species, and were not attributed to any effect of the serum. Goodpasture (131) has attributed similar lesions in rabbits to Encephalitozoon cuniculi.

BRAIN: A widespread vasculitis in association with focal inflammatory lesions (Figs. 50 and 51) in approximately one-half the treated and control animals was seen. The lesions have been described in detail by Bender (129), and Goodpasture (131) claims to have isolated the Encephalitozoon cuniculi as the causative organism. The treated group did not show a significant increase in arteritis over those of the control group.

LIVER: Focal lesions of cocciodiosis were noted in numerous of the control and treated animals. In view of the attention that has been given by some authors to small areas of focal necrosis, and increase in connective tissue and cell content of the portal triads, representative illustrations of these lesions from several control animals have been included. In some animals, the portal triads were almost devoid of connective tissue and there was no evidence whatsoever of excessive cellularity or bile duct proliferation (Fig. 52). In others the portal tracts were rendered unusually prominent by the presence of excessive amounts of cellular connective tissue, rich in lymphocytes and large mononuclear cells. The bile ducts of these animals were frequently somewhat dilated and lined with hyperplastic epithelium which showed a pronounced tendency to proliferate and produce new ducts (Figs. 53 and 55). Small areas of focal necrosis were infrequently seen in which the liver cells had been replaced by dense clusters of large and small mononuclear cells (Fig.54). There was no evidence that treatment with serum significantly increased the incidence of these spontaneous lesions.

KIDNEYS: An acute arteritis was not seen in any of the renal vessels, nor was any evidence of alteration from the normal seen in the renal glomeruli. Proliferation of the capillary endothelium or any other changes reminiscent of acute glomerulonephritis were not found. Numerous animals of both treated and control groups showed foci of interstial inflammation. Sometimes these acute inflammatory changes were associated with necrosis of tubules and a mixed leucocytic exudate (Fig. 56). In other instances the lesions appeared to be

of a more chronic character, showing some atrophy and dilatation of tubules, and proliferation of fixed tissue elements, with a sprinkling of mononuclear leucocytes (Fig. 57). Treatment of the animals with large doses of horse serum did not appear to affect the incidence of such lesions and they were not attributed to the experimentally induced hypersensitivity.

KNEE JOINTS: Knee joints were not routinely examined during the first portion of the experiment, and as a consequence, the knee joints from only 14 control and 53 treated animals are reported on here.

Difficulty in assessing the effectiveness of the treatment in causing inflammatory lesions in joints was experienced because of the presence of tissue alterations in both control and treated animals. When present, the changes were found most frequently in folds of synovial membrane at the base of the semilunar cartilages (Fig. 58) and in the angle formed by the fusion of joint capsule with periosteum. Six illustrations taken from three of the control animals have been utilized to illustrate the maximum changes which were found in the joints of untreated rabbits (Figs. 58, 59, 60, 61, 62 and 63).

The changes seen in control animals consisted primarily in slight proliferative tendencies in the lining cells of the synovial membrane, together with a slight increase in density and cellularity of the underlying connective tissue. Occasionally a few capillaries added to the general appearance of slight proliferative activity. An inflammatory exudate of leucocytes was never encountered and constituted one of the chief points which served to distinguish the degenerative changes of untreated animals from the definite inflammatory response seen in the joints of 6 of the treated series (Figs. 64, 65, 66, 67, 68 and 69).

The tissue alterations of treated animals which were attributed to hypersensitivity were much more than the most extensive changes of the control group. The lining synovial cells were piled layers deep in several cases.

The underlying collagen fibres showed swelling and hyalinization, and the increased vascularity was more severe than any seen in the control series. The inflammatory exudate consisted of lymphocytes and large and small mononuclear cells. They showed no tendency to cluster into granulomatous nodules. Proliferative activity of fibroblasts was conspicuous. In the most severe lesions, of which Fig. 64 is representative, the general reaction was of a more acute, diffuse inflammatory nature. The subsynovial tissues were edematous, hyperemic and diffusely infiltrated with inflammatory cells, and definite villus formation had occurred. A few fragmented pyknotic nuclei were seen, and the occasional pseudo-eosinophilic leucocyte. The small arterioles appeared swollen and prominent, but definite degenerative or inflammatory changes were not observed in their walls.

. These microscopic alterations in the articular and periarticular tissues were, for the most part, discernible only on microscopic examination. In one instance, however, (#27) the changes were severe enough to be visible in the gross specimen. In this single case there appeared to be distortion and disorganization of both knee and elbow joints associated with slight limitation of movement. The cartilage showed pink, pitted erosions and pannus formation.

DISCUSSION

In assessing the significance of histologic abnormalities which were observed in the treated animals, two fundamental questions always arose. The first was concerned with the pathogenesis of the alterations which were encountered. Of the lesions seen, which should be attributed beyond reasonable doubt to serotherapy and its resultant hypersensitivity; which should be considered as possibly resulting from hypersensitivity; and which should be recognized as manifestations of spontaneous disease in the species, having no direct relationship to serotherapy whatsoever. The second was concerned

with the extent of the morphologic similarities or dissimilarities between and experimentally induced lesions and those of human disease entities which are widely suspected as being of allergic origin. Included in this group are acute rheumatic fever, periarteritis nodosa, rheumatoid arthritis, dermato-myositis and acute diffuse glomerulonephritis.

The observations of the preceding experiments constitute very strong evidence that one or more massive intravenous injections of horse serum will consistently produce in rabbits an arteritis that is basically similar to the lesions of periarteritis nodosa in man. It is true that an acute generalized arteritis with lesions not unlike those of periarteritis nodosa have been observed and reported as occurring spontaneously in axis deer, cattle, swine, dogs and rats. However, it has not yet been reported as occurring spontaneously in cats, rabbits or guinea-pigs (132). The occurrence of an arteritis in 47 of the 77 treated rabbits in our series confirms the findings of Klinge (89) and Vaubel (90) and others (100) (101) (105) (110) who have described similar lesions in rabbits subsequent to parenteral injections of foreign protein. Failure to confirm these results on the part of Smith and Zeek (117) may be attributed in part to the inadequate number of animals treated, and in part to the technique of treatment employed by them. The relatively small series of Rich and Gregory (110) and the low incidence of arterial lesions in the series of Vaubel (90) detract somewhat from the significance of their findings. The high incidence of arterial lesions (61.1%) in our relatively long series of 77 animals leaves no doubt whatsoever that they may be attributed to hypersensitivity.

The basic morphologic similarity between the experimentally induced arteritis and that seen in periarteritis nodosa was striking, and has already been emphasized (110) (116) (120) (89) (90). Slight differences between the

experimental and human lesions were noted, however. The inflammatory changes along the vessels of the experimental animals were segmental in character, but never became large enough to be visible to the naked eye, never resulted in aneurysm formation, and never became progressively severe enough to cause illness and death in any affected animal. The experimental lesions differed from the human too, in their restricted distribution. In rabbits, under the conditions of this experiment, the great majority of inflammed arteries were found to reside in the heart, whilst those of the kidney, spleen and skeletal muscle remained conspicuously free from such involvement. In man, the organs exhibiting arterial lesions in periarteritis nodosa are substantially different (133). These appear to be minor quantitative rather than qualitative differences, and may well be explained, in part, at least, on the basis of species differences. It would appear also, that such factors in the technique of treatment as the type of antigen employed, and the quantity used, exert a very significant influence on the types of lesions which may result, and the severity of the reaction which is invoked. When small shocking doses of serum were utilized, Fox and Jones (114) reported that an arteritis was produced which was acute, but restricted to the heart, and something less than necrotizing in its characteristics. These results are comparable to the rather mild arteritis seen by Hawn and Janeway (120) when purified bovine serum albumin was utilized as antigen. Our results confirm the observations of Rich and Gregory (110), Junghans (101) and Hawn and Janeway (120) that when whole serum is used as antigen, in large amounts, the resulting arteritis may be generalized in its distribution, and necrotizing in character.

The morphologic similarities between the experimental lesions and the human disease which strongly suggest that the latter may have its pathogenesis in hypersensitivity, are further supported by the frequent association in humans of a polyarteritis with other definite hypersensitive conditions such as asthma (134), and by the observation of the lesions of periarteritis

nodosa in cases of drug hypersensitivity (56) (135) (125), and serum sickness (54). These latter facts do not define the etiology of periarteritis nodosa. On the contrary, the suggestion is that several different etiologic agents may effect the disease through the common pathogenetic mechanism of hypersensitivity.

Lesions in the hearts of experimental animals have been observed by many investigators (89) (90) (111) (116) who claim to have produced the changes by means of experimentally induced hypersensitivity. These authors have emphasized the similarity of these alterations to the cardiac lesions of acute rheumatic fever. Not all of the reports are completely convincing, and a careful analysis of their work casts some doubt on the validity of the conclusions drawn from some of these experiments. Klinge (89) used only 5 rabbits in that portion of the experiment where he describes a carditis. The only convincing photograph is that of an Aschoff-like perivascular scar, while the illustration of the valve lesion shows nothing more than mucoid swelling found in many normal stock rabbits. Vaubel (90) who lists changes in the heart muscle in 33 of 47 treated rabbits, does not present any relative illustrations, and much of the description suggests changes now known to be present in normal untreated rabbits (130). The incidence and precise character of cardiac lesions in the series of Hopps and Wissler (116) seems open to question since their illustrations of lesions which they attribute to hypersensitivity include the obvious spontaneous myocarditis of rabbits reported by Miller (130). However, the pictures of valve lesions and Aschoff-like lesions in the myocardium illustrated by Rich and Gregory (111) are rather convincing of the analogy of these lesions to those of rheumatic carditis. They found cardiac lesions in 19 of 51 treated rabbits, but failed to report on the incidence of such lesions in control rabbits living in the animal house at the same time and under the same conditions. This latter consideration is important in view of the fact that some of these lesions occurred in the control animals of the

present series.

In our material, in both treated and control animals, the myocardium showed foci of spontaneous myocarditis in approximately the same incidence as that reported by Miller (130). Inflammatory foci were found in the valve rings of 10 treated animals. These bore some resemblance to Aschoff-bodies in that there was swelling and necrosis of collagen fibres, palisading of nuclei and even the formation of giant cells in small numbers. It is true that similar lesions were not found in the valve rings of the 25 control animals. However, in 2 of the control animals, and in 6 of the treated group, inflammatory foci of mononuclear leucocytes and lymphocytes, and occasionally well-formed granulomata were seen. While the valve rings and valve cusps were considered separately in the tabulation of results, it is not reasonable to regard them as dissociated functional and anatomic entities when the pathogenesis of the inflammatory lesions seen there is to be considered. Thus the total incidence of the inflammatory valve lesions is 2 in the 25 controls (8.0%) and 16 in the 77 treated animals (20.7%). While this higher percentage incidence in the treated group is suggestive, the difference is not actually statistically significant. Moreover, while it is true that isolated cardiac lesions in the experimental animal do resemble those of rheumatic fever, it is also true, and probably more important, that the great majority of such lesions exhibit but a superficial resemblance to those of the human disease. In all fairness it must be recognized that species differences may well account for the considerable morphologic dissimilarities between the two, and that a suitable variant of this experimental procedure might appreciably reduce the dissimilarity. However, the fact remains that under the conditions of these experiments, the observations have failed to prove that lesions with the characteristics of rheumatic carditis can be produced experimentally in rabbits by this method. Moreover, I do not feel that any other experiments have as yet presented unmistakable proof that such lesions may be induced in hypersensitive animals.

In the lungs, the vascular lesions which were observed were morphologically of three kinds. The first consisted of an acute or subacute arteritis no different from that seen in the aorta and vessels of other viscera. The second type of change was characterised by the presence of dense perivascular and intimal collections of large mononuclear cells. This lesion was found in 5 of the treated animals, and was not seen in any of the control group. Pentimalli (91) described and illustrated similar proliferative lesions in the lungs of rabbits following the parenteral administration of foreign serum, and Oeller (136) reported changes not unlike these in guinea-pigs following the administration of chicken serum. Gerlach and Finkelday (137), however, were unable to confirm the latter's results. While the percentage incidence of this type of lesion in this series was so small as to place some reasonable doubt on their hypersensitive pathogenesis, I did feel that hypersensitivity could not be ruled out as the pathogenic agent in their production until the study of larger series and a further consideration of the results of other investigators was made. The third type of lesion consisted of changes involving pulmonary capillaries. In a series of 26 animals, Knepper and Waaler (105) reported the occurrence of hyaline thrombi in the pulmonary capillaries of 4 animals dying of anaphylactic shock, and Vaubel (90) observed similar changes in 3 animals out of a group of 8 which received repeated intravenous injections of serum. Two of the 3 animals observed by Vaubel died in anaphylactic shock. Gregory and Rich (113) studied the lungs of 56 rabbits which had received large intravenous injections of serum, and described the presence of focal capillary damage in 10 cases, 9 of which showed widespread capillary thrombosis. These authors felt that their lesions were quite comparable to those of pneumonitis in acute rheumatic fever and to the pulmonic changes in cases of sulfonamide hypersensitivity. In their experimental material, 2 animals died in acute anaphylactic shock, and 4 more died within 24 hours. In our series, 8 animals died of the effects of anaphylactic

shock, and of these, one showed capillary plugs of hyaline material not unlike thrombus. Comparable changes were not seen in any other animals. In some of the lesions illustrated by Gregory and Rich (113) an early slight leucocytic response is seen. Our isolated case showed no such inflammatory reaction. If this histopathologic alteration is to be regarded as a part of the hypersensitivity reaction of tissues, the evidence suggests that it must be with the proviso that its occurrence is only associated with severe anaphylactic reactions, frequently involving the death of the animal.

In addition to the massive necrosis of heart muscle which was observed in 7 animals, and necrosis of skeletal muscle in 15 cases, necrotizing lesions of smooth muscle were observed in the stomach wall in 2 cases and intestinal muscle in 1 case. The leukocytic reaction in the skeletal muscle lesions was more pronounced than that seen in the heart and intestine, but it was felt that if some allowance was made for tissue differences, the three lesions might beconsidered to be vasically the same. The illustrations of Klinge (89) and Vaubel (90), who produced these lesions in small numbers in skeletal muscle following multiple serum injections, are qualitatively similar, although apparently not as extensive as those seen in the present series. Thus the evidence indicates that focal necrosis of muscle is a further morphologic change attributable to a generalized hypersensitivity reaction. In this respect it is interesting that Steiner, Freund, Leichentritt and Maun (127) have recently described focal inflammatory lesions of skeletal muscle and peripheral nerves in cases of rheumatoid arthritis. The morphologic appearances of these clinical lesions have many points of similarity with those of the experimental animal., They are focal in character, and often induce relatively mild inflammatory reactions, with mononuclear leucocytes and occasionally giant cells, so that a granulomatous appearance results. The morphologic similarities between the experimentally induced lesions and those seen in skeletal muscle in typical cases of dermatomyositis are even more striking,

and the possibility of a common pathogenesis in hypersensitivity is further enhanced by the observation of an acute arteritis with fibrinoid changes and leucocytic infiltrations in human cases of dermatomyositis (138).

Many opinions exist, some supported by experimental observations, regarding the possibility that hypersensitivity may play a causative role in the production of rheumatoid arthritis (139) (140) (141) (142) (120) (89) (90). The present experimental findings show that hypersensitivity may well induce the development of a chronic inflammatory arthritis in rabbits. While the majority of lesions described were non-specific and represented only a quantitative change from abnormalities seen in control joints, it is probably worth while to note that one animal presented clinical and pathological features similar to those of well advanced rheumatoid arthritis in man. In this case, there was pannus formation, erosion of the articular cartilage, limitation of movement and distortion and disorganization of the joint. Unfortunately, the low percentage incidence of these results do not warrant any absolute conclusion concerning the relationship of the experimentally induced hypersensitivity to the occurrence of the joint lesions. In the human, Turnbull's description (143) of the remarkable improvement in many cases of rheumatoid arthritis when put on diets free of foods to which they were sensitive indicates strongly the importance of hypersensitivity in the complex etiology of rheumatoid arthritis.

In these experiments, there was a complete failure to reproduce the lesions of acute glomerulonephritis as described in the experiments of Rich and Gregory (110). It is possible that further procedures employing purified antigens in the manner of Hawn and Janeway (120) will be more successful. The lesions of the latter group appear from their illustrations to be quite early, and though acute, quite capable of undergoing complete resolution, leaving no evidence whatsoever of residual damage. The lesions seem to be more comparable to the focal capillary damage seen in pulmonary capillaries than to the lesions of typical human glomerulonephritis. It is noteworthy that

Vaubel (90) described capillary damage with the formation of hyaline plugs of thrombus in the glomeruli of several of his animals.. The frequent finding of non-specific focal inflammatory lesions in the kidneys of both treated and control rabbits would seem to indicate that such lesions, which were attributed by Longcope (85) to serotherapy, are really lesions of spontaneous disease in rabbits.

It is altogether possible that hypersensitivity may cause focal necrosis of the liver and induce proliferative activity of the portal tracts, but the tremendous variation in the morphologic appearances in the livers of apparently healthy untreated rabbits renders them unsuitable for the purpose of studies of this nature. In like manner, the frequent occurrence of inflammatory arterial lesions and granulomatous foci in the brain substance rabbits renders them quite unsuitable for experimental morphologic studies of this particular kind.

SUMMARY AND CONCLUSIONS

When rabbits are treated with large doses of whole horse serum given intravenously, an acute necrotizing arteritis is consistently produced. While minor points of difference occur, these lesions are morphologically very similar to those of periarteritis nodosa in the human, which fact constitutes very strong evidence that the human disease has its pathogenesis in a hypersensitivity reaction in these tissues.

Diffuse and focal inflammatory lesions of smooth, cardiac and skeletal muscle are also produced in rabbits as a result of serotherapy and generalized hypersensitivity. The lesions of skeletal muscle are morphologically quite similar to the muscle lesions of dermatomyositis, and not unlike the muscle changes associated with rheumatoid arthritis.

Chronic inflammatory lesions in joints which resemble rheumatoid arthritis appear to result from serotherapy and generalized hypersensitivity. While not seen in the control animals, the percentage incidence of such lesions

in the treated group was quite small, which detracted somewhat from the significance of these observations.

Subacute inflammatory reactions were seen in the intimal layer of the aorta, and in small numbers in the intima and adventitia of the pulmonary vessels, and appeared to be a part of the generalized hypersensitivity reaction. These lesions have no morphologic counterpart in spontaneous human disease, although the aortic intimal changes have been observed in humans following serotherapy.

Lesions which had many points in common with the cardiac changes of rheumatic fever were seen in the heart. The lesions did not precisely simulate those of rheumatic fever, but the analogy was close enough to strongly suggest that the tissue changes in the human disease may well have their origin in a hypersensitivity tissue reaction. Apart from the dissimilarities between the human and experimental lesions, which might well be reduced by suitable variations in experimental technique, the significance of the findings were vitiated somewhat by the observation of rather similar lesions in a small number of control animals. Thus from the results of these experiments, it was easier to note that cardiac lesions quite similar to those of rheumatic fever had been observed in experimental animals than to state that hypersensitivity had been proven to be responsible for their production.

Lesions simulating in any way those of acute glomerulonephritis were not produced in these rabbits under the conditions of this experiment.

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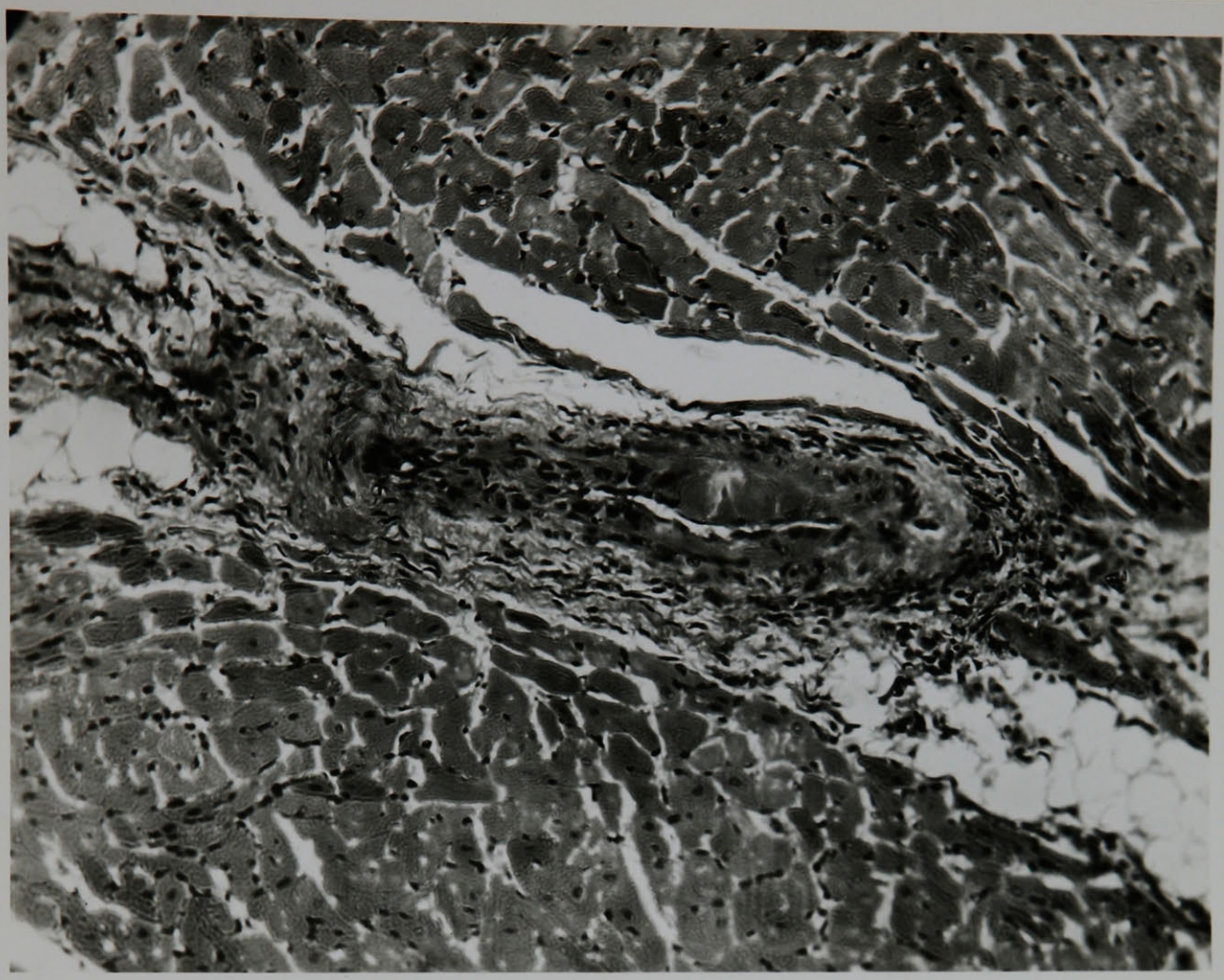
Fig. 1. Coronary artery of rabbit # 19.
Acute arteritis in which a segment of the wall shows an eosinophilic, swollen, homogeneous, acellular area in which there is loss of nuclei and cell detail. Two injections of serum. X 205.
This, and all subsequent illustrations, are of sections stained with Haematoxylin and Eosin.

Fig. 2. Coronary artery of rabbit # 51.
Acute arteritis. The media and adventitial connective tissue show swelling and fibrinoid necrosis. The majority of the inflammatory cells are lymphocytes, with a small admixture of large mononuclear cells. Two injections. X 225.



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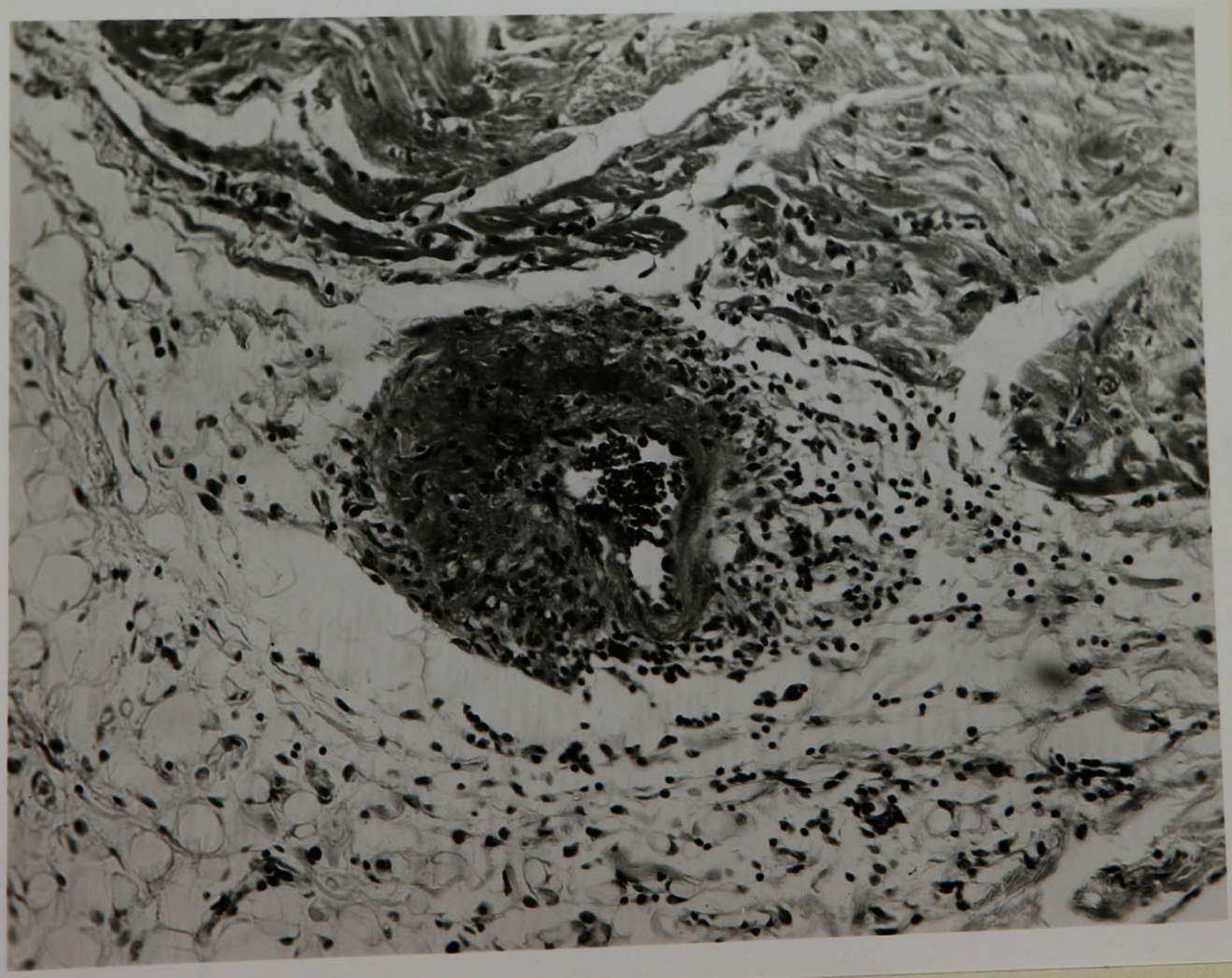

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Fig. 3. Mesenteric artery of rabbit # 27.
Chronic arteritis. The subendothelial layer is thickened with cellular connective tissue and the media shows slight distortion due to scarring. The endothelial cells tend to be swollen, and lymphocytes and large mononuclear cells are plentifully sprinkled throughout the rather dense collagenous adventitia. Eight injections. X 82.

Fig. 4. Mesenteric artery of rabbit # 48.
Necrotizing arteritis. The segmental character of the inflammatory reaction is well shown, with destruction of the full thickness of the wall in some areas. Two injections. X 76.

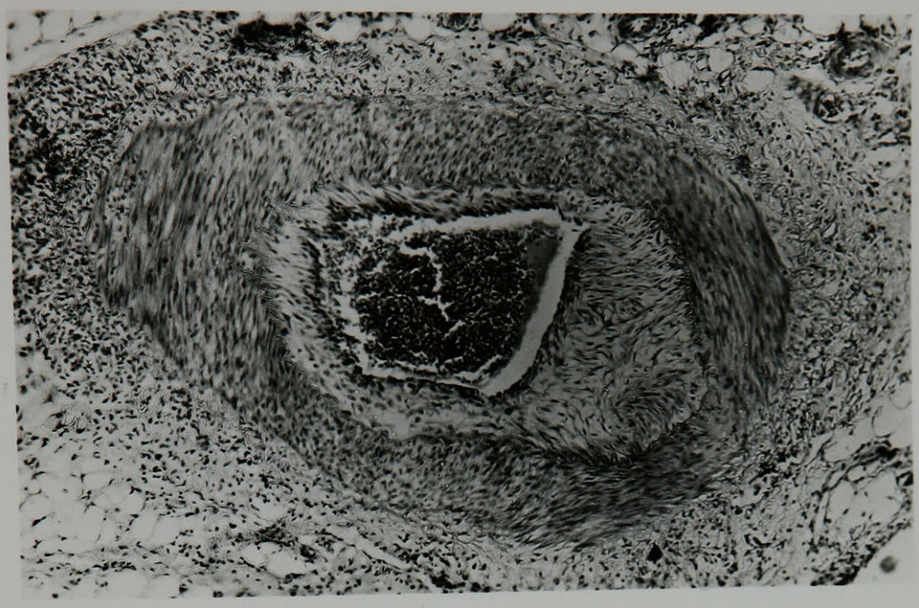

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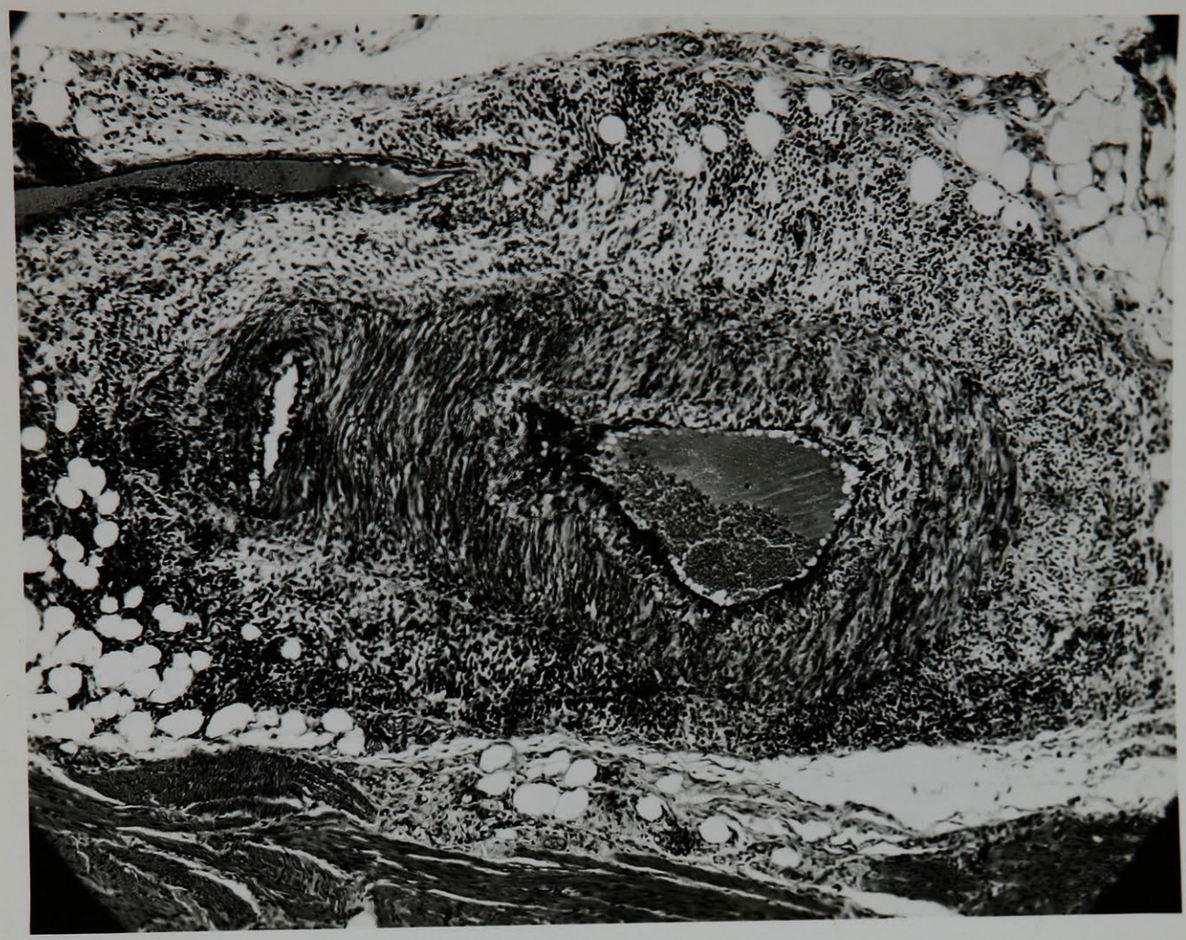
Fig. 5. Gastric artery of rabbit # 6.
The entire vessel wall shows swelling and separation of the cellular elements by an inflammatory exudate rich in polymorphonuclear leucocytes. Extensive fibrinoid necrosis of the media is present. Two injections. X 87.

Fig. 6. Aorta of rabbit # 58.
A diffuse inflammatory reaction in the subendothelial tissue of the aorta composed largely of an infiltrate of lymphocytes and large mononuclear cells is seen. Two injections. X 100.



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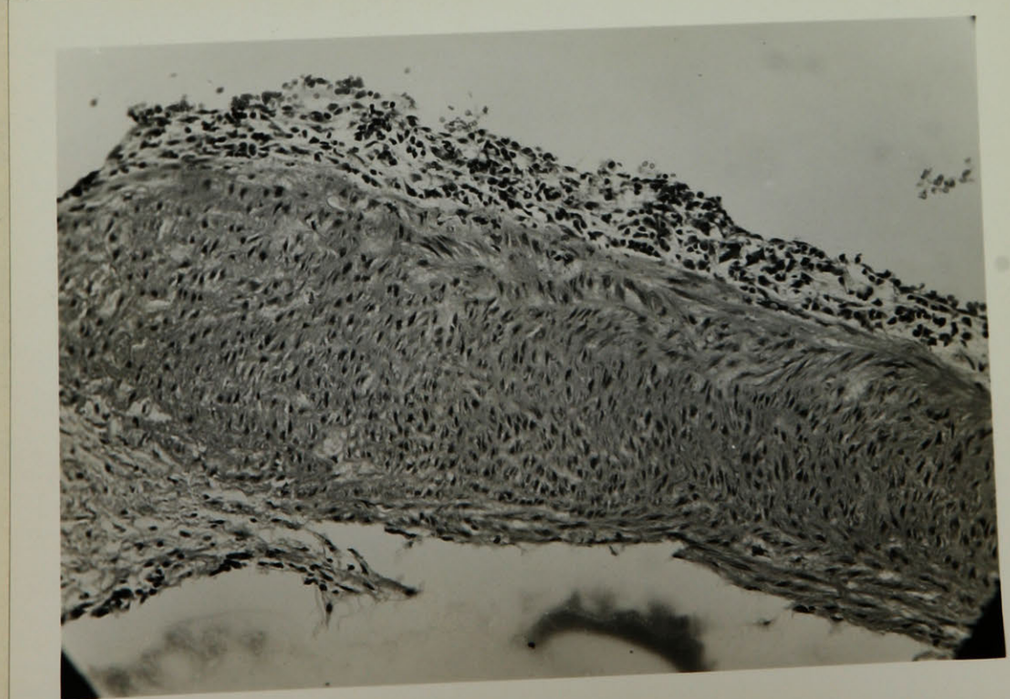

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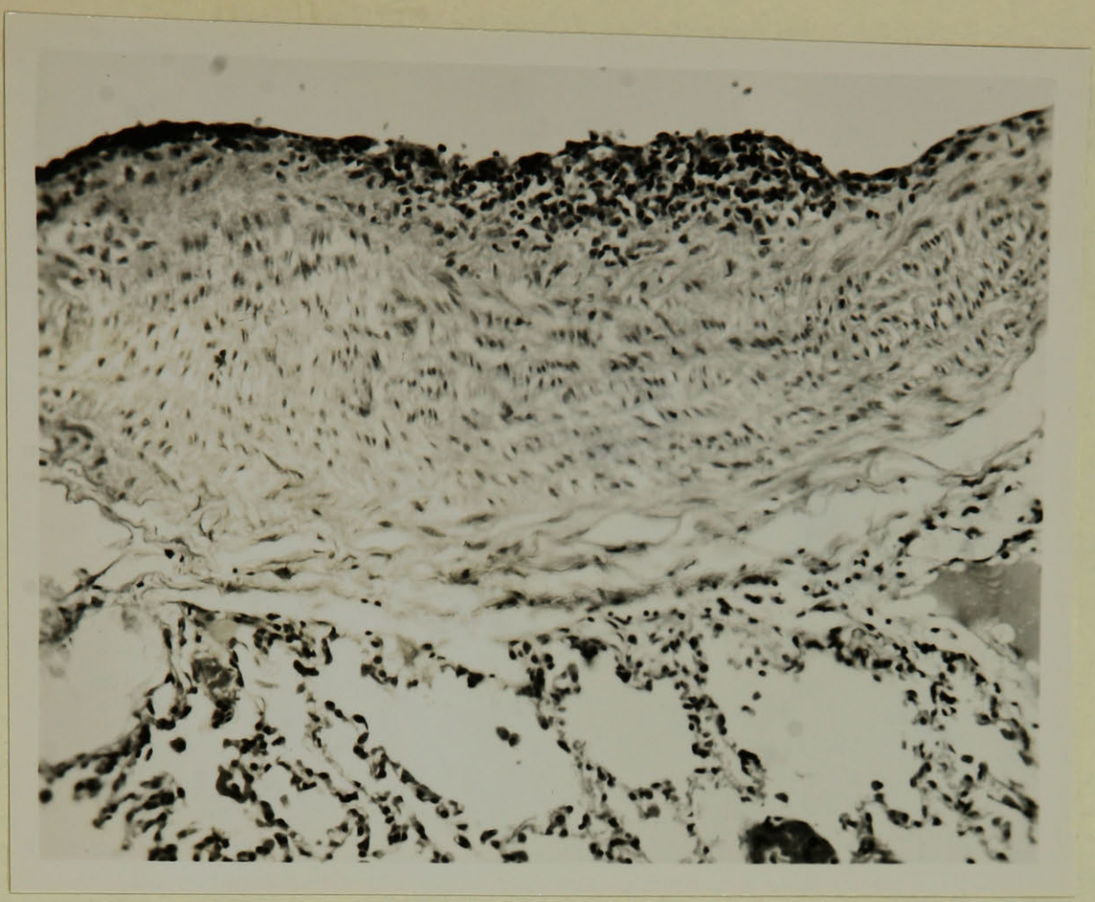
Fig. 7. Pulmonary artery of rabbit #53.
The subintimal tissues are thickened by proliferating histiocytes and an infiltrate of mononuclear leycocytes. The lining endothelial cells are swollen and prominent. Two injections. X 190.

Fig. 8. Pulmonary artery of rabbit #59.
The subintimal tissues are uniformly thickened by edema and an infiltrate of lymphocytes and mononuclear leucocytes. The smooth lining of endothelial cells is no longer continuous. Two injections. X 190.



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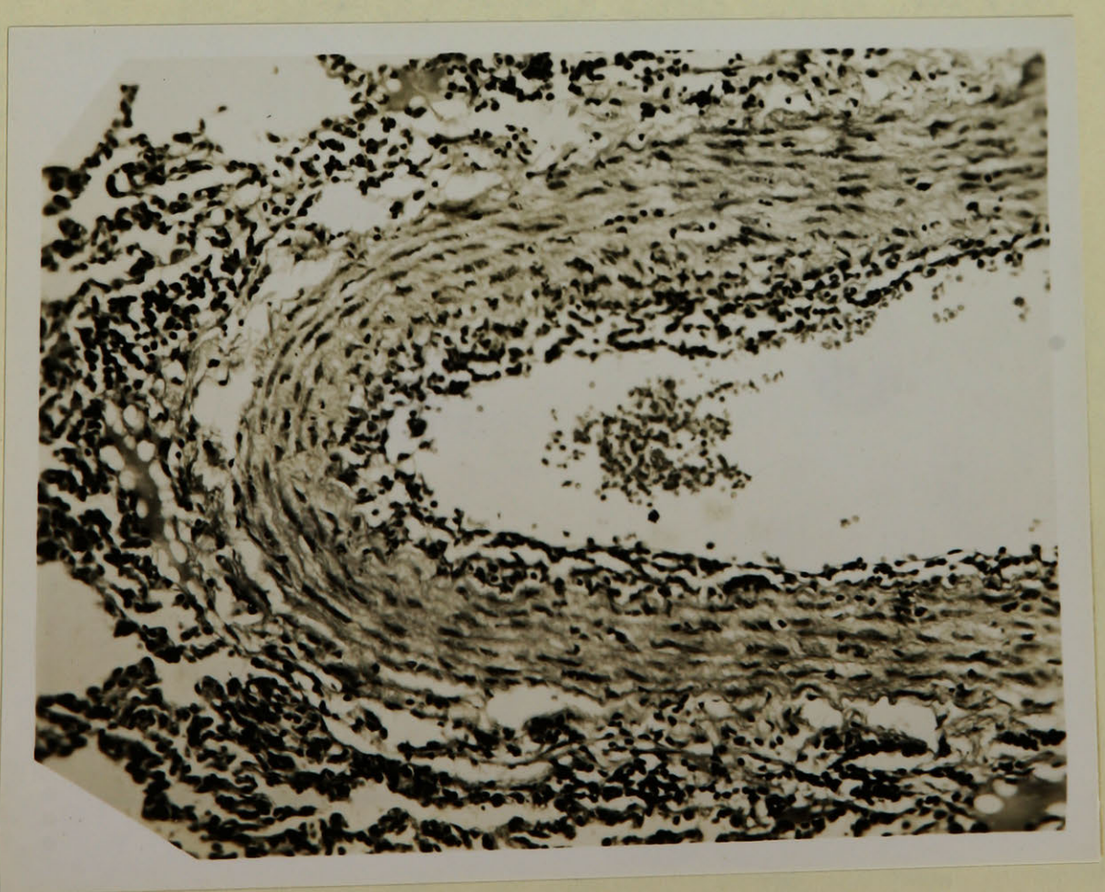

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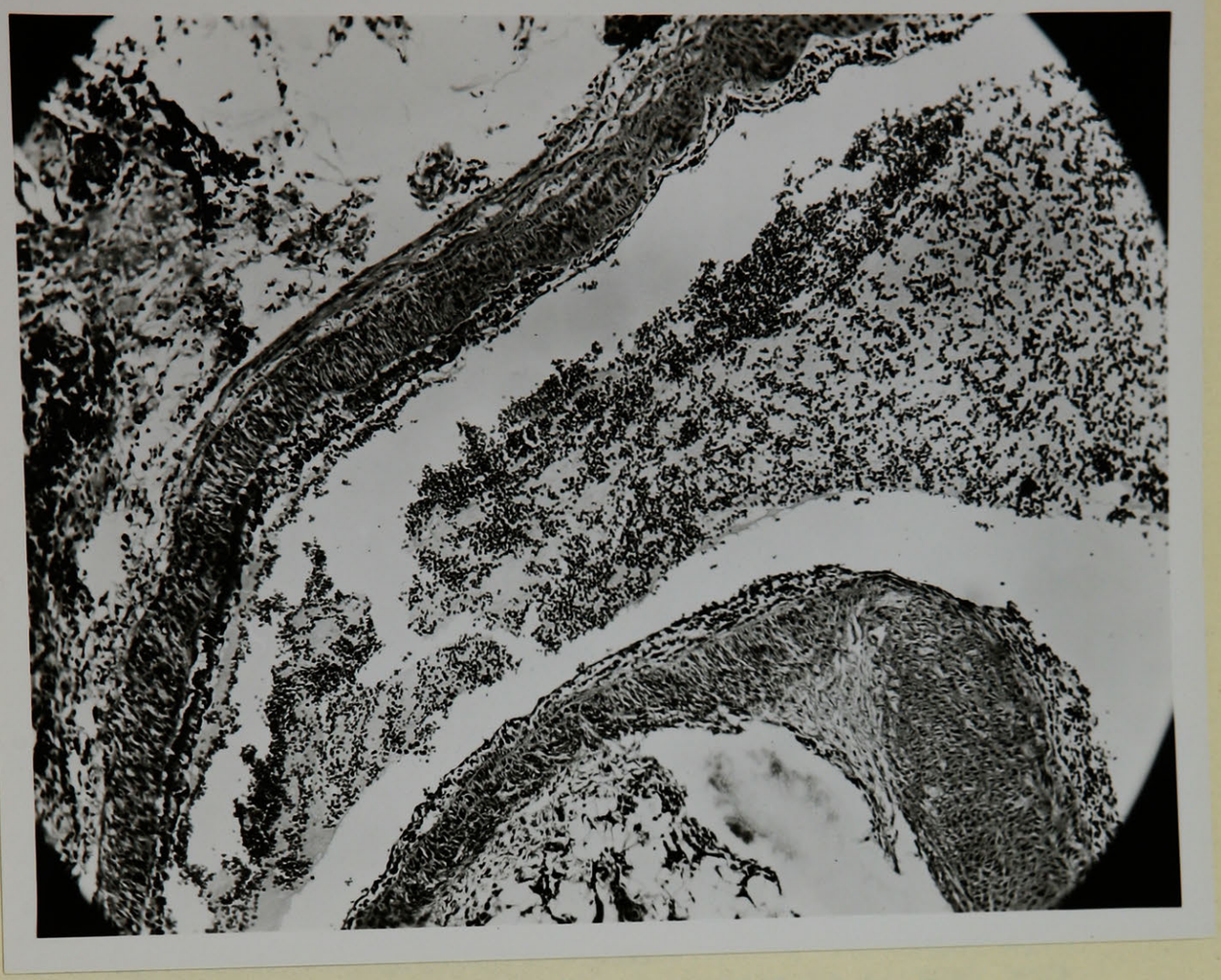
Fig. 9. Ostium of coronary artery of rabbit #58.
A diffuse inflammatory reaction in the intimal layer of the aorta is seen to be continuous with a necrotizing arteritis involving the entire thickness of the wall of the first portion of a coronary artery. Two injections. X 84.

Fig. 10. Gastric artery of rabbit # 6.
Subacute arteritis. The lining endothelial cells are swollen, and at one side show considerable proliferative activity. The media shows slight hyaline swelling of one segment, and a cluster of large mononuclear leucocytes and proliferating fibroblasts is seen in the adventitia. Two injections. X 680.



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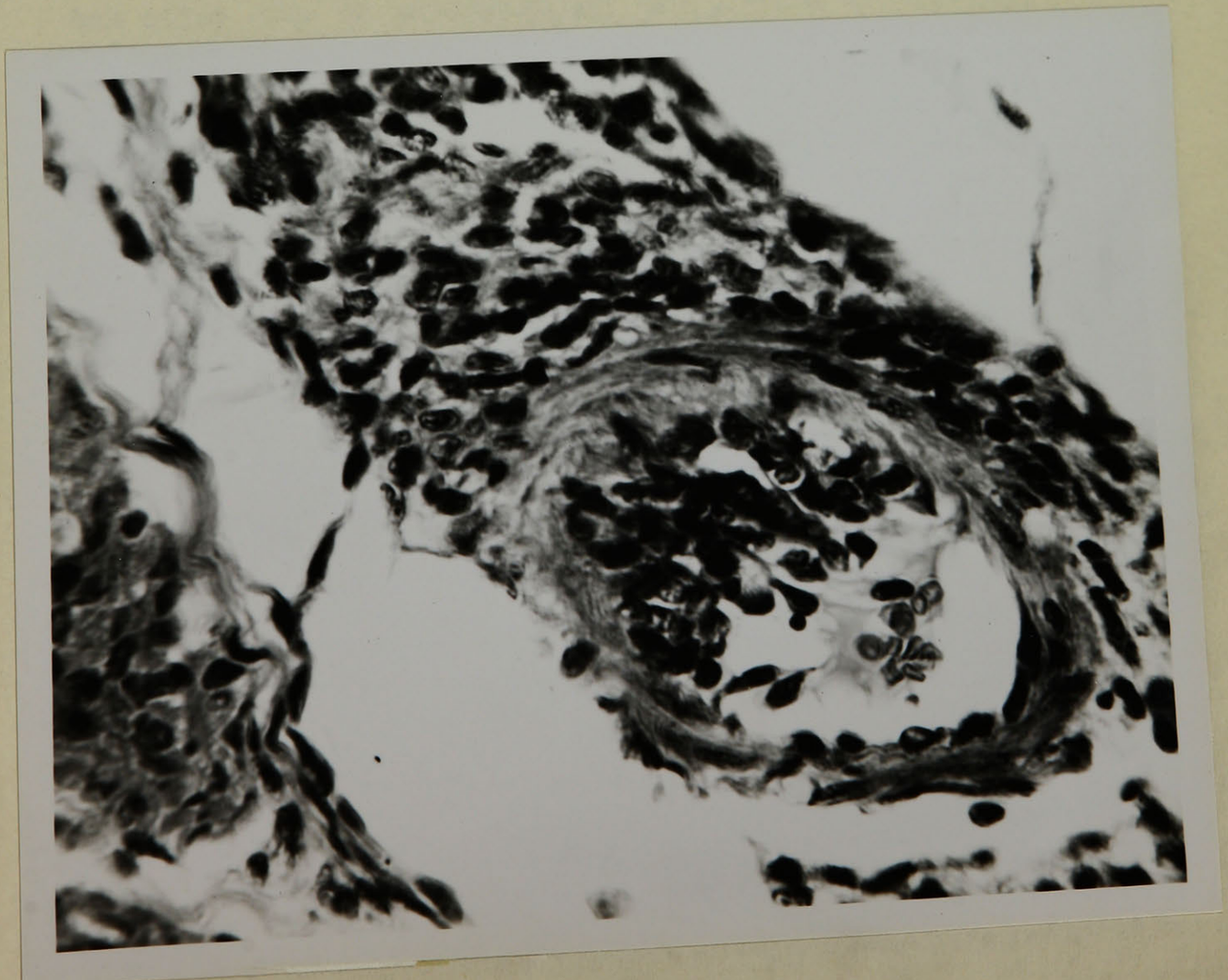

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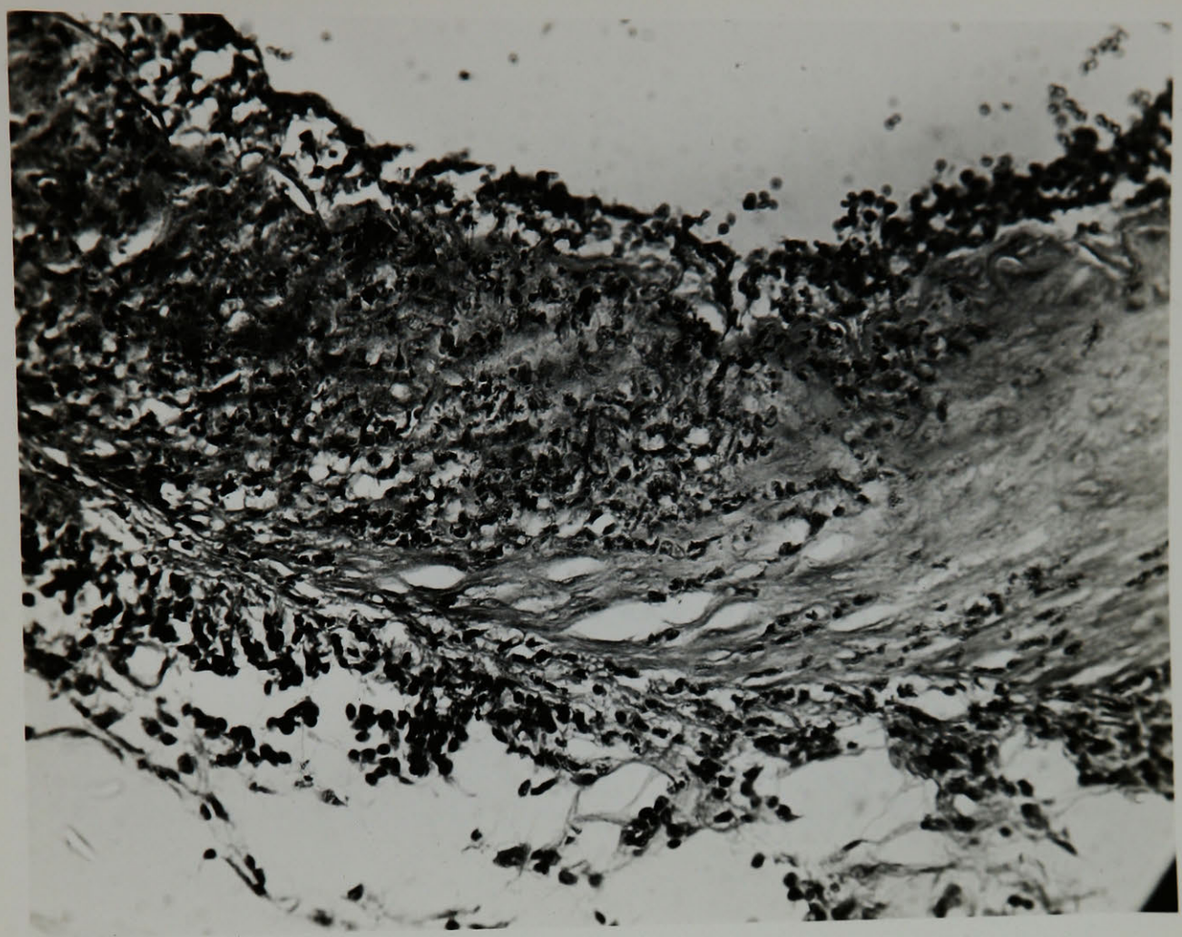
Fig. 11. Mesenteric artery of rabbit # 48.
High magnification of segment of artery shown in Fig. 4 to illustrate details of fibrinoid necrosis in the media with a purulent exudate, and proliferative changes in the endothelium. Two injections. X 310.

Fig. 12. Gastric artery of rabbit # 6.
High magnification of segment of artery shown in Fig. 5 to show details of necrotizing inflammatory reaction. There is pyknosis of nuclei, and fragmentation of both cells and nuclei in the media. The fibres are widely separated by fluid. The intimal endothelium has been raised by edema fluid, and shows swelling and proliferative changes. The adventitia contains a dense infiltrate of large mononuclear leucocytes. Two injections. X 300.



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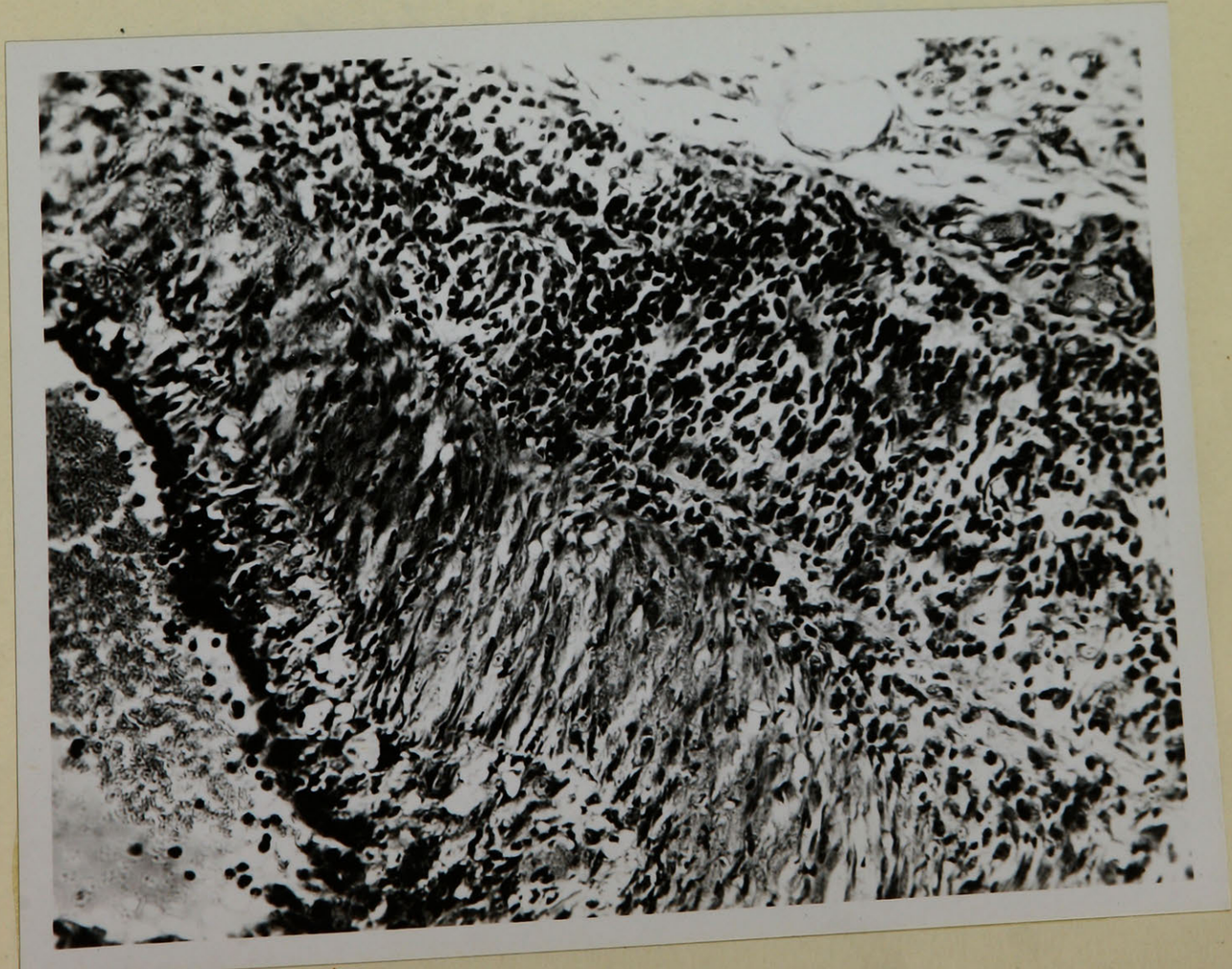

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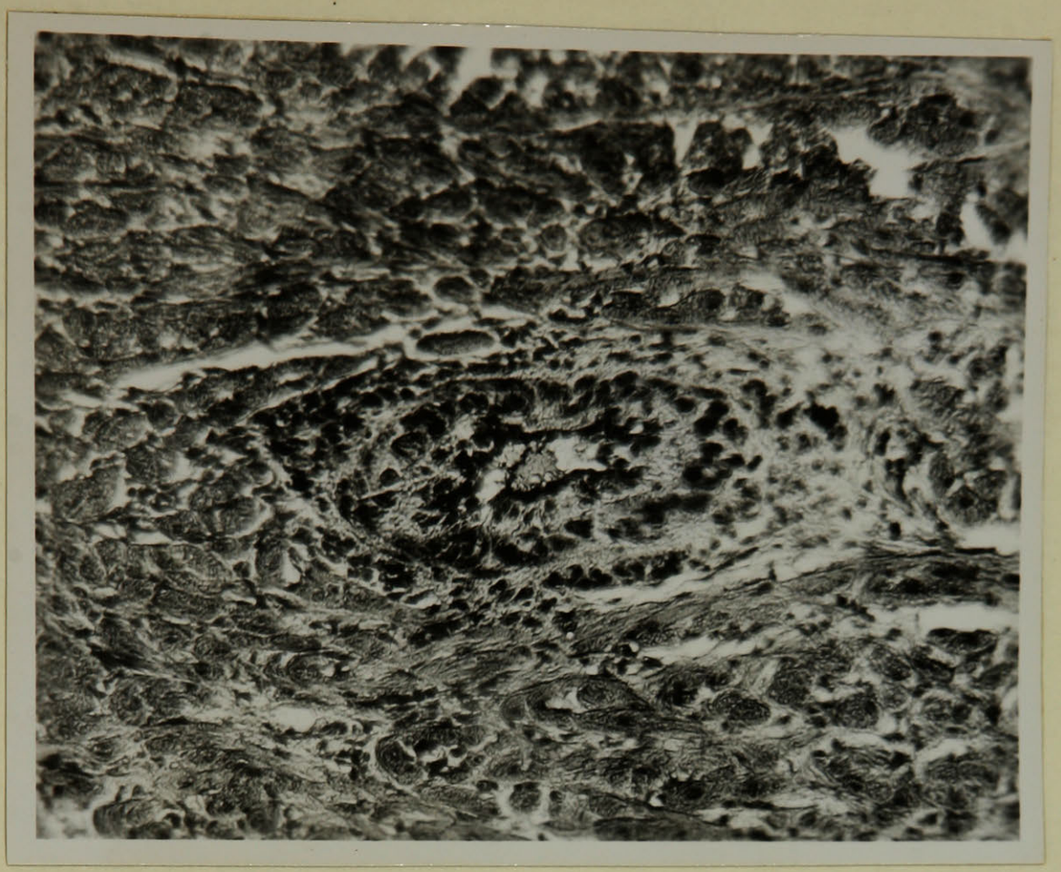
Fig. 13. Coronary artery of rabbit # 94.
Subacute arteritis. The media shows swelling and hyalinization of its fibres which obscures the cell outlines. The endothelial cells are swollen and prominent, and collections of mononuclear leucocytes in the adventitia vaguely resemble Aschoff-bodies. Two injections. X 220.

Fig. 14. Coronary artery of rabbit # 94.
Subacute arteritis. Note the paravascular collection of lymphocytes and large mononuclear cells. Two injections. X 220.



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14.

Fig. 15. Heart valve of rabbit #15.
The valve cusp is swollen, and the connective tissue has a translucent almost mucoid appearance. There is a slight proliferative tendency in the endothelial lining cells. Three injections. X 150.

Fig. 16. Heart valve of control rabbit # 66.
The valve cusp shows a hyaline swelling of the connective tissue similar to that seen in Fig. 15. There is no cellular exudate. These lesions were encountered in both treated and control animals in approximately the same proportion of cases, and were not attributed to hypersensitivity. No injections. X 225.

15

16.

Fig. 17. Heart valve of control rabbit # 119.
The illustration is reversed. An inflammatory nodule is present on the inferior surface of the cusp. The central portion of the nodule is pale with edema fluid which separates attenuated collagen fibres. Lymphocytes and swollen histiocytes are found in the periphery of the lesion. This animal was not treated in any way. X 240.

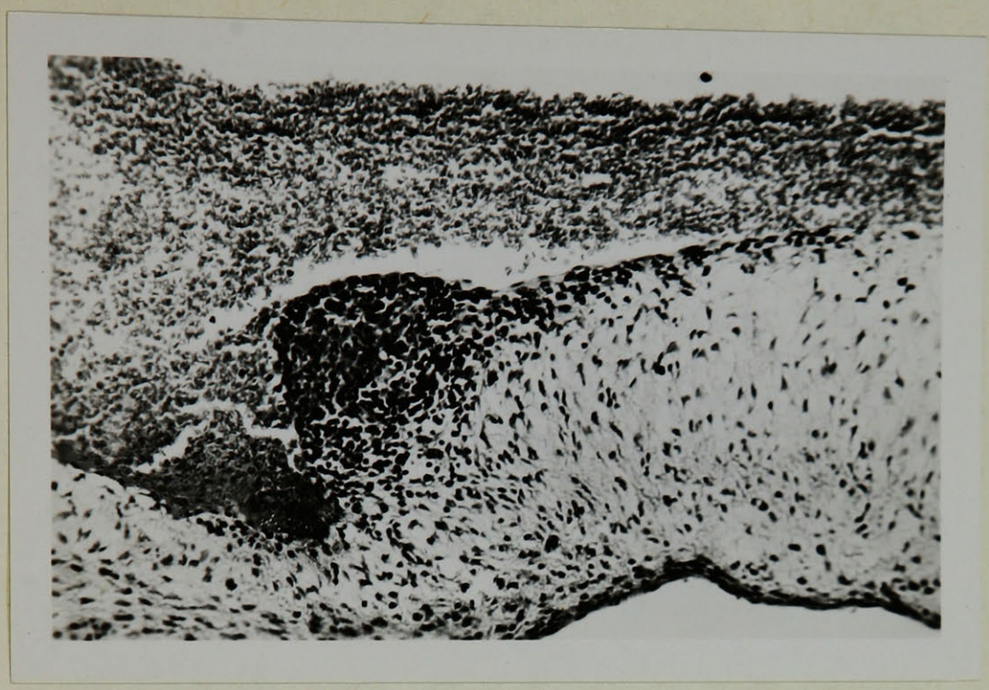
Fig. 18. Heart valve of rabbit #40.
A focal inflammatory reaction in the valve cusp is seen, with a dense collection of small round cells. The valve is edematous, pale and swollen to a fusiform outline. Six injections. X 178.

17

18

Fig. 19. Heart valve of control rabbit # 87.
The valve cusp appears thickened and pale with edema fluid. The connective tissue fibres are attenuated and widely separated. On the inferior surface of the valve is a dense collection of inflammatory cells, mainly lymphocytes. This animal received no treatment. X 170.

Fig. 20. Heart valve of rabbit # 27.
The free end of the valve cusp shows a marked degree of diffuse thickening with some fibroblastic activity, excessive deposits of hyaline connective tissue and a few rather diffuse collections of lymphocytes and mononuclear cells. Eight injections. X 75.



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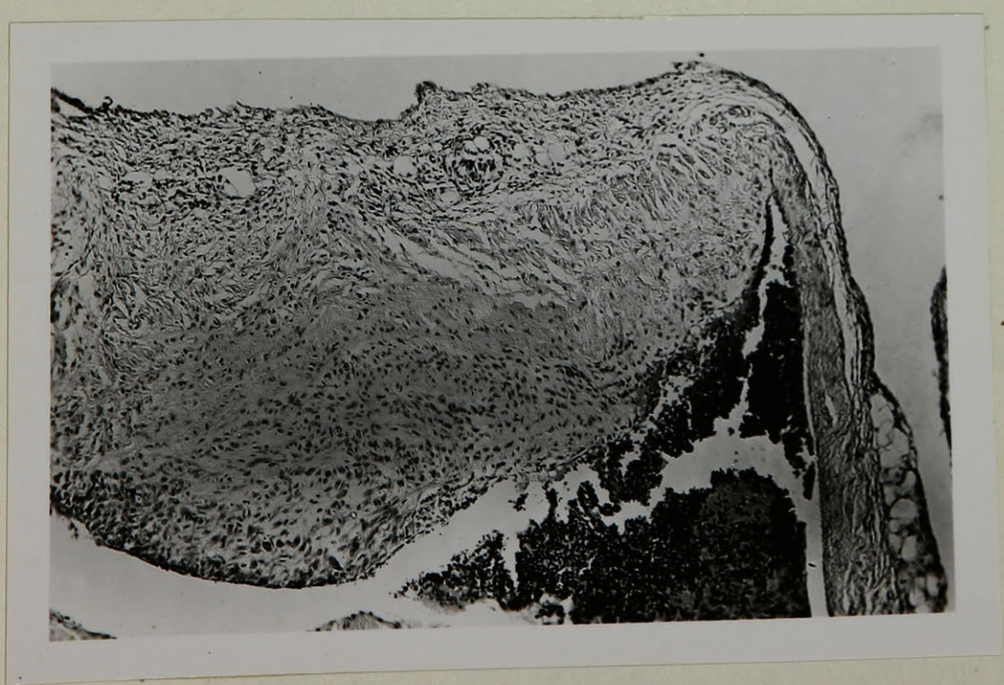

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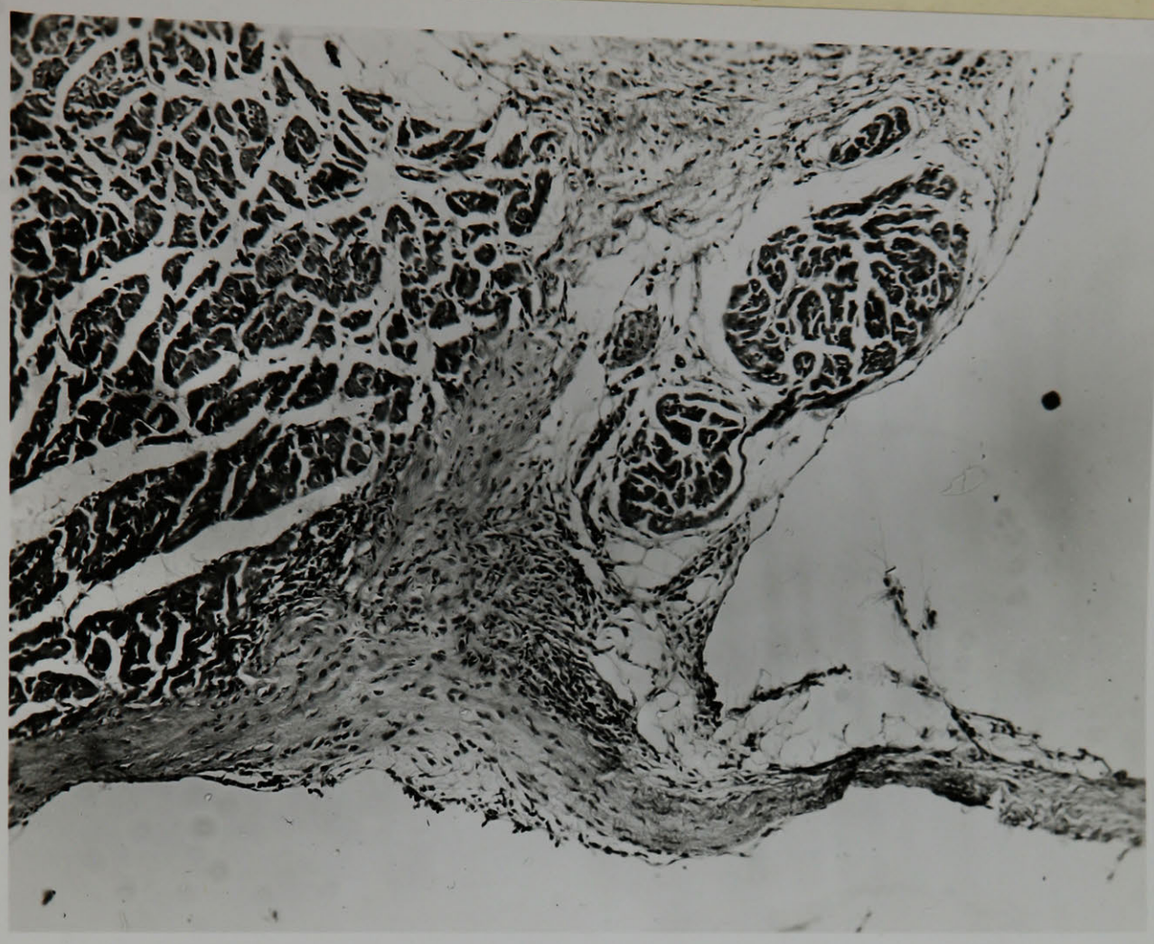
Fig. 21. Valve ring of rabbit # 41.
A diffuse inflammatory reaction of rather low intensity is seen to be involving the greater portion of the valve ring in cross-section. Four injections. X 112.

Fig. 22. Valve ring of rabbit # 41.
A higher magnification of Fig. 21 shows hyaline collagenous tissues with diffuse collections of large mononuclear cells, together with swelling and proliferative activity on the part of fibroblasts. Four injections. X 250.



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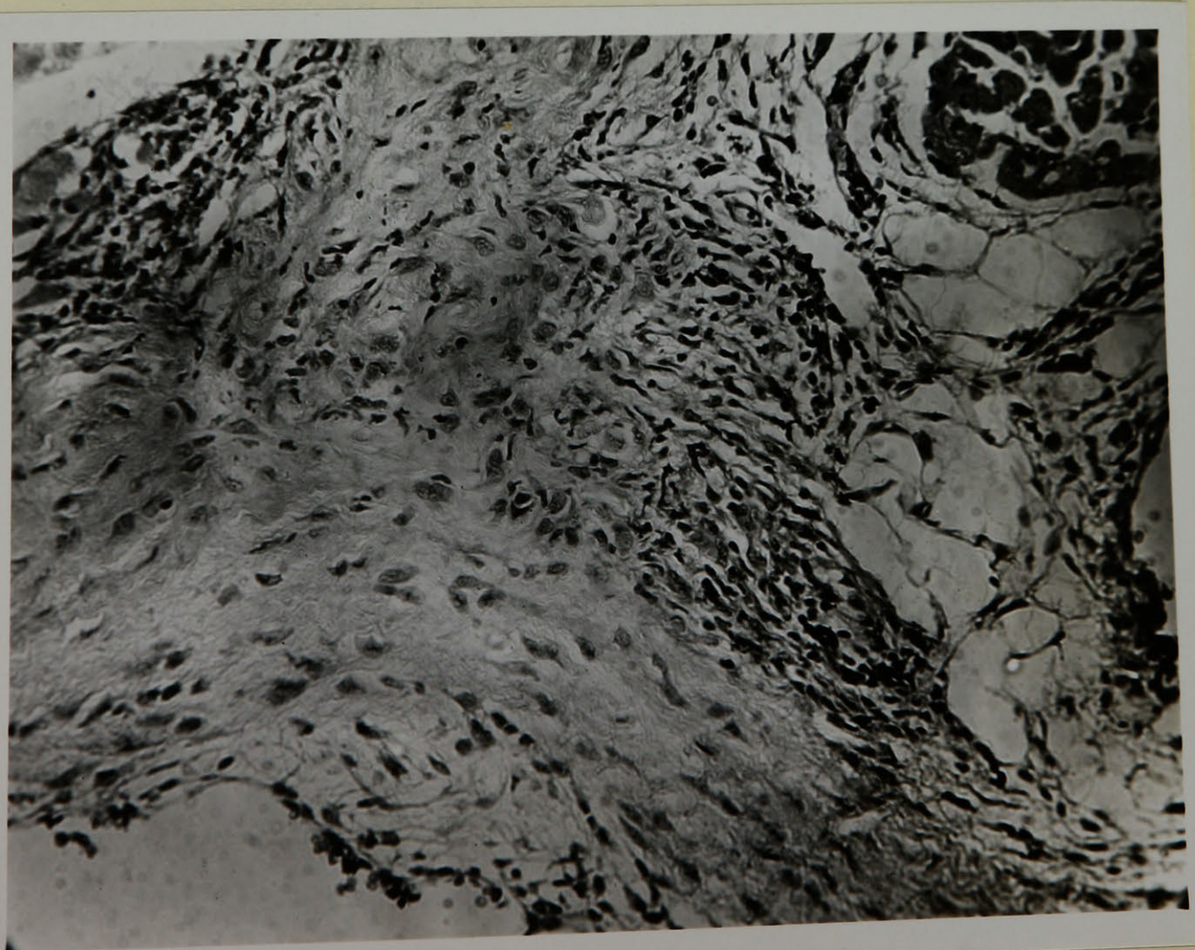

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Fig. 23. Aorta and aortic valve of rabbit # 15. Topographical section to show a granulomatous lesion on the upper surface of the aortic valve at its insertion into the first portion of the aorta. Three injections. X 100.

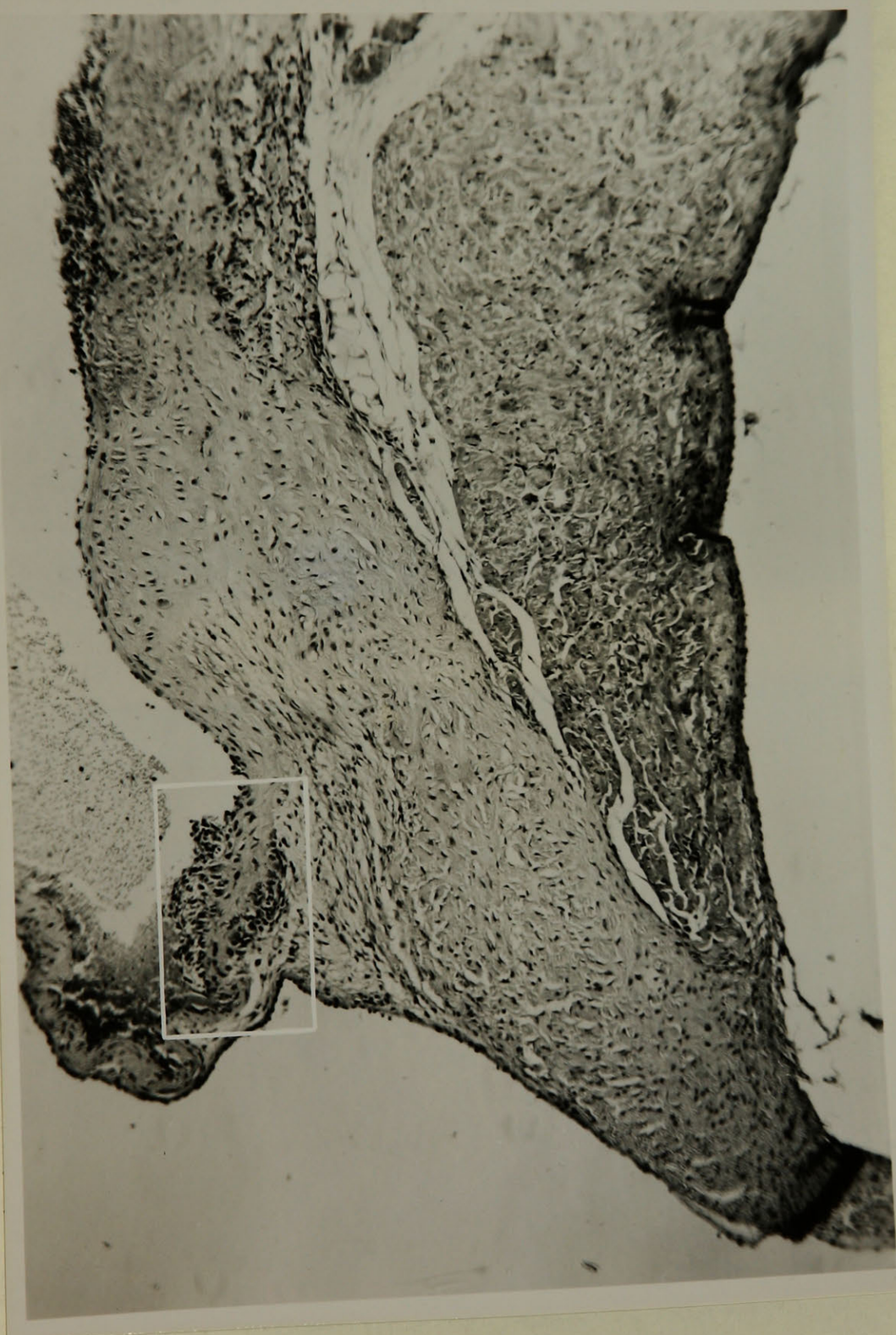

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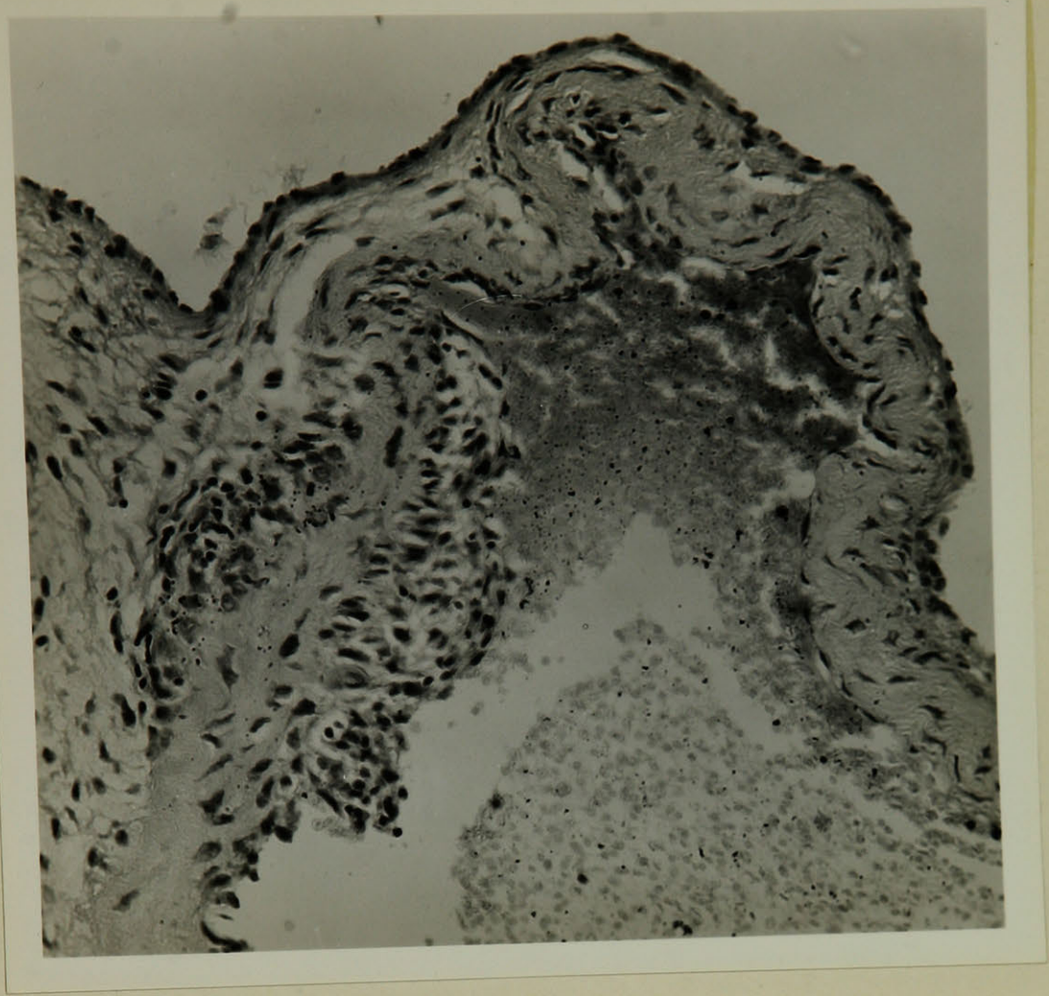
Fig. 24. Base of aortic valve of rabbit # 15.
A higher magnification of the lesion in Fig. 23.
Swollen histiocytes and proliferating fibroblasts are clustered in a fusiform fashion about a focus of hyaline collagen. Palisading of nuclei is quite prominent.
Three injections. X 235.

Fig. 25. Valve ring of rabbit # 110.
The collagenous fibres are coarse and hyalinized. Near the endocardial surface may be seen numerous mononuclear leucocytes and large pale histiocytes, together with a few multinucleated giant cells. Two injections. X 220.



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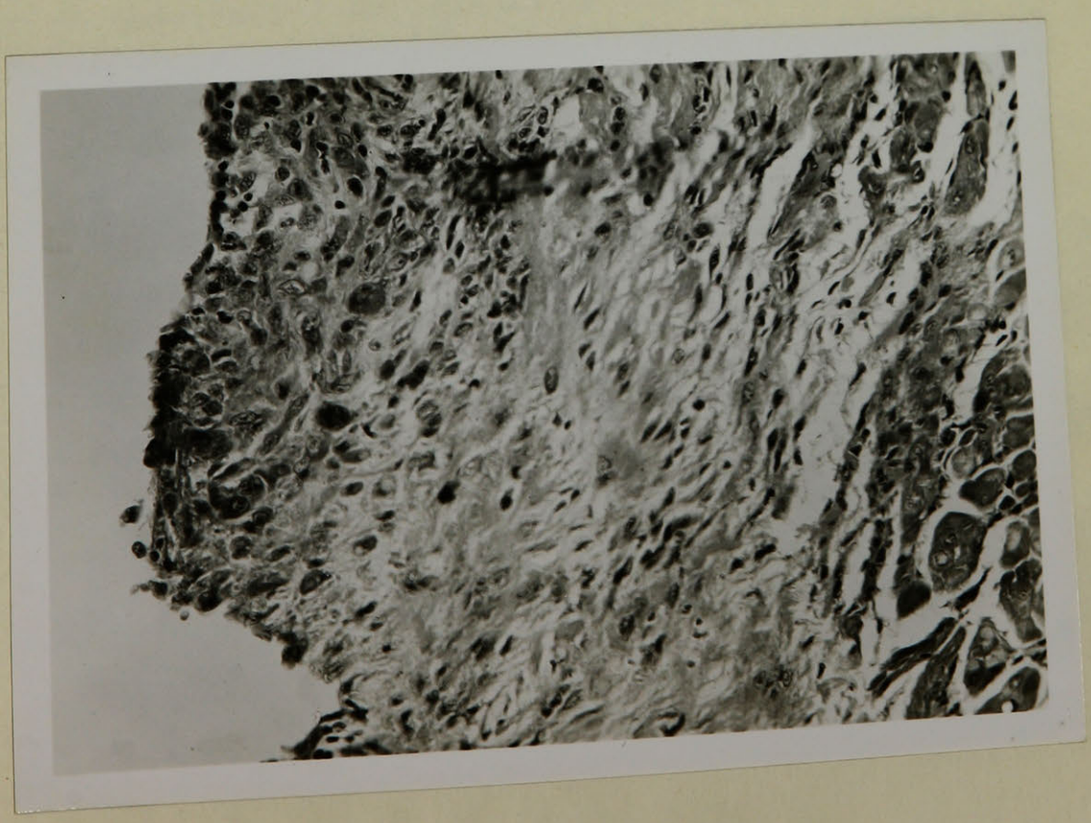


25.

Fig. 26. Valve ring in heart of rabbit # 15.
The lesion shows a rather diffuse inflammatory change with large and small mononuclear leucocytes infiltrating the connective tissue ring, and the fibrocytes showing swelling and proliferative tendencies. Three injections. X 187.

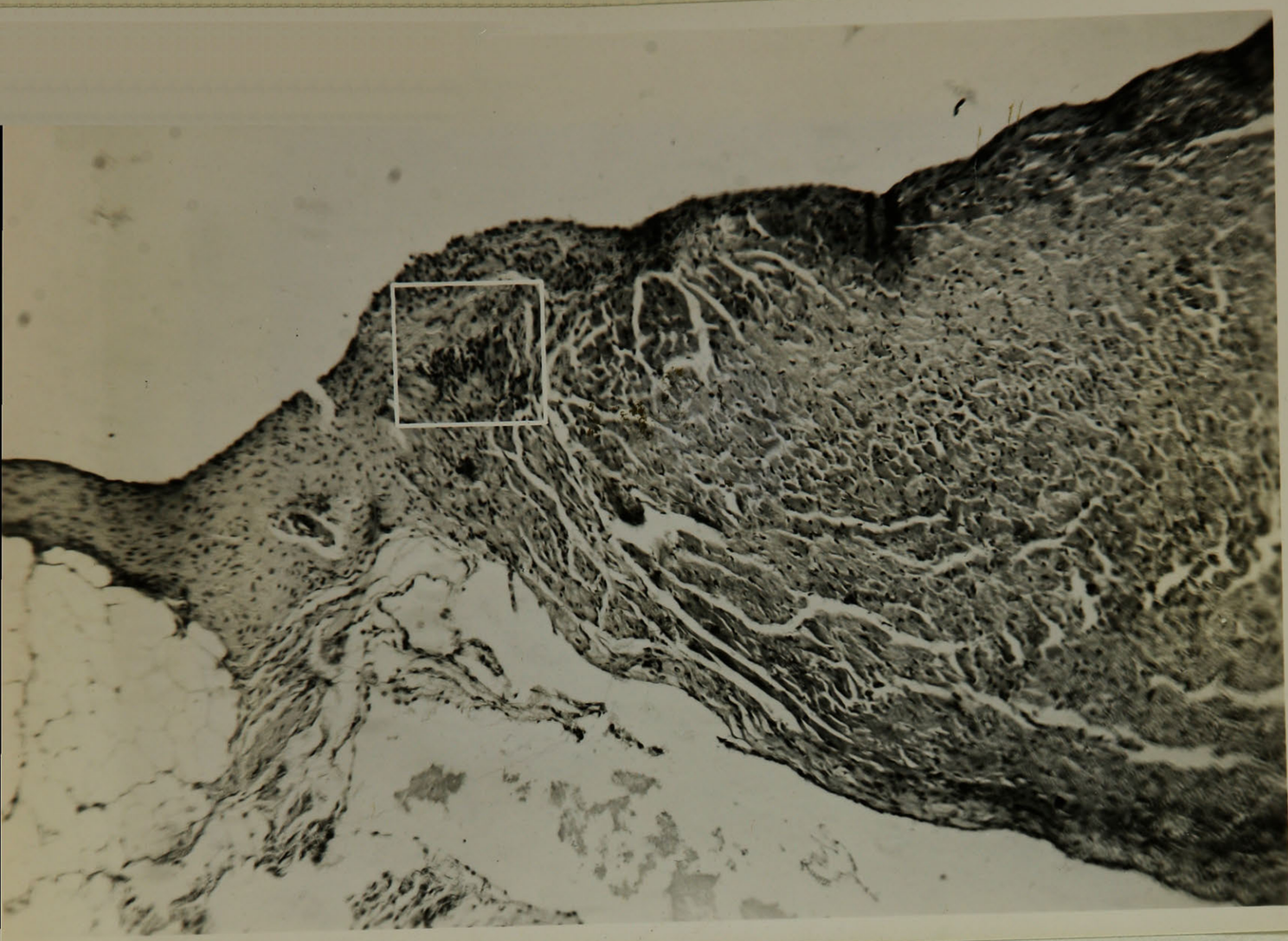
Fig. 27. Heart valve of control rabbit # 119.
A higher magnification of the lesion illustrated in Fig. 17 to show the detail of this granulomatous focus. This animal was not treated in any way. X 415.

26

27.

Fig. 28. Valve ring in heart of rabbit # 15.
A topographical section showing the relative size of a focus of chronic inflammatory cells in the valve ring. Three injections. X 115.

Fig. 29. Valve ring in heart of rabbit # 15.
A slightly higher magnification of Fig. 28 to show the characteristics of the inflammatory reaction. The infiltrate consists mainly of hymphocytes and a few large mononuclear leucocytes. Three injections. X 185.



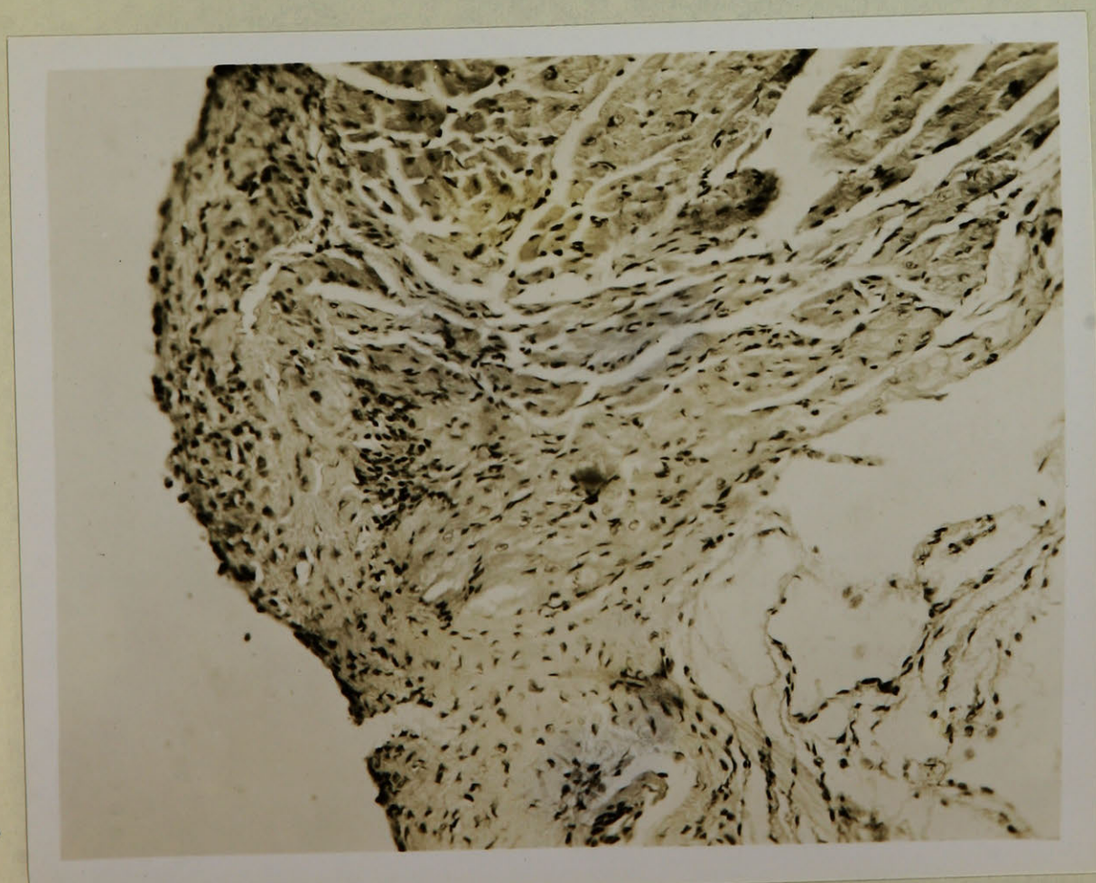


Fig. 30. Heart valve of rabbit # 46.
A few mononuclear leucocytes and lymphocytes are scattered throughout the cusp, and the living endothelial cells are swollen and prominent. This degree of change occurred in both treated and control animals and was not included in the score of lesions attributed to hypersensitivity. Two injections. X 117.

Fig. 31. Heart valve of rabbit # 24.
A diffuse inflammatory reaction with proliferating fibroblasts and a dense infiltrate of mononuclear leucocytes and lymphocytes is present on the under surface of the valve. Eight injections. X 240.

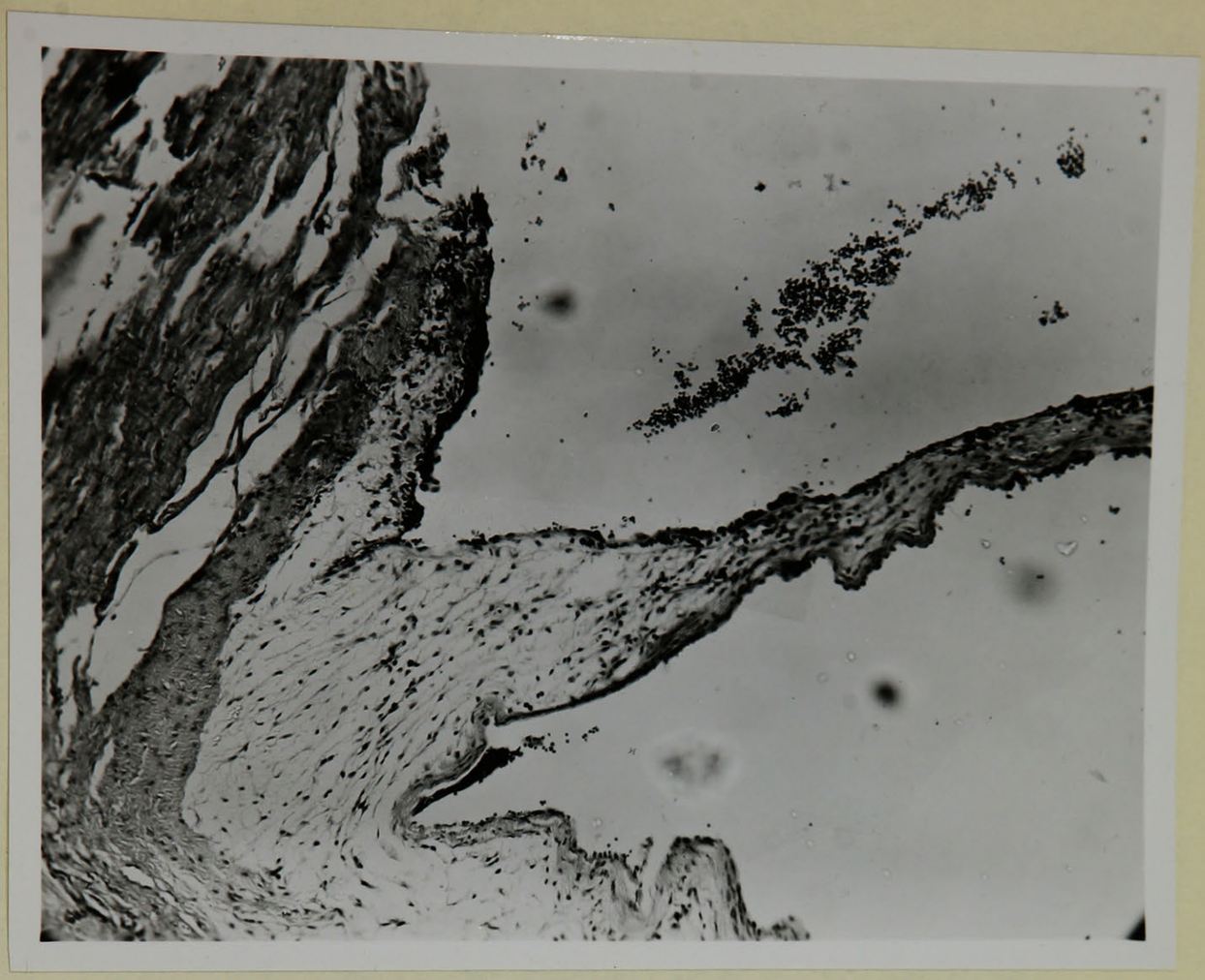


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Fig. 32. Myocardium of control rabbit # 119. The illustration shows a small coronary artery with a collection of mononuclear leucocytes and lymphocytes in the adventitia. This is representative of the inflammatory reaction that can occur in relation to vessels in the hearts of untreated rabbits. No injections. X 230.

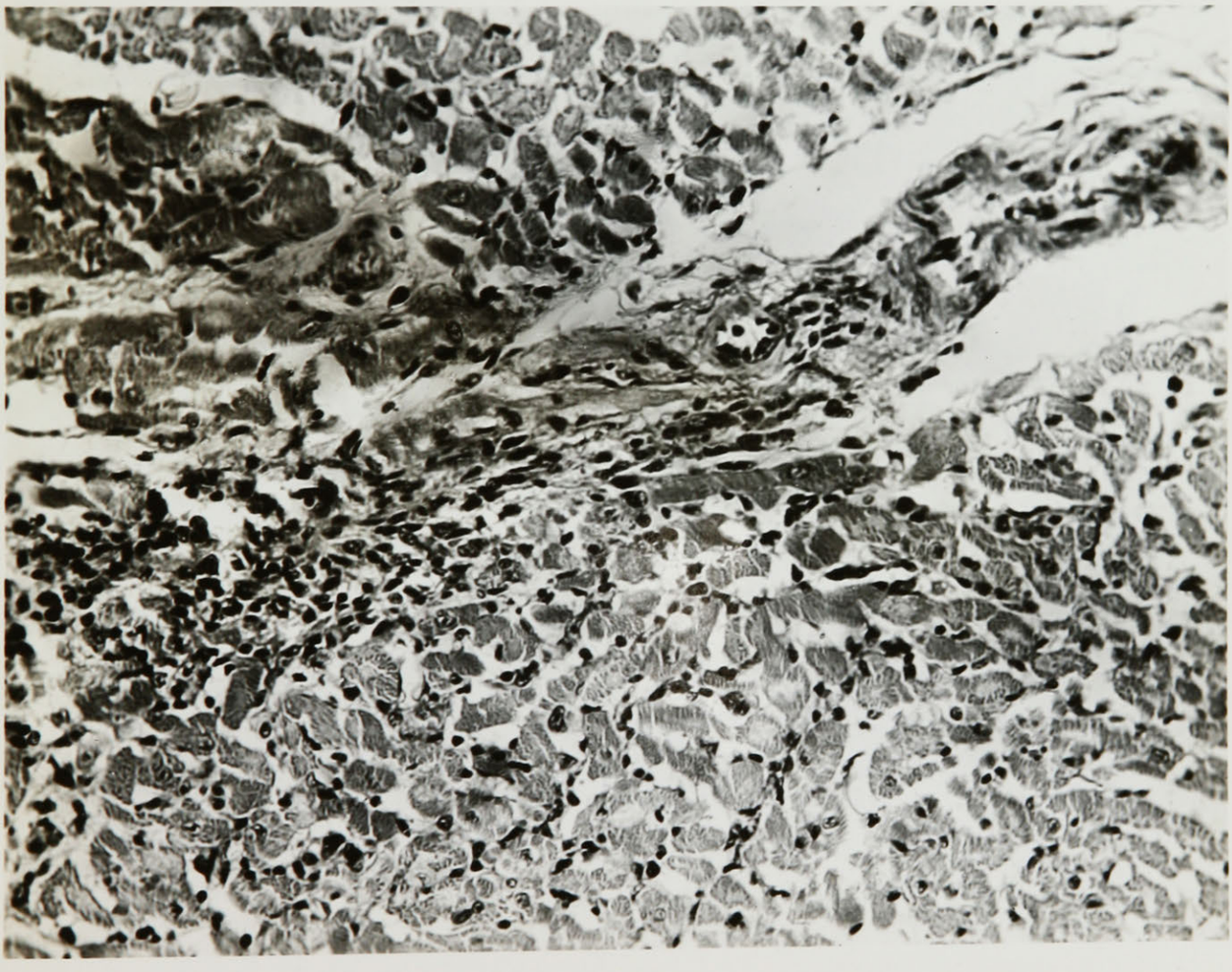

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Fig. 33. Myocardium of control rabbit # 64.
A small collection of lymphocytes with a few large mononuclear cells is seen among the muscle fibres. Cell aggregates such as this occurred frequently in both treated and control animals. No injections. X 280.

Fig. 34. Myocardium of control rabbit # 123.
Adjacent to the normal arteriole is a small vessel whose walls are disorganized and infiltrated by a mixed leucocytic exudate. A few of the neighbouring muscle fibres appear to have dropped out. No treatment. X 290.

33.

34.

Fig. 35. Myocardium of control rabbit # 120.
A rather diffuse collection of lymphocytes is seen in this area of heart muscle, which is otherwise normal. This was the largest spontaneous lesion seen in the hearts of the control series. No injections. X 240.

Fig. 36. Myocardium of rabbit # 44.
Virtually all of the muscle fibres in the upper half of the field show acute degenerative changes with swelling, loss of striations and nuclei, and intense basophilia due to excessuve deposits of calcium salts. The cellular exudate is sparse and composed mainly of lymphocytes with a few monocytes. Two injections. X 210.

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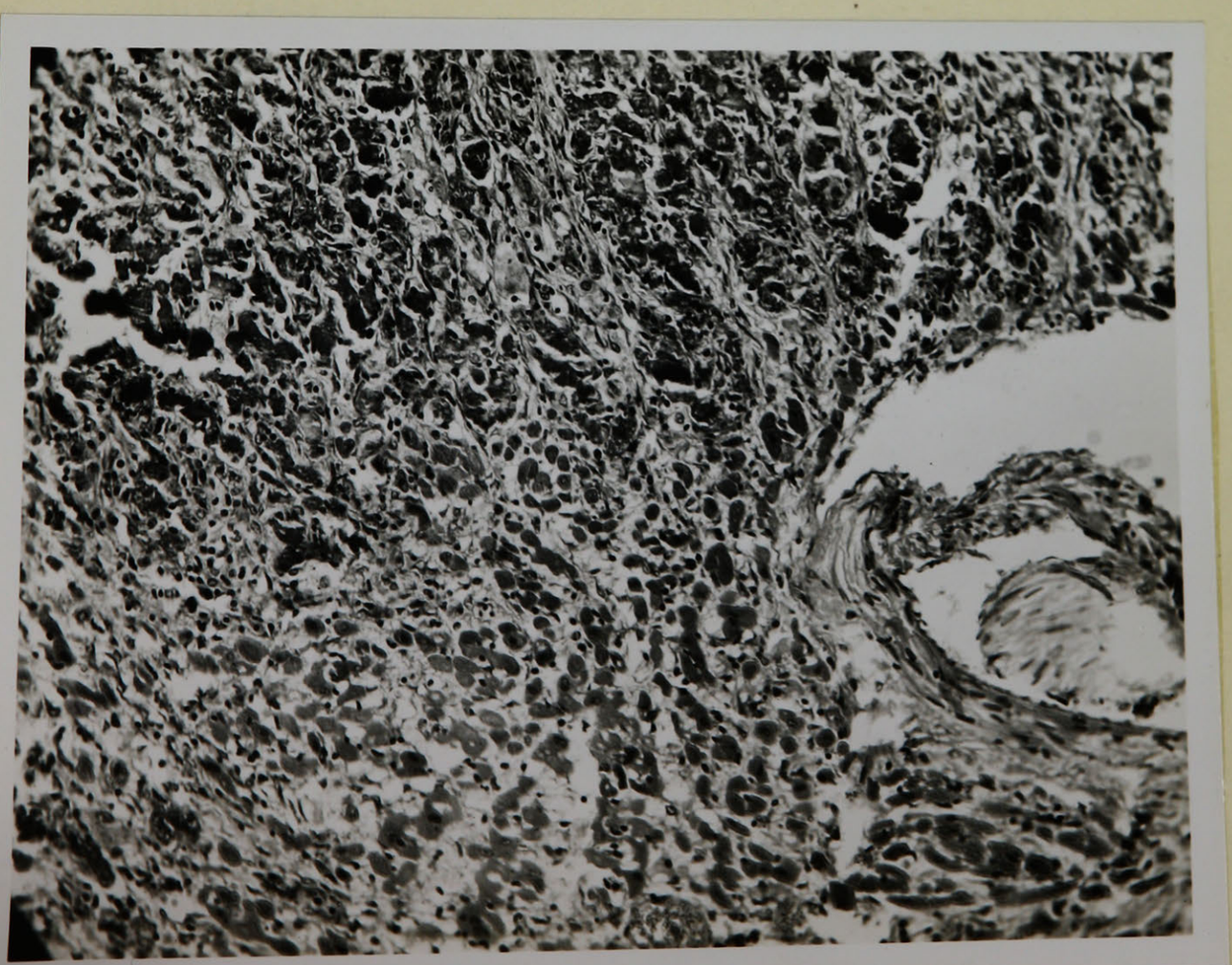

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Fig. 37. Myocardium of rabbit # 19.
Same lesion as that shown in Fig. 36 at a somewhat higher magnification. The line of demarcation between normal and necrotic muscle runs transversely slightly below centre. Two injections. X 300.

Fig. 38. Heart muscle of rabbit # 32.
Necrosis with fragmentation and calcification of muscle is present in the upper left hand corner of the field. A diffuse inflammatory reaction of low grade intensity is seen in the remainder of the area. The muscle fibres are separated by edema fluid, histiocytes are swollen and prominent, and a sparse sprinkling of lymphocytes and mononuclear leucocytes is present. Two injections. X 205.

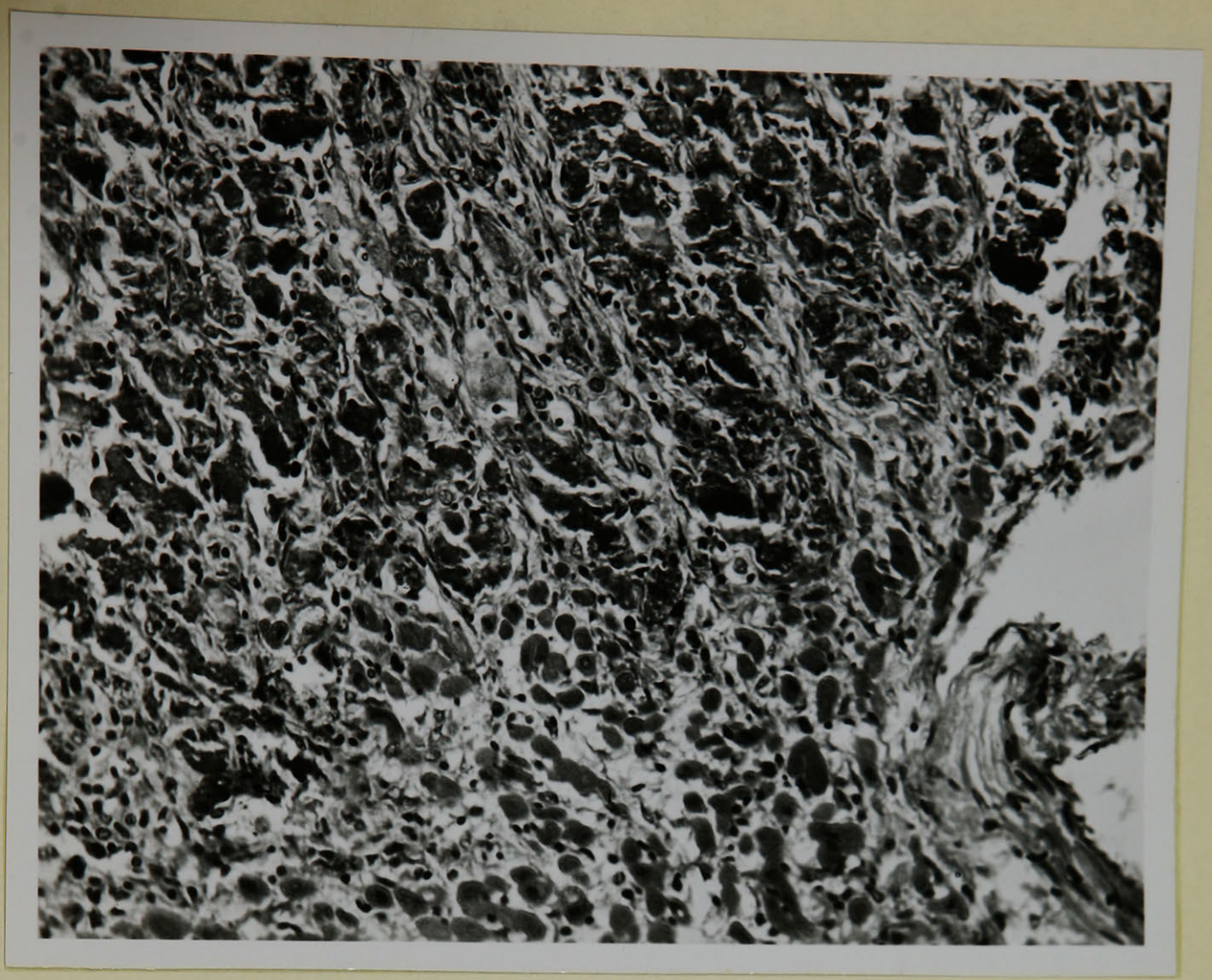

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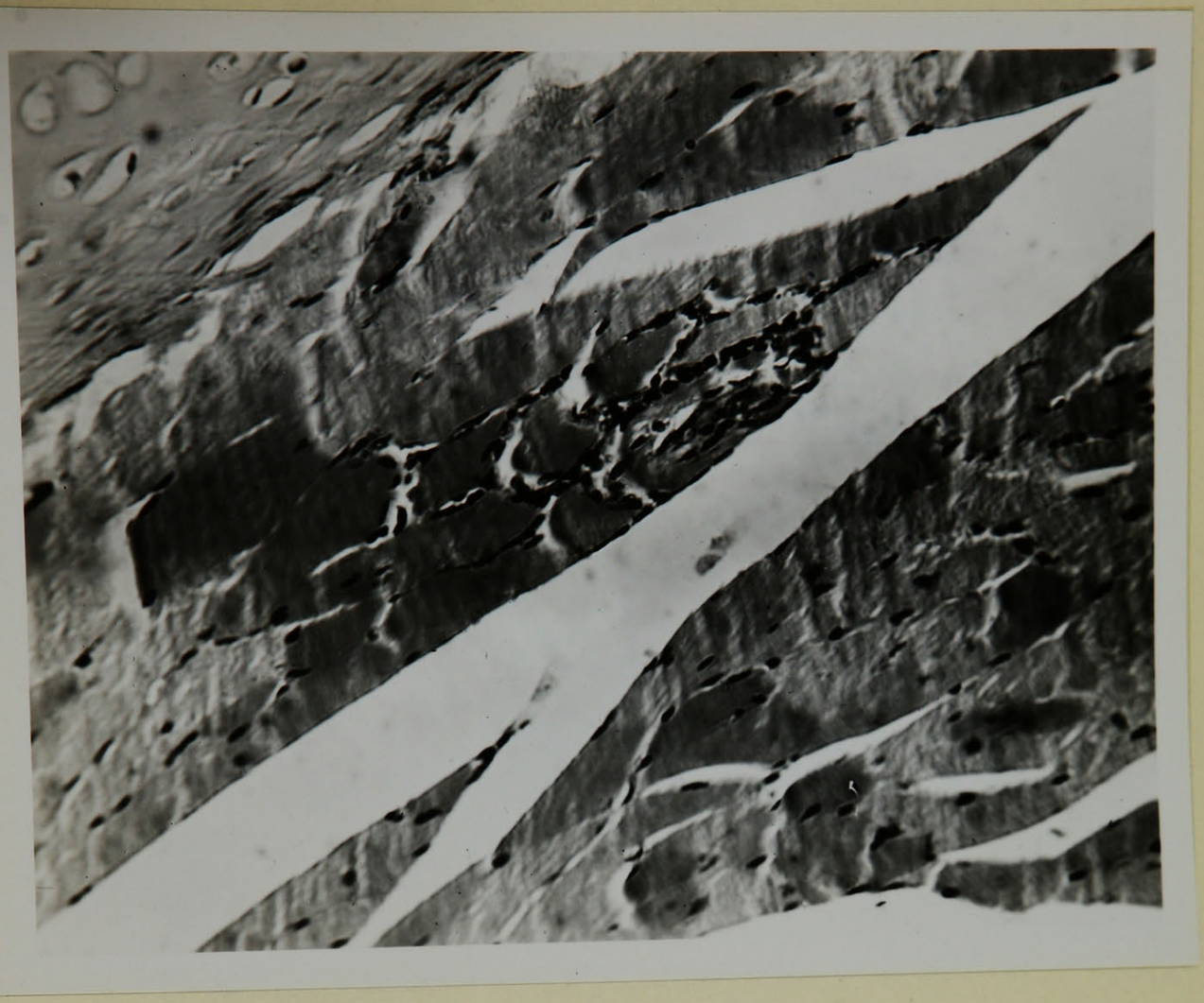
Fig. 39. Skeletal muscle of rabbit # 28.
The affected fibres show pronounced basophilic and loss of striations. Proliferation of the sarcolemmal nuclei and beginning fragmentation of a few fibres are also seen. Three injections. X 330.

Fig. 40. Skeletal muscle of rabbit # 44.
Numerous small clusters of fibres are affected. The changes range from slight swelling and basophilia, to complete fragmentation and opaque basophilia with a rather dense leucocytic exudate. Two injections. X 170.



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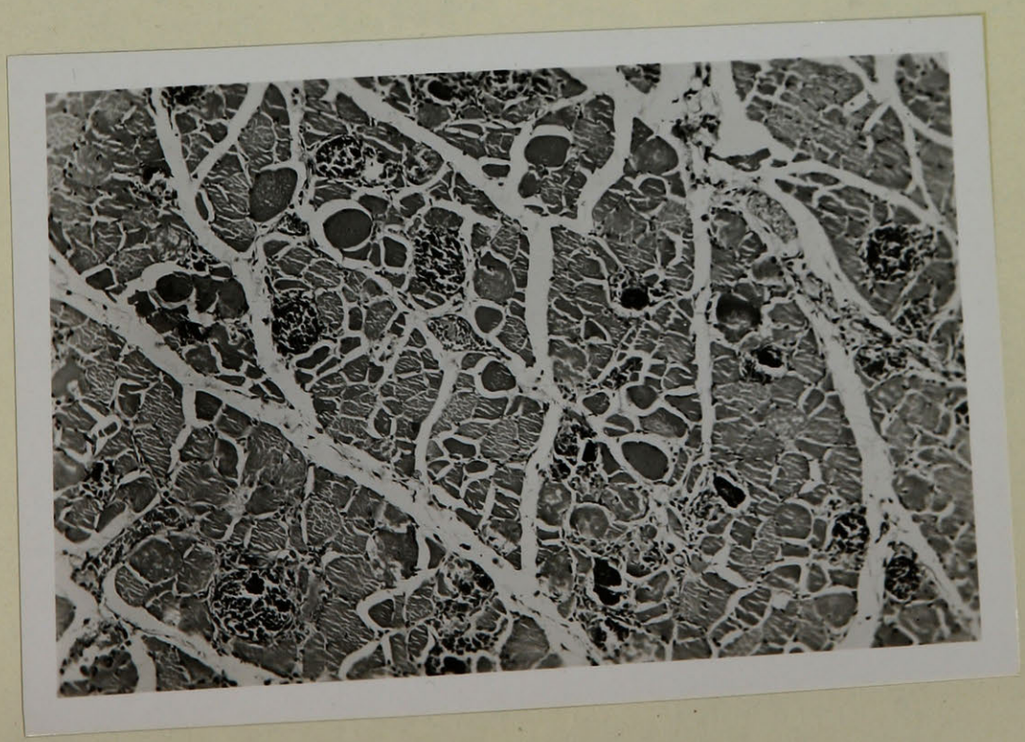
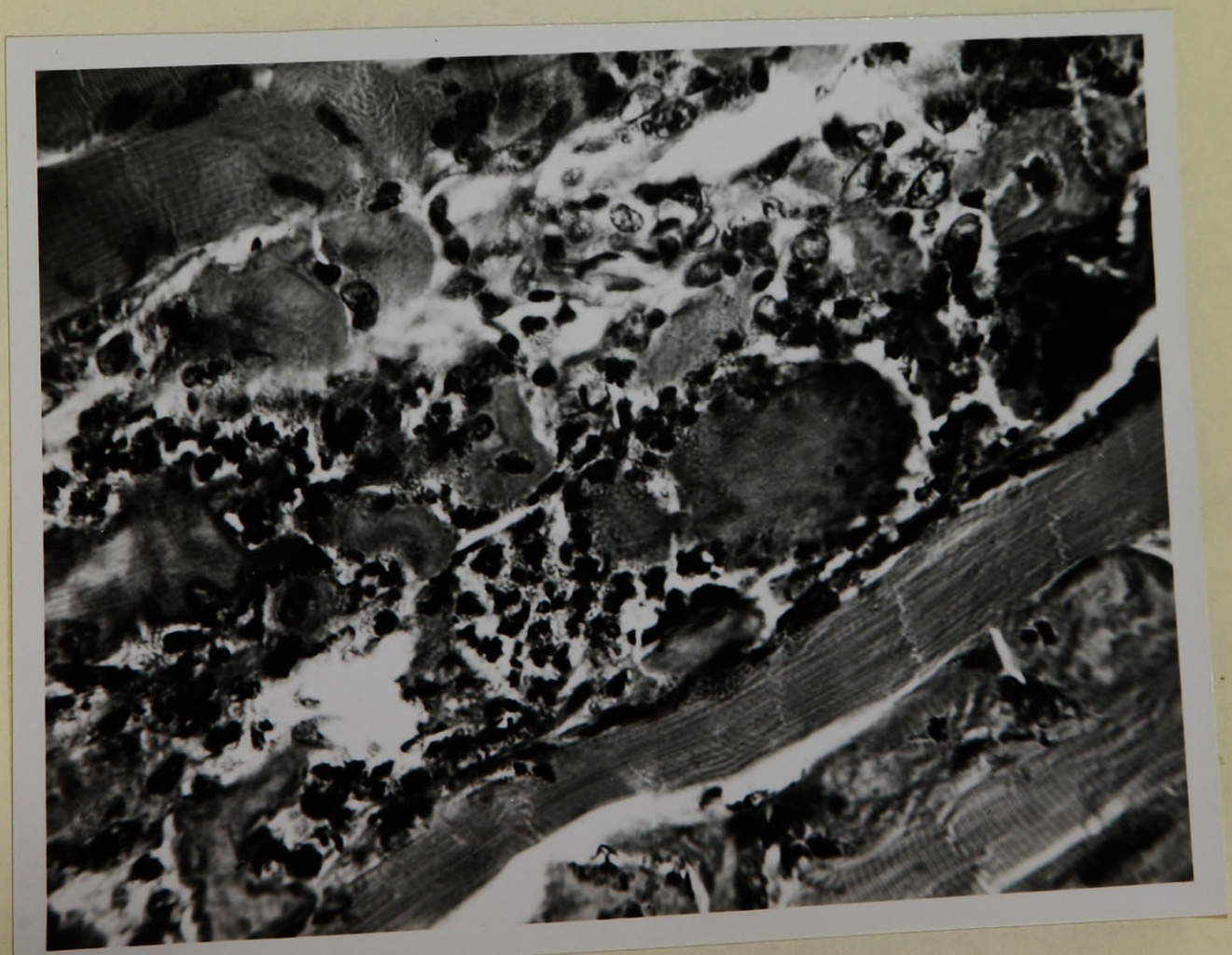


40.

Fig. 41. Skeletal muscle of rabbit # 7.
This animal exhibited the severest lesions of skeletal muscle found in this series. The focal character of the lesions is still apparent in the upper portion of the field. In the lower portion, the necrosis has become confluent. Four injections. X 180.

Fig. 42. Skeletal muscle of rabbit # 7.
Higher magnification of lower part of field in Fig. 41. Fragmentation of muscle fibres, and the purulent nature of the exudate is well seen. Four injections. X 620.

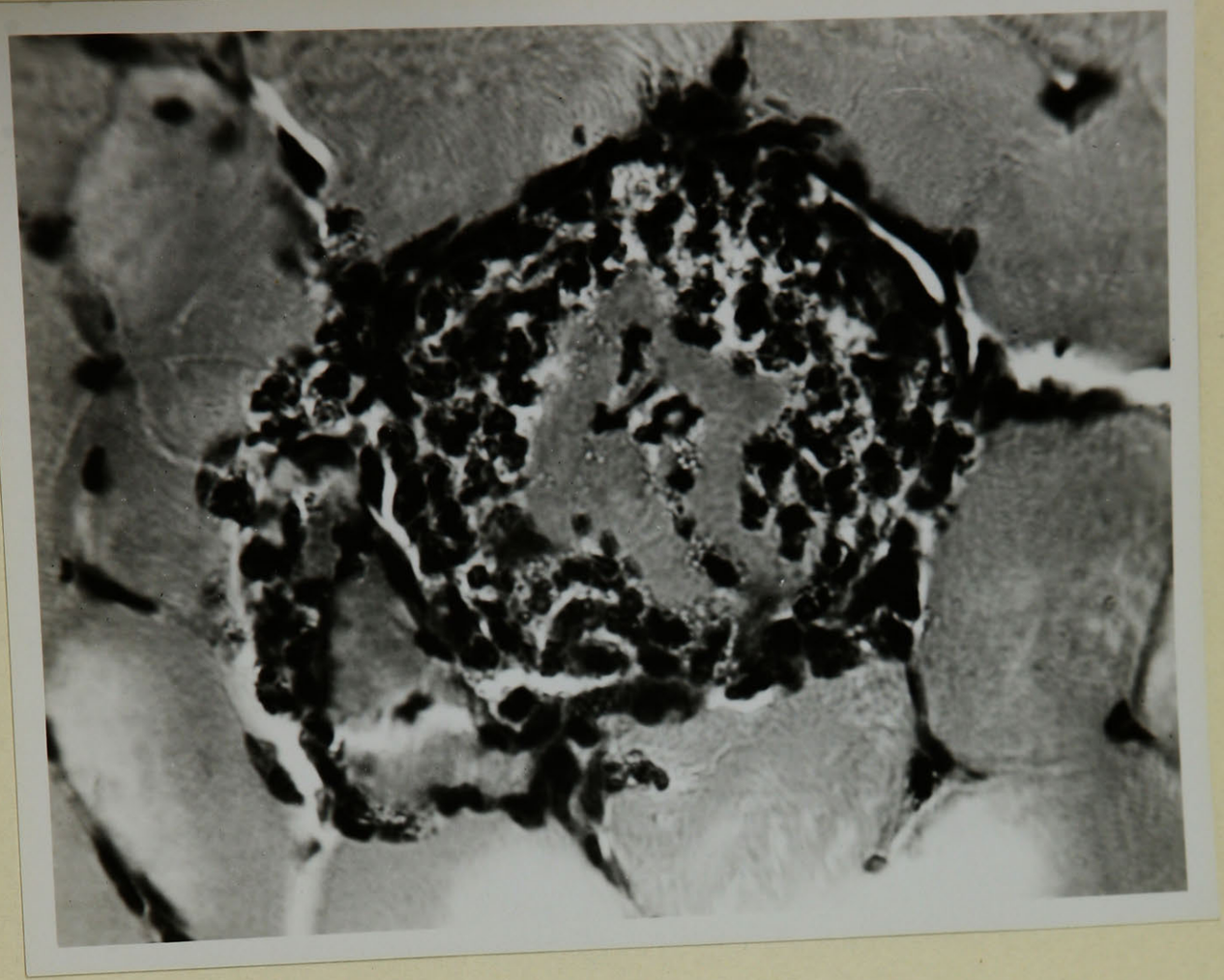
41.



42.

Fig. 43. Skeletal muscle of rabbit # 28.
Two necrotic muscle fibres show fragmentation and a dense infiltrate of polymorphonuclear leucocytes. Three injections. X 1030.

Fig. 44. Skeletal muscle of rabbit # 7.
A higher magnification from the upper portion of the field in Fig. 41. The necrotic fibre to the left of the field is particularly dense with calcific deposits. The associated inflammatory response is somewhat granulomatous with proliferation of histiocytes and sarcolemmal nuclei, and the formation of several multinucleated giant cells. Four injections. X 690.



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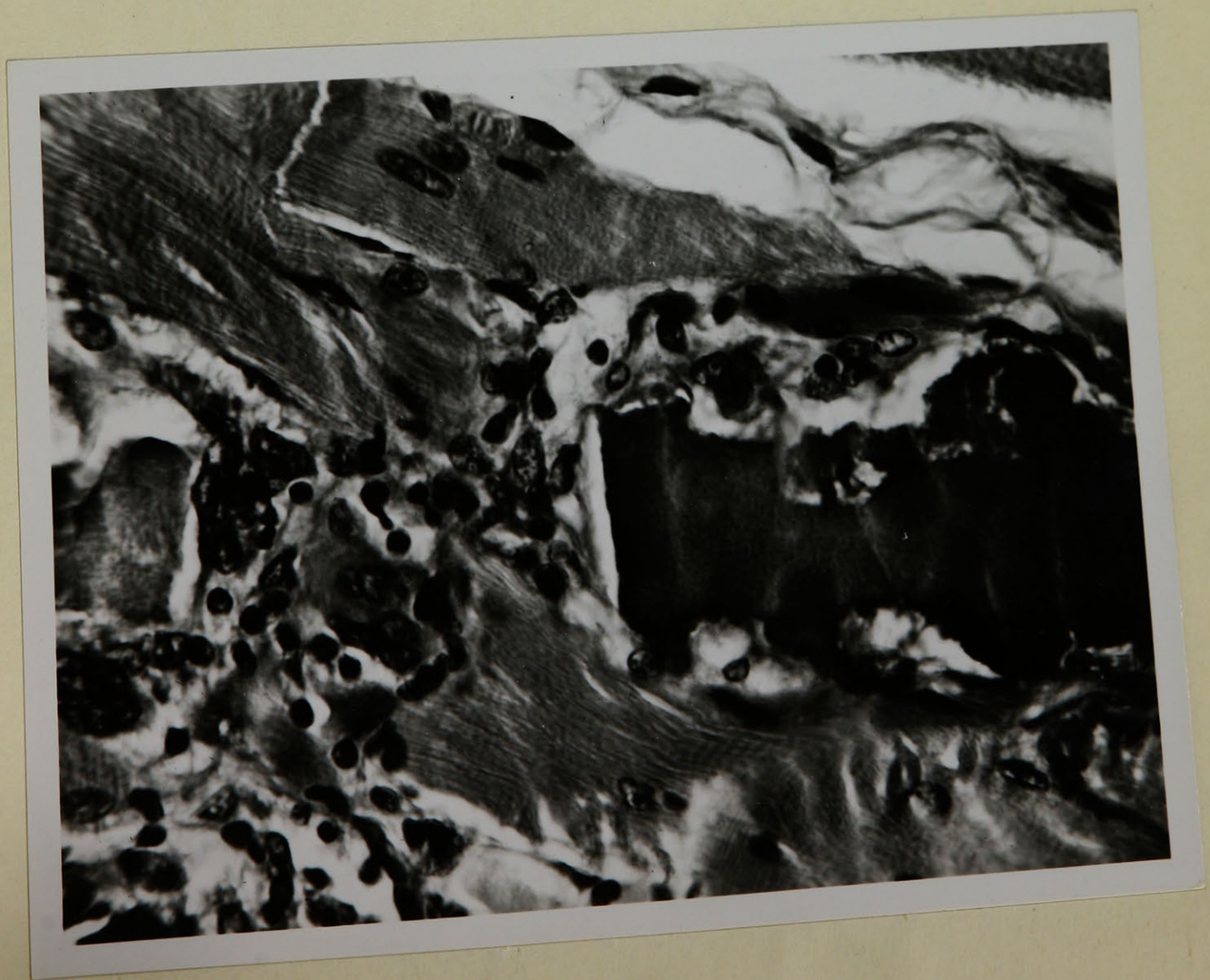
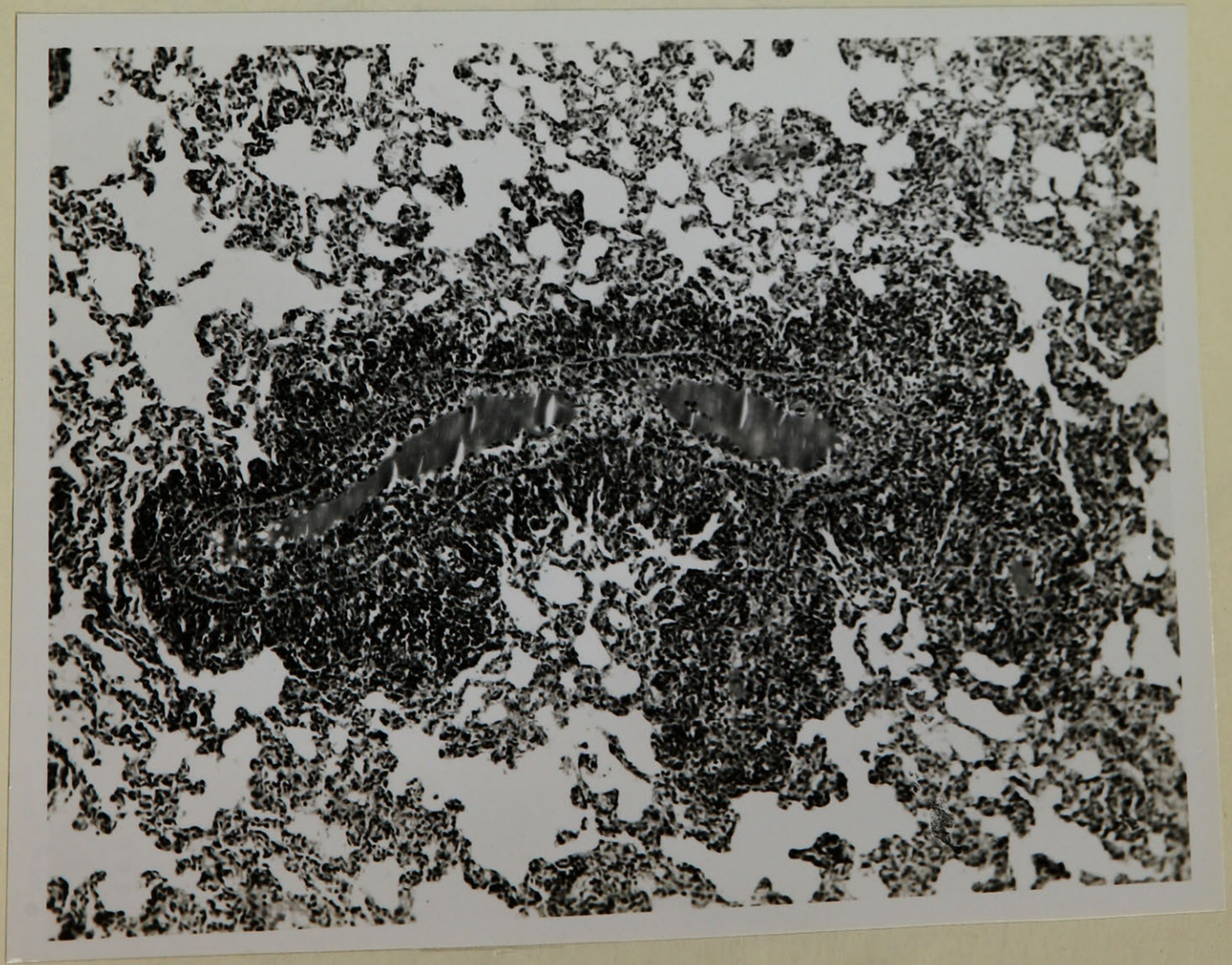


44.

Fig. 45. Lung of rabbit # 55.
A topographical section to show the frequency and distribution of collars of chronic inflammatory cells and proliferating adventitial cells in vessels of the lung. Two injections. X 49.

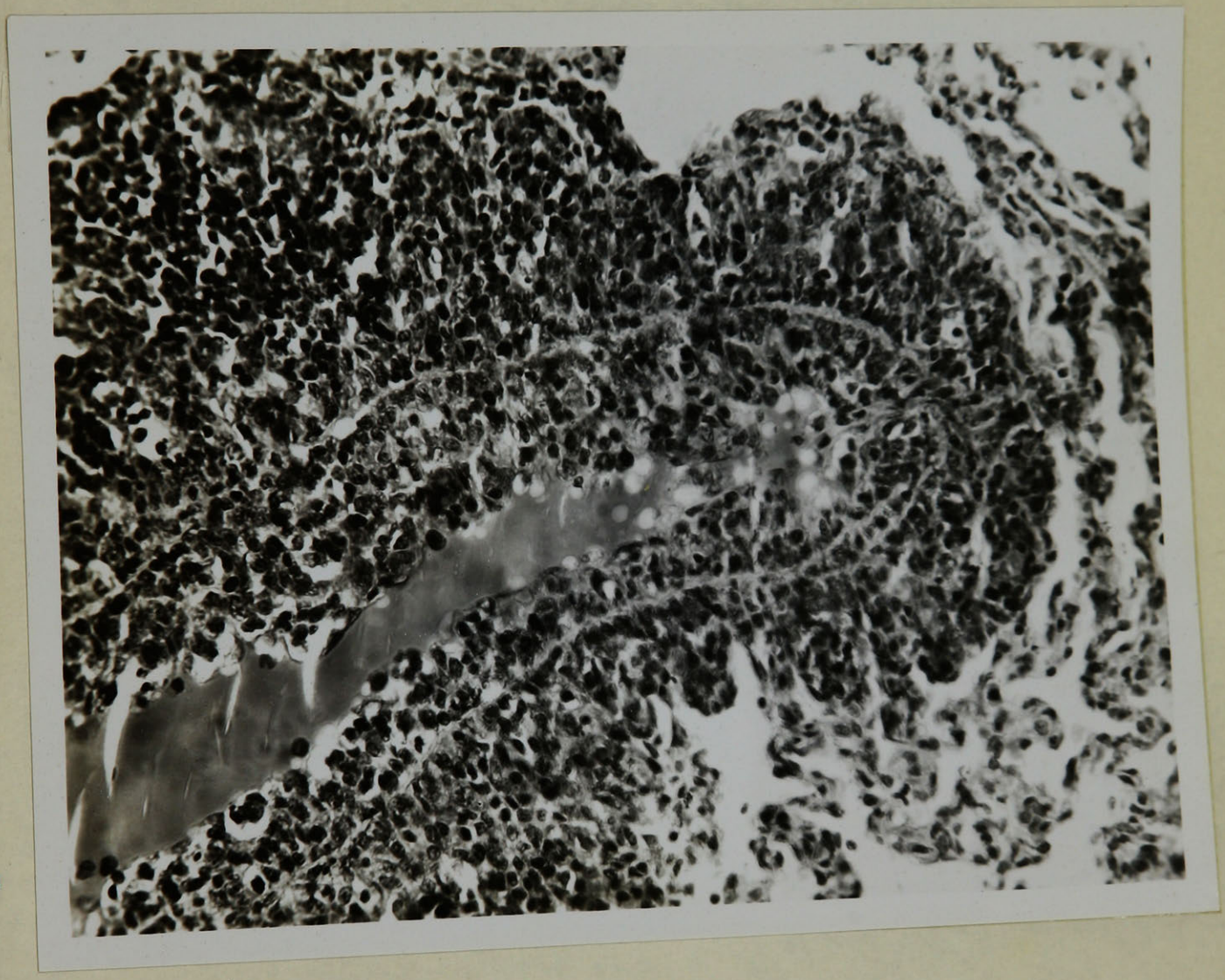
Fig. 46. Lung of rabbit # 55.
Slightly higher magnification of vessel from rabbit # 55.
X 110.

45.



46.

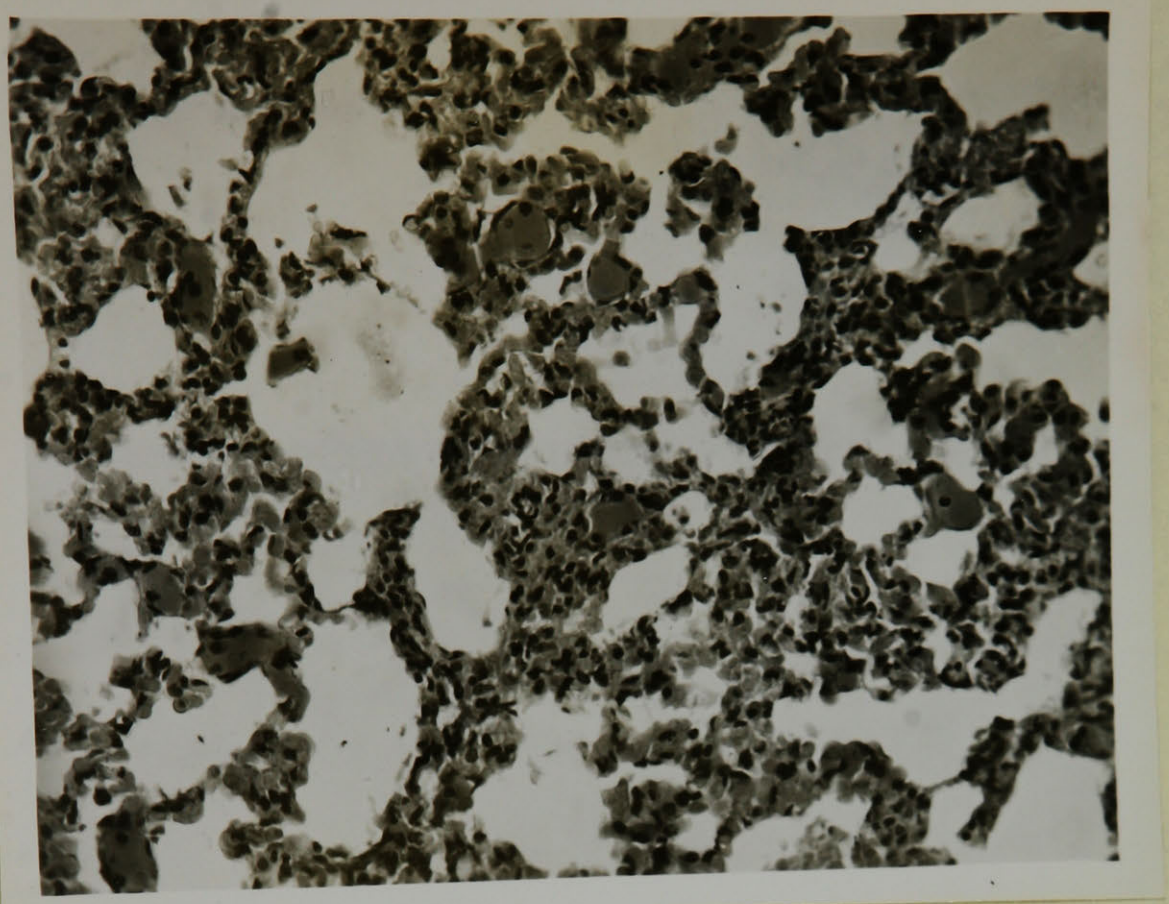
Fig. 47. Pulmonary vein in rabbit # 55.
A higher magnification of Fig. 45 to show the details of the cellular reaction. Both intimal and adventitial layers are diffusely and heavily infiltrated with lymphocytes and large mononuclear leucocytes. In addition, there are large numbers of mononuclear cells, somewhat fusiform or polygonal in outline, with abundant pale basophilic cytoplasm and vesicular nuclei to be found in large numbers in both the intimal and adventitial layers. The lining of smooth intimal endothelium has been interrupted. Fibrinoid necrosis is not present. Two injections. X 335.



47.

Fig. 48. Lung of rabbit # 49.
Many of the capillaries in this field are distended with hyaline plugs of what appears to be thrombus material. This animal was one of eight which died in acute anaphylactic shock. It received 7 injections of serum and died immediately after the last. X 270.

Fig. 49. Lung of rabbit # 49.
A higher magnification of Fig. 48 to show the detail of the capillary thrombi. A few of the septa are somewhat thickened and cellular, but this change is no more marked than that seen in numerous control animals. The hyaline plugs of thrombus do not appear to be associated with any obvious changes in the capillary walls, or leucocytic exudate whatsoever. X 450.



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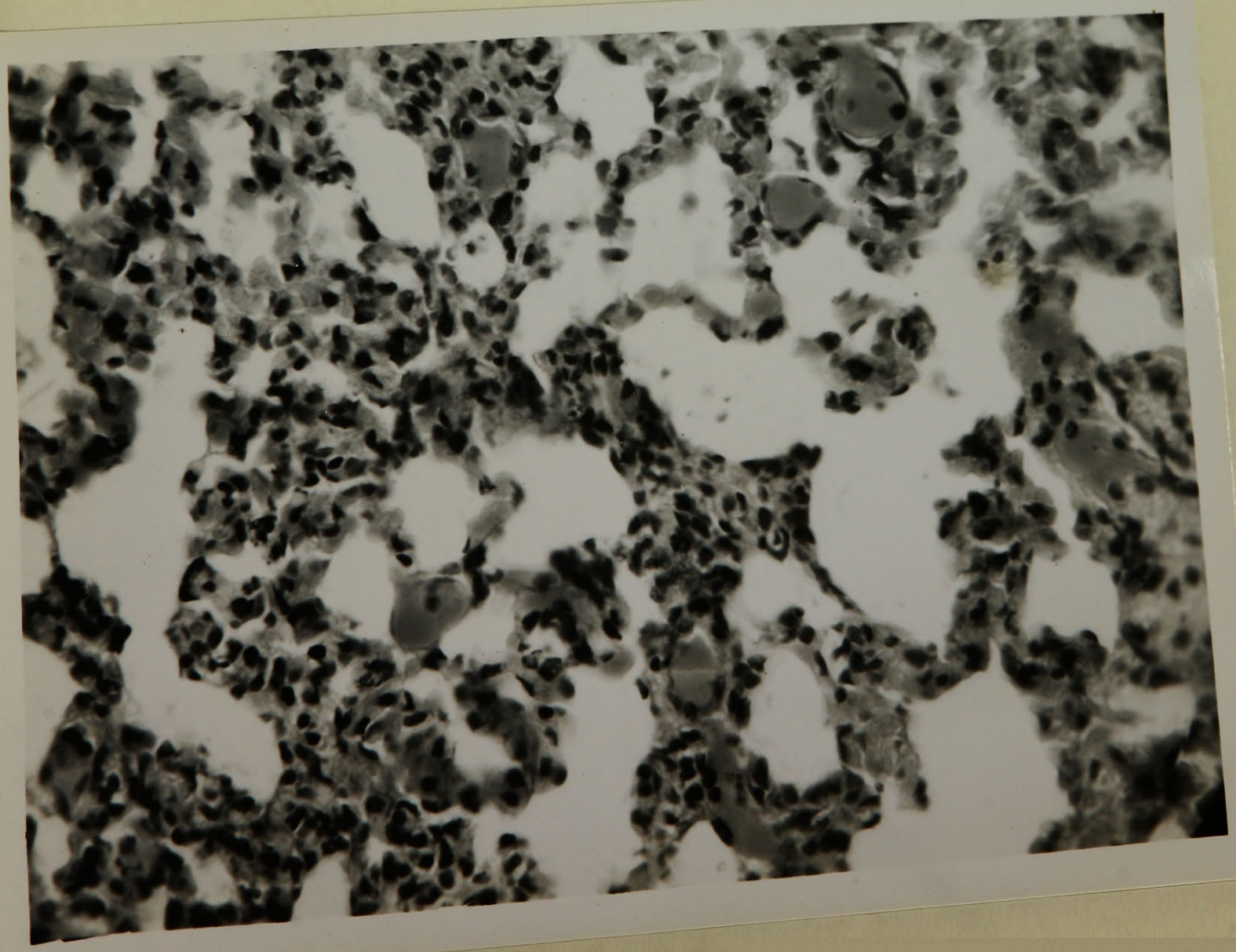
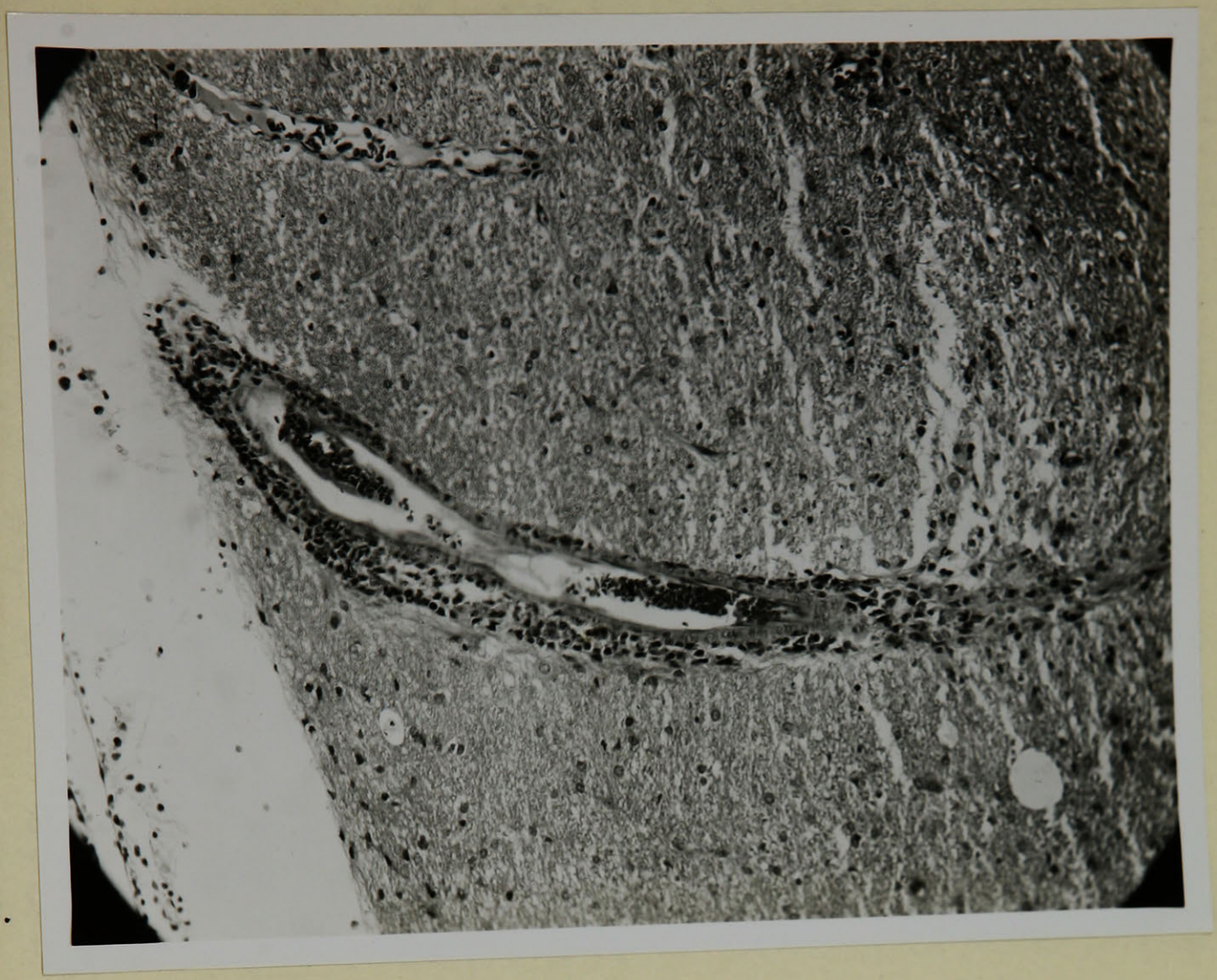


9.

Fig. 50. Brain of control rabbit # 64.
The small cerebral artery is surrounded by a cuff of chronic inflammatory cells, most of which are lymphocytes. No injections. X 190.

Fig. 51. Brain of control rabbit # 87.
The vessels show collars of closely packed lymphocytes together with a few mononuclear leucocytes. In the brain substance adjacent to the vessels is a small, well formed granuloma. These spontaneous lesions occurred in approximately one-half the treated and control animals. No treatment. X 212.



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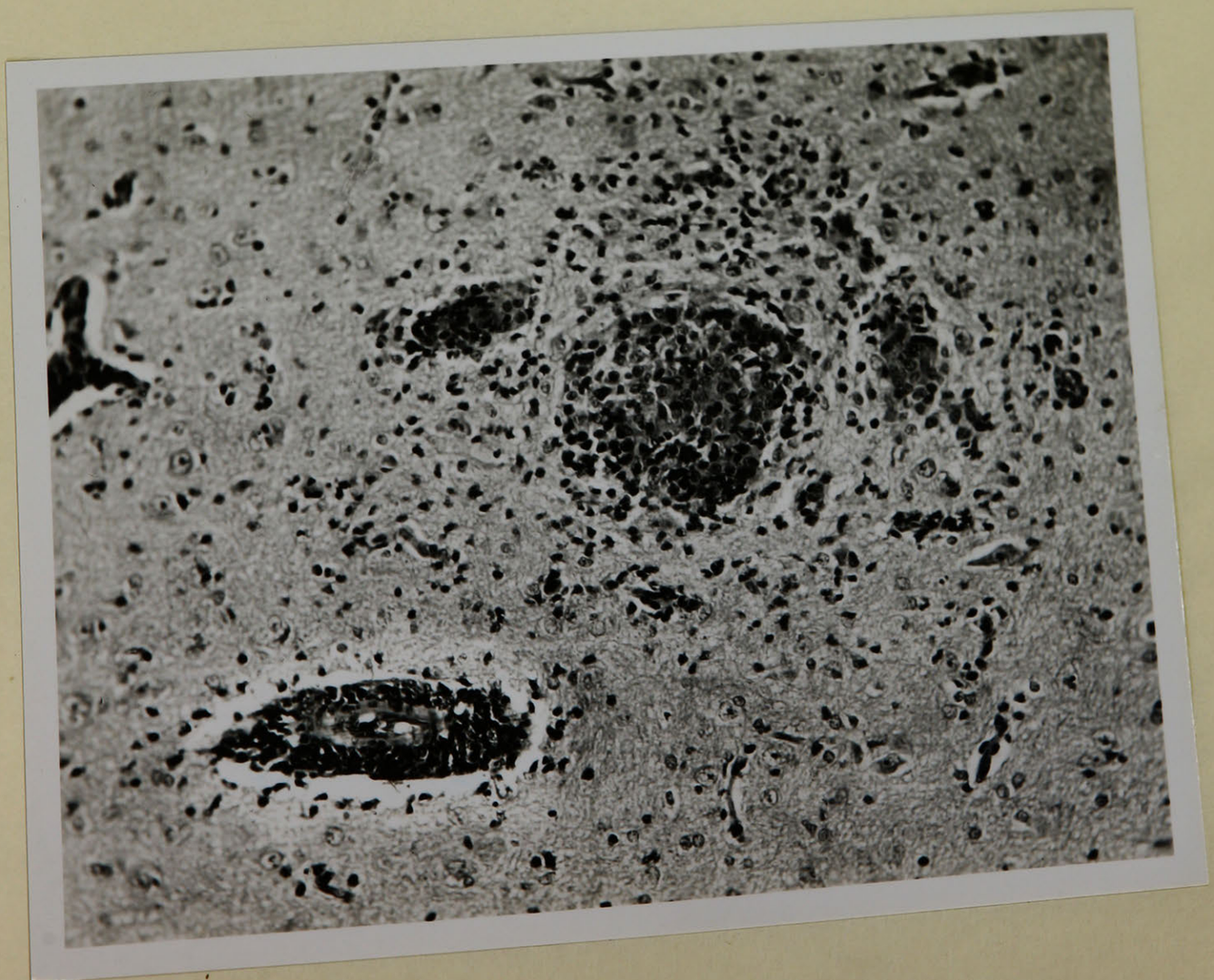
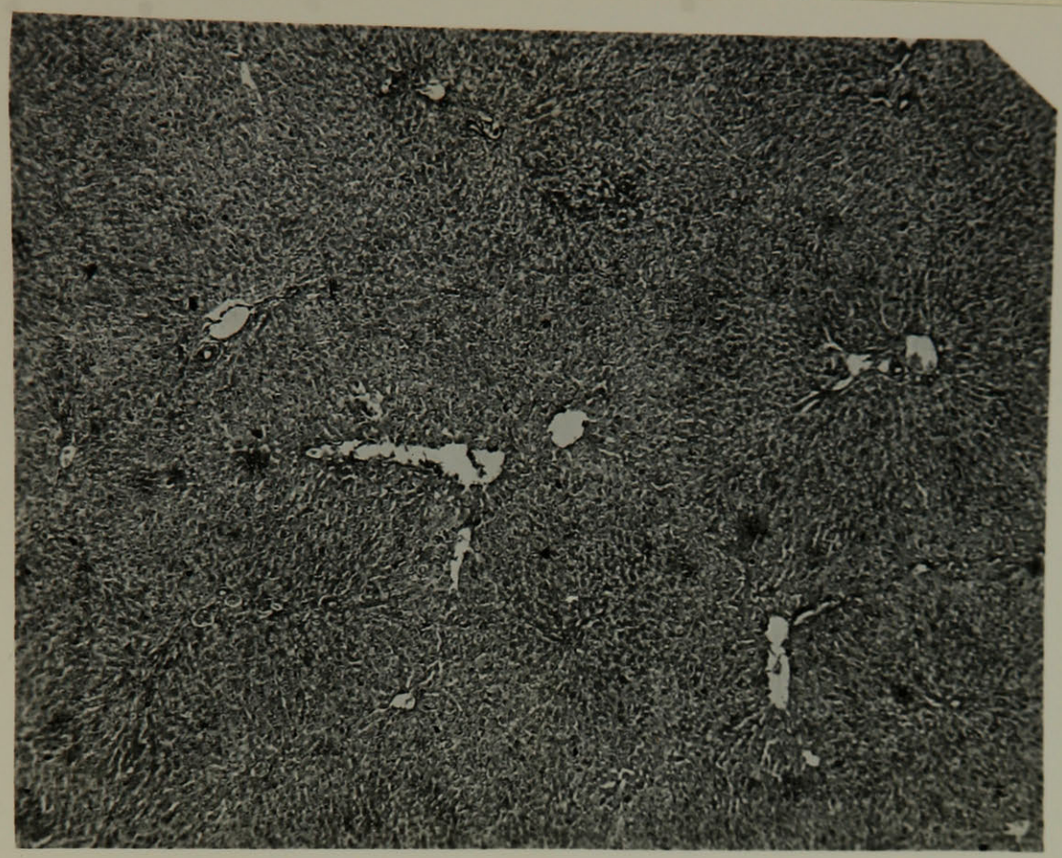


51.

Fig. 52. Liver of control rabbit # 19.
Section of normal liver. The portal tracts are quite inconspicuous. No treatment. X 37.

Fig. 53. Liver of control rabbit # 87.
The illustration demonstrates the most marked degree of change in the livers of untreated rabbits in this series. The portal triads are rendered unusually prominent with cellular connective tissue. No treatment. X 37.



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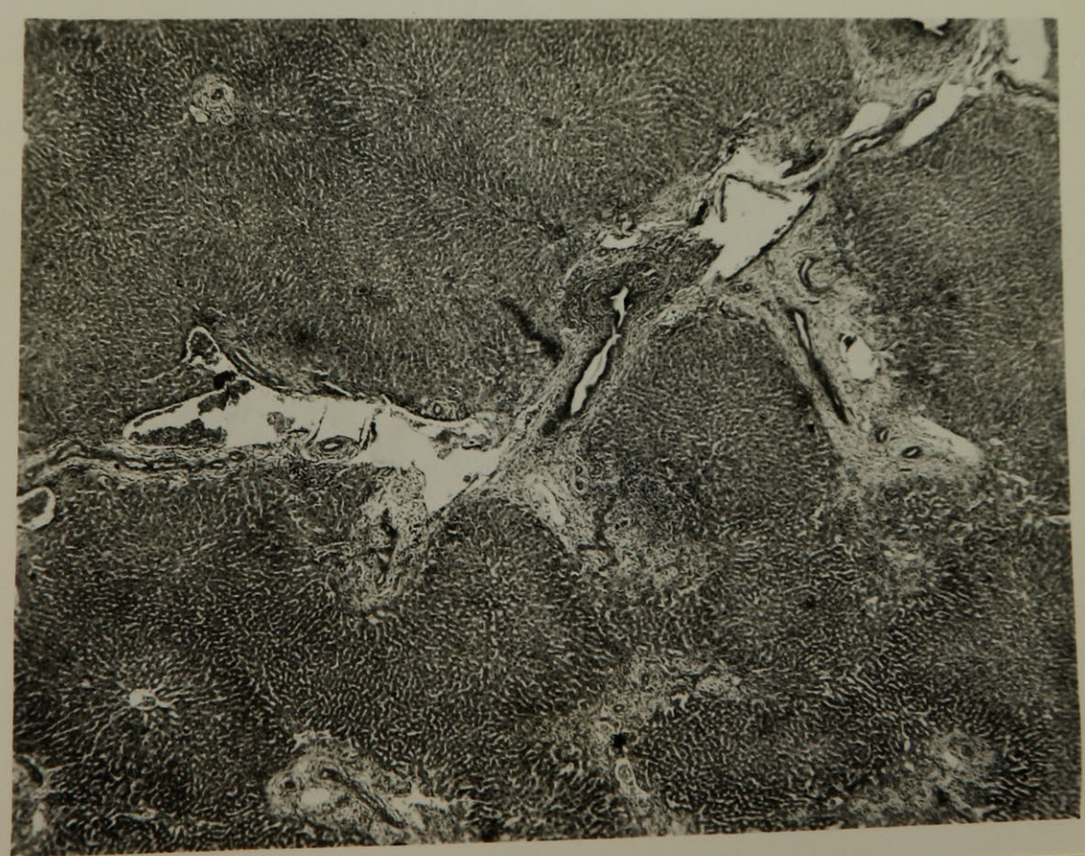
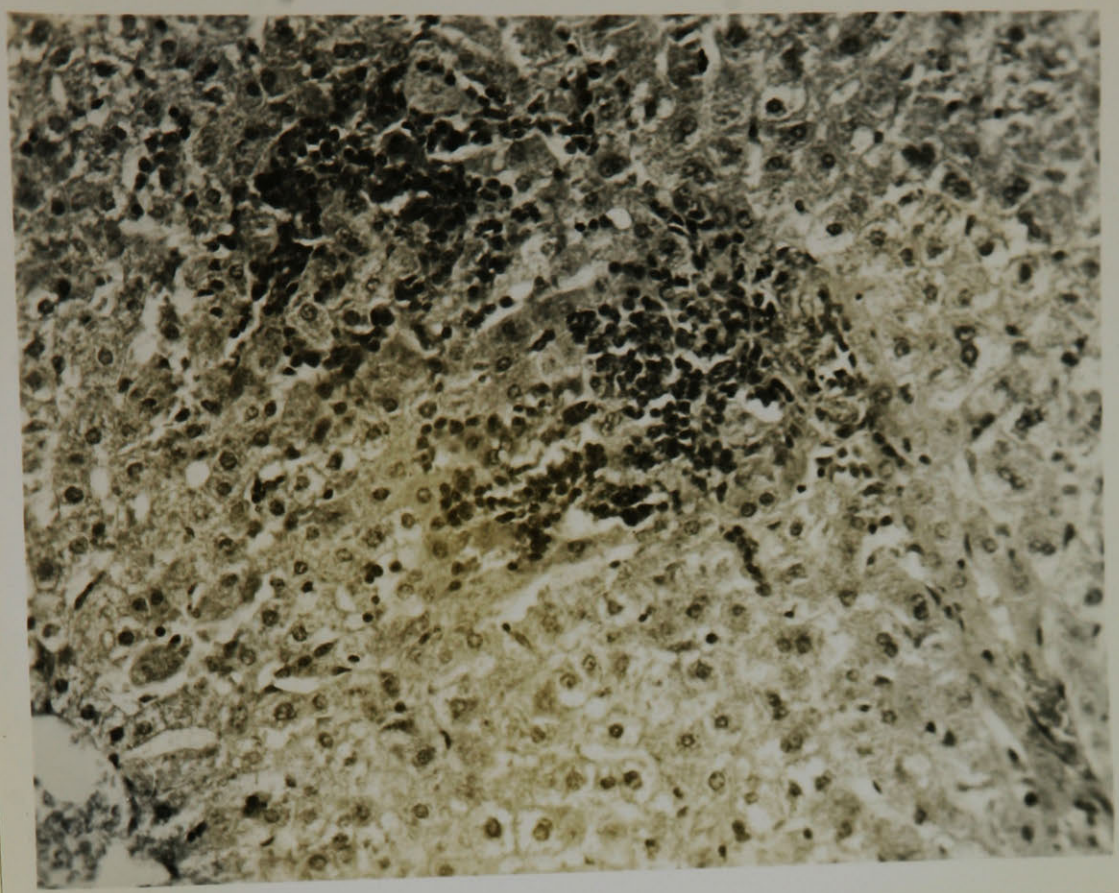


53.

Fig. 54. Liver of control rabbit # 117.
The illustration shows a small area of focal necrosis. The hepatic cells have been replaced by a mixed leucocytic exudate. Such lesions are not infrequently encountered in untreated rabbits, and cast some doubt on experimental procedures which profess to produce them. No injections. X 250.

Fig. 55. Liver of control rabbit # 87.
Slightly higher magnification of portal tract from Fig. 53. The abundant connective tissue is infiltrated with lymphocytes and monocytes, and the bile ducts are dilated and lined with hyperplastic epithelium. No treatment. X 190.



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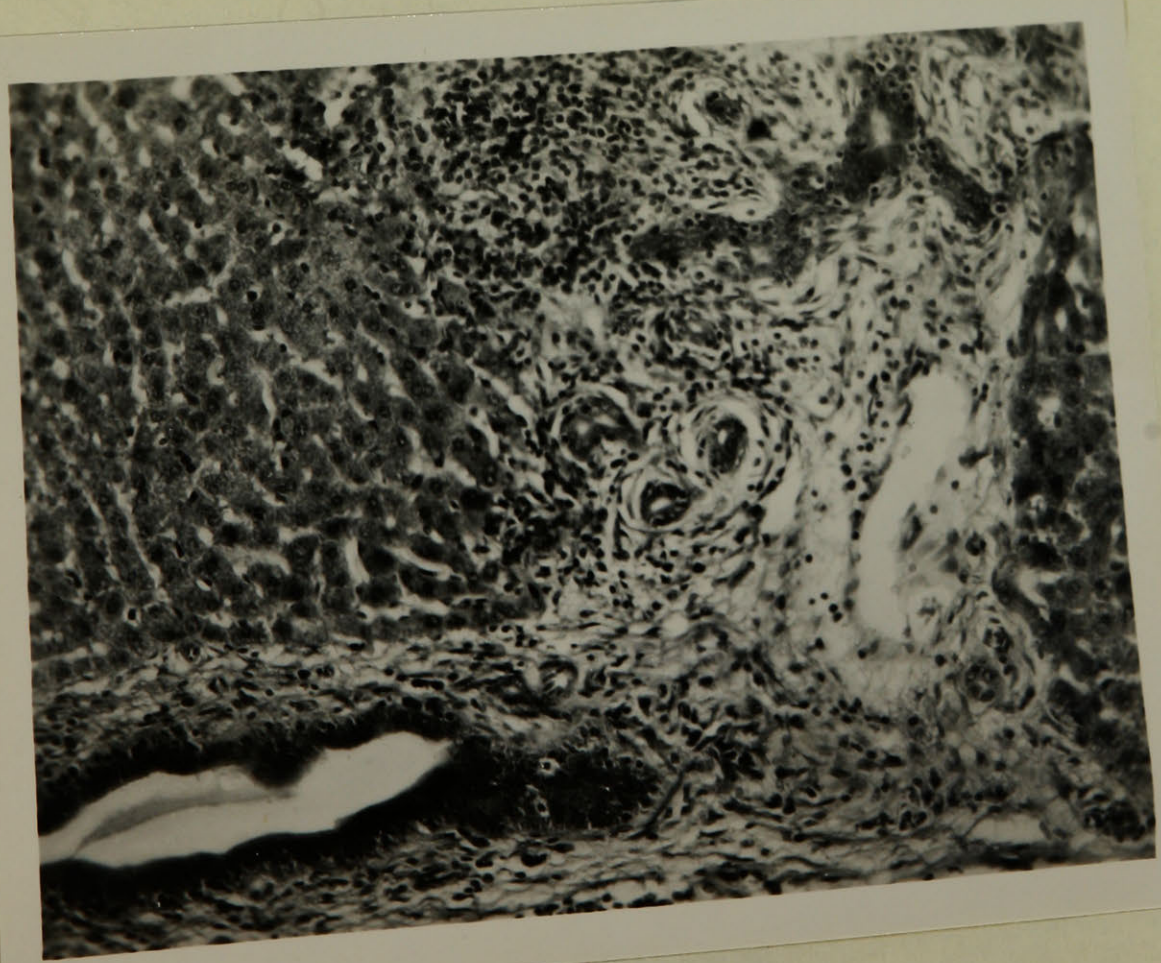
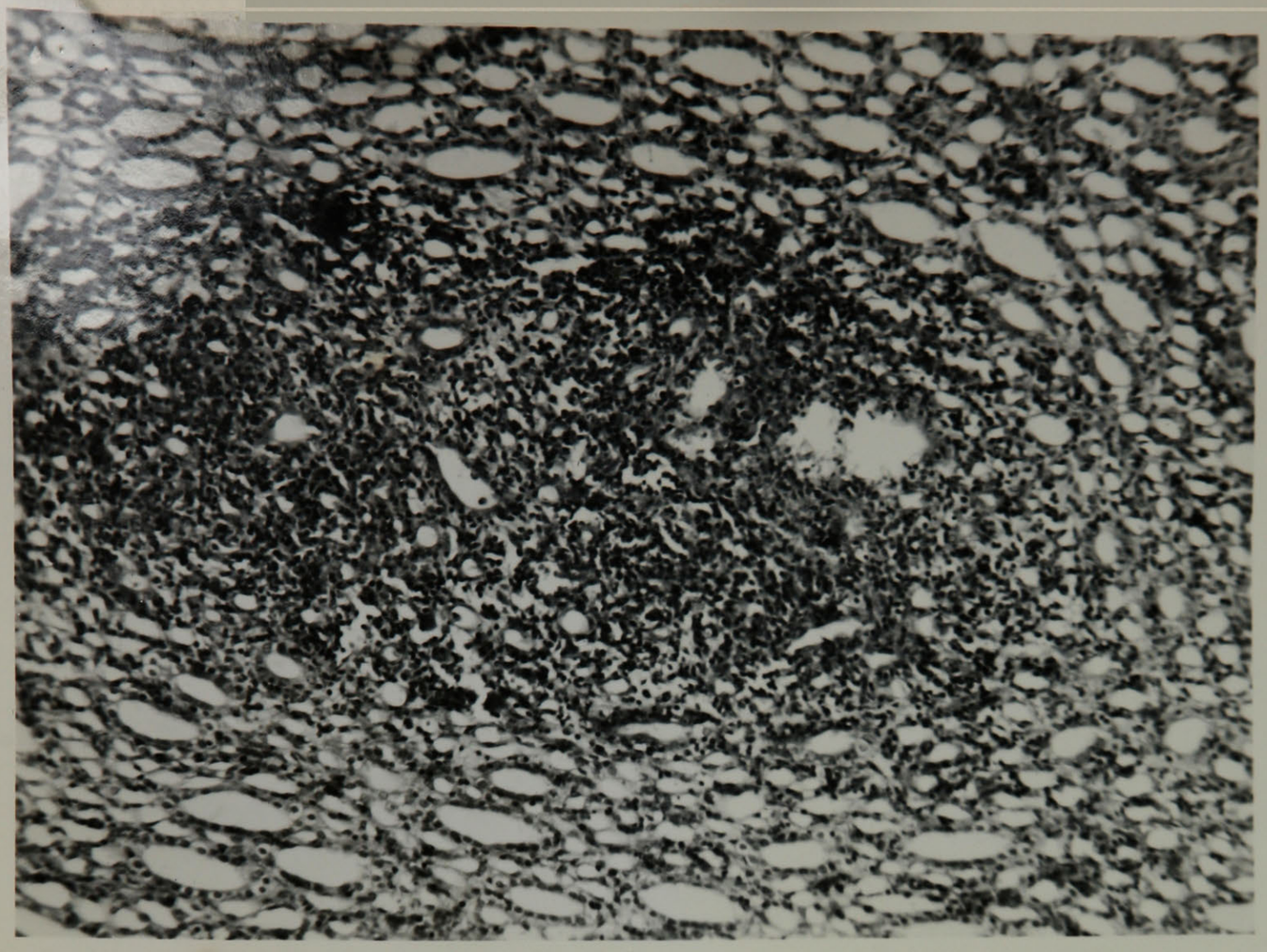


55.

Fig. 56. Kidney of control rabbit # 64.
A large focus of acute interstitial nephritis in the medulla is seen, with necrosis of tubules and a mixed leucocytic exudate, including many polymorphonuclear leucocytes. No treatment. X 110.

Fig. 57. Kidney of rabbit # 44.
The illustration shows a focus of interstitial nephritis in the cortex which is less acute than that pictured in Fig. 58. Atrophy and dilatation of tubules are seen, and interstitial scarring. Comparable lesions were frequently seen in control rabbits, and serotherapy did not increase their incidence in the treated group. X 230.



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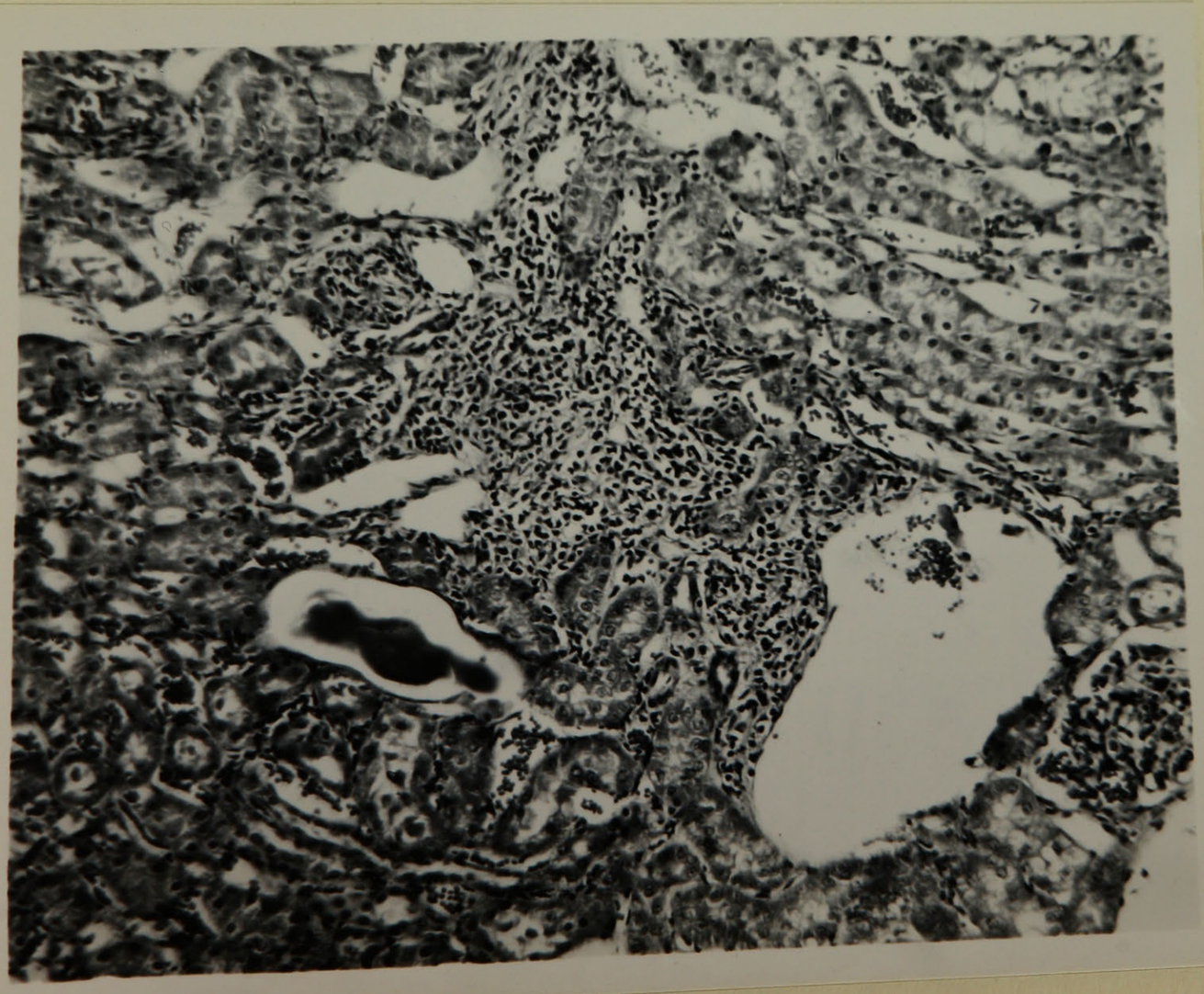


57.

Fig. 58. Knee joint of control rabbit # 13.
A topographical section to show the relationship of semilunar cartilage, joint capsule and articular cartilages. When inflammatory changes in the synovial membrane occurred, they were most frequently found in folds near the base of the semilunar cartilage, or in the angle formed by fusion of joint capsule and periosteum. No treatment. X 70.

Fig. 59. Knee joint of control rabbit # 13.
Slightly higher magnification of fold of synovial membrane from joint of Fig. 60. The synovial cells are somewhat swollen and prominent, and the underlying connective tissue is a little denser than usual. There is no inflammatory exudate. This is representative of the maximum change which was found in the joints of untreated rabbits. X 260.

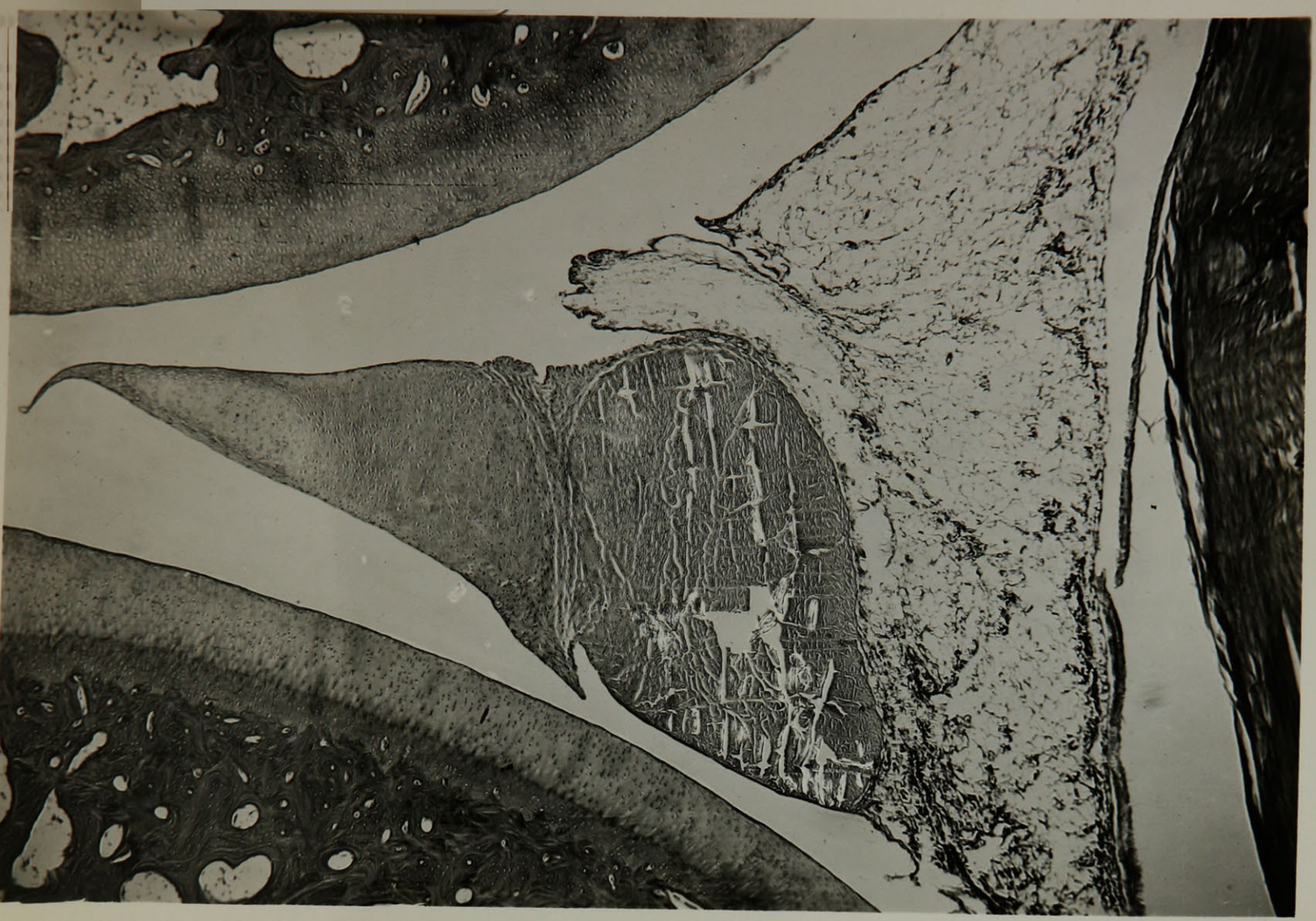
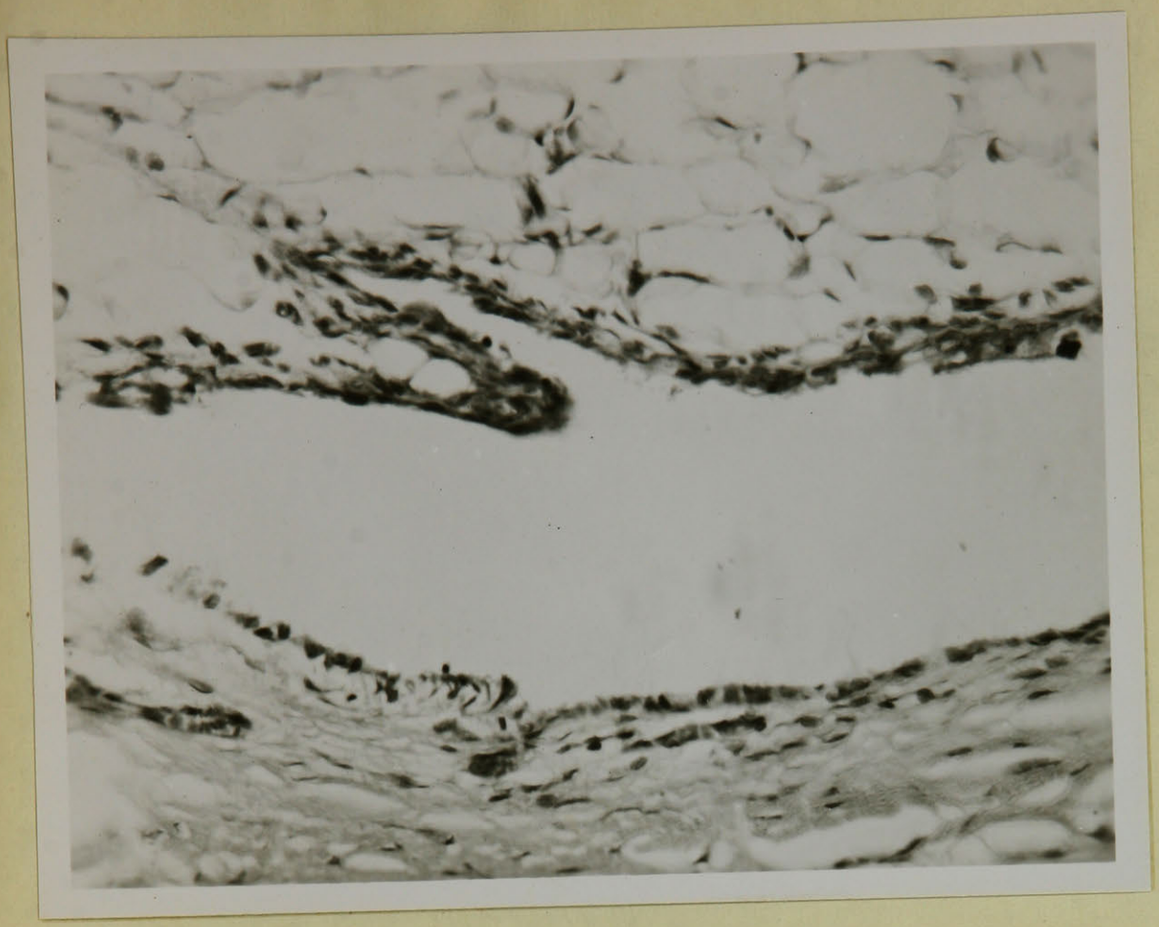


Fig. 60. Synovial membrane from joint of control rabbit # 118. The synovial lining cells are slightly swollen and prominent. No treatment. X 260.

Fig. 61. Synovial membrane from joint of control rabbit # 118. A villus fold whose attachment to the synovial membrane is not included in the section. No treatment. X 260.



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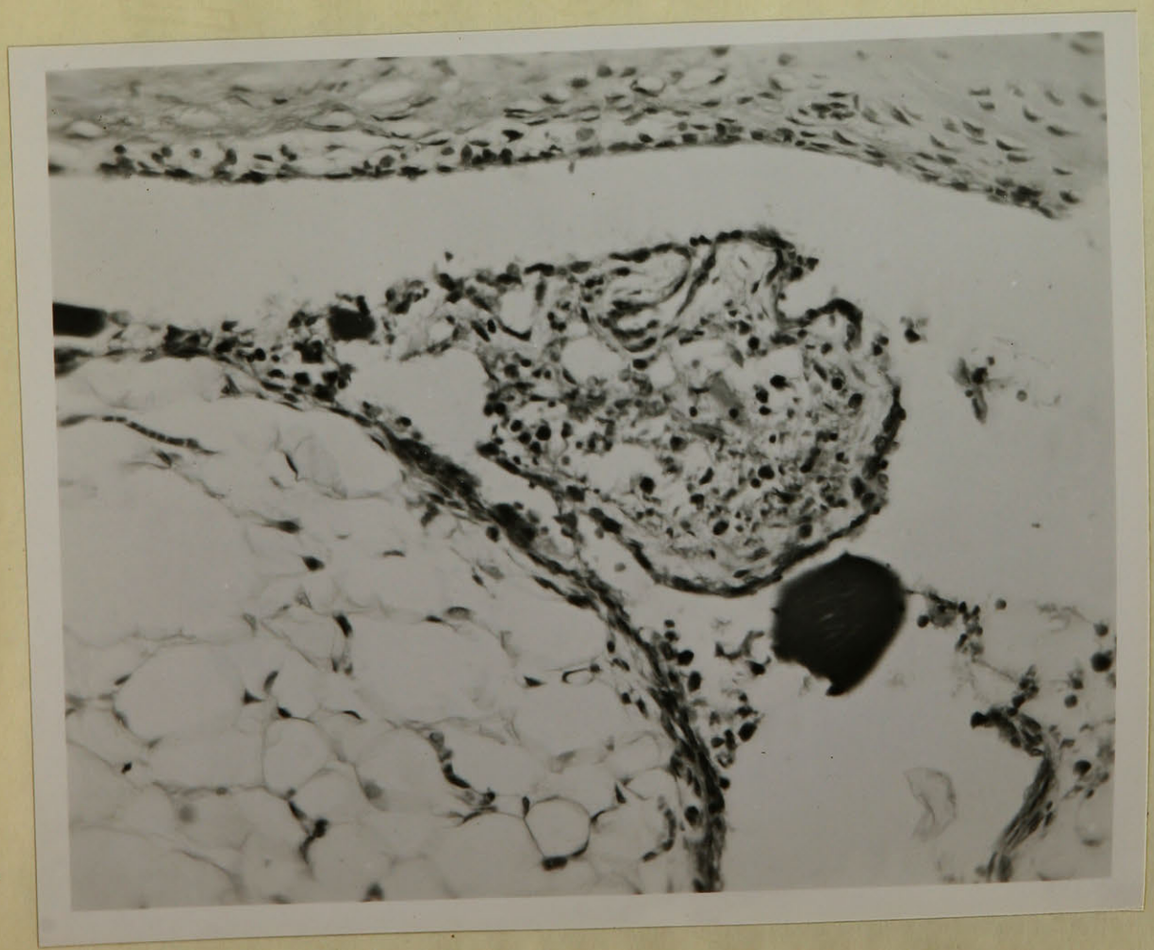
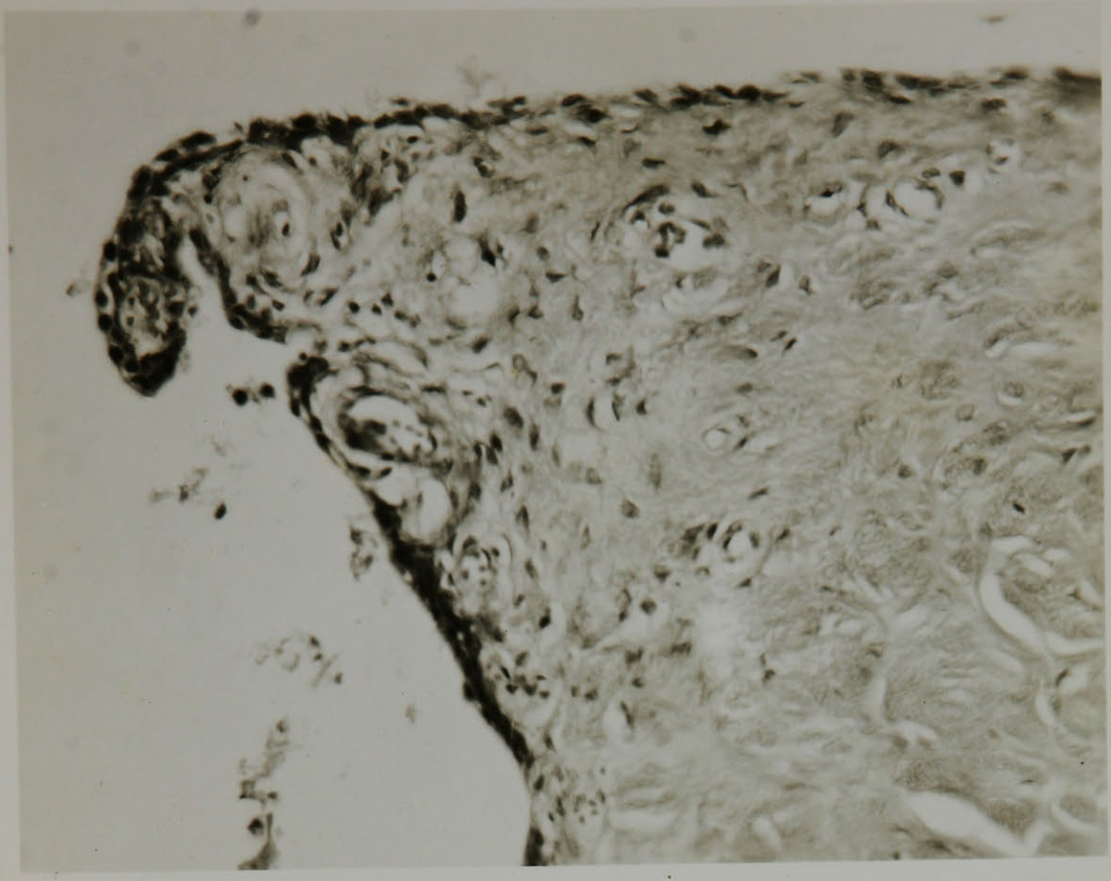


61.

Fig. 62. Base of semilunar cartilage from control rabbit # 121. The synovial cells are swollen and hyperchromatic, and the fibrocytes also appear swollen and increased in number. A few capillaries add to the general appearance of slight proliferative activity. No treatment. X 260.

Fig. 63. Synovial membrane from joint of control rabbit # 13. Slight condensation and increased cellularity of the subsynovial connective tissue are seen. No treatment. X 260.



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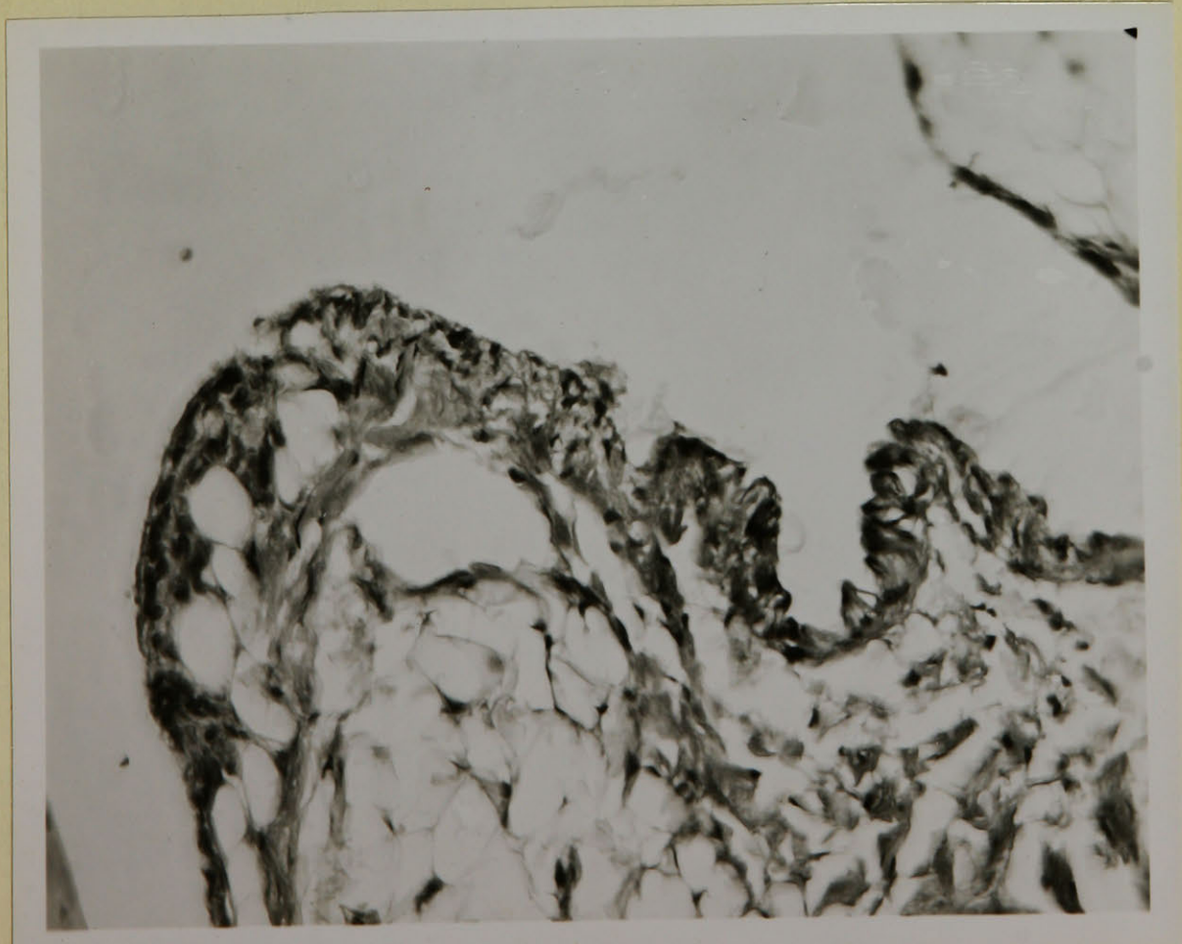
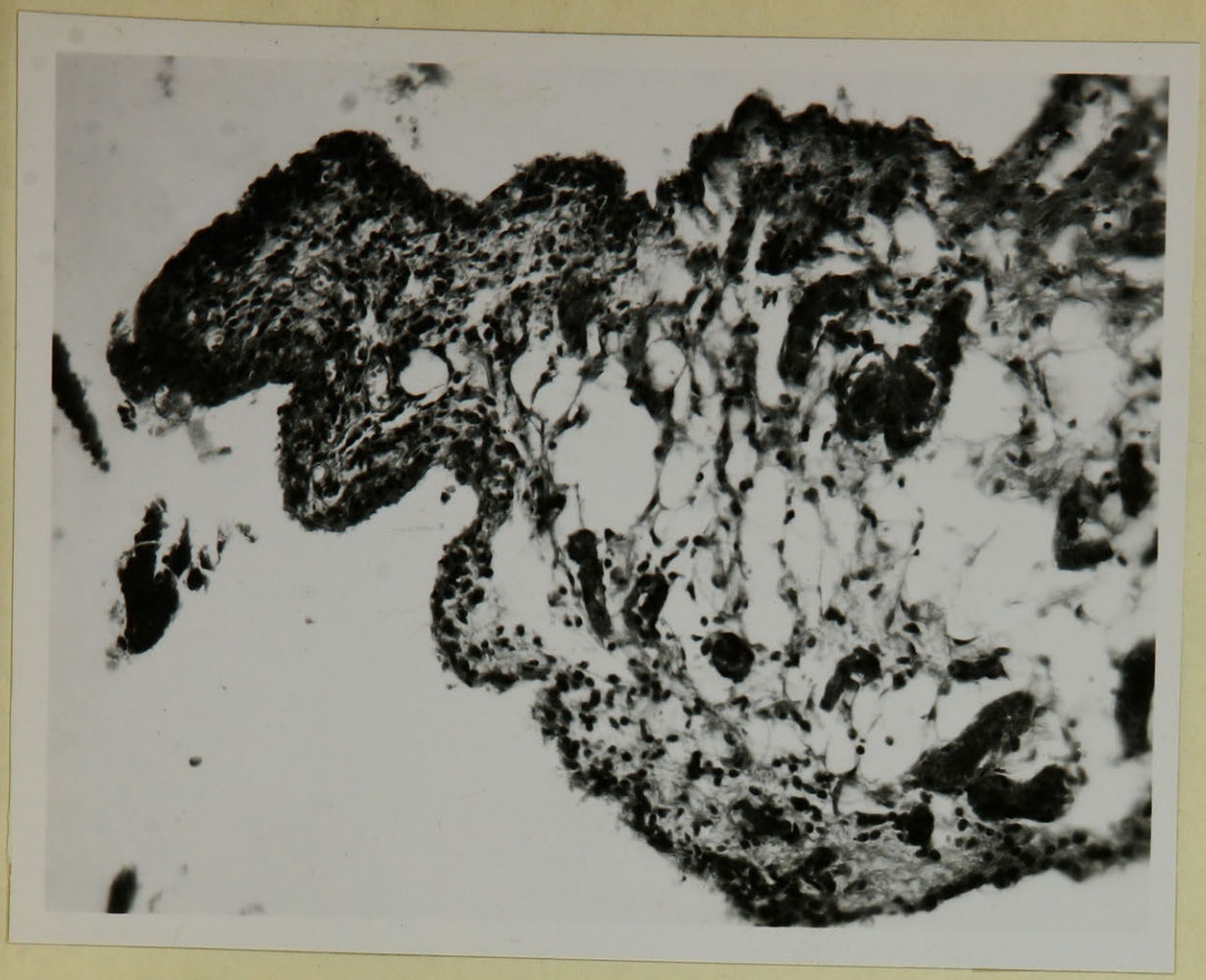


63.

Fig. 64. Villus fold in joint of rabbit # 72.
The synovial lining cells are swollen and show definite proliferative activity. The numerous capillaries of the loose connective tissue are intensely congested, and a definite leucocytic exudate is present. The majority of the inflammatory cells are lymphocytes and monocytes, although a few polymorphonuclear leucocytes are also included. Two injections. X 210.

Fig. 65. Synovial membrane in joint of rabbit # 18. The redundant synovial folds are very vascular and contain small numbers of mononuclear leucocytes. Six injections. X 210.



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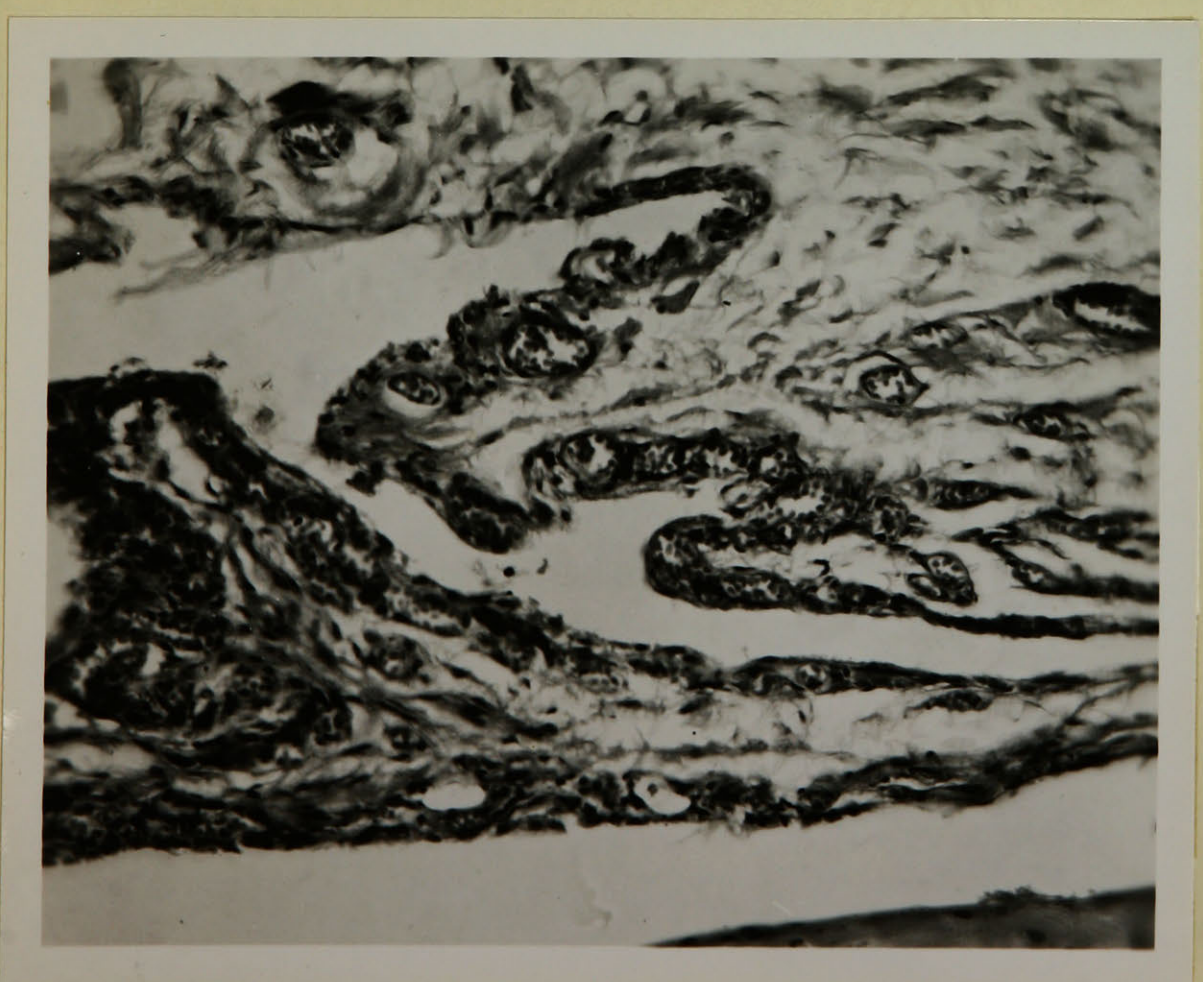

65.

Fig. 66. Synovial membrane from joint of rabbit # 112. The collagenous connective tissue is swollen and hyaline, and numerous capillaries are present. A mixed cellular exudate, including a few polymorphonuclear leucocytes, is seen. Two injections. X 210.

Fig. 67. Synovial membrane from joint rabbit # 1. The villus fold is edematous and contains a small number of lymphocytes and mononuclear leucocytes. The synovial cells are swollen and hyperchromatic. Two injections. X 210.

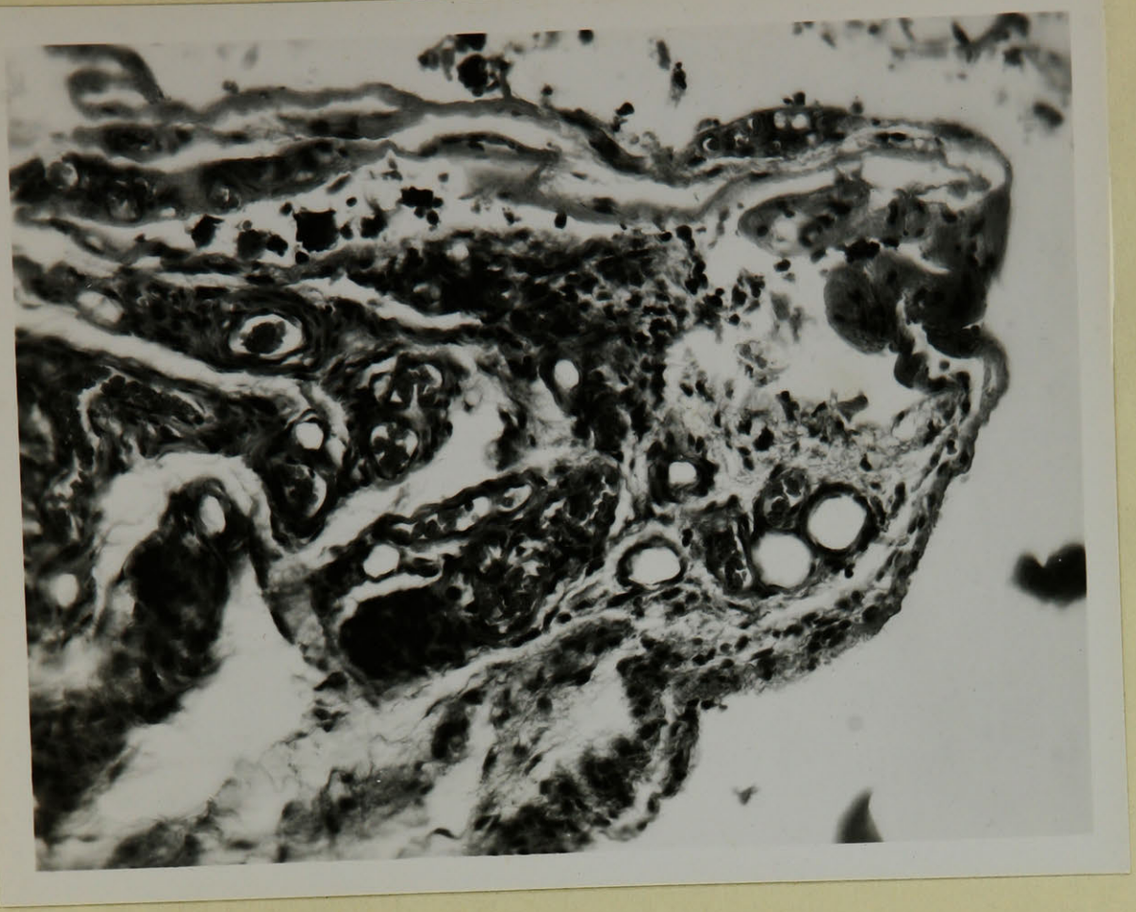

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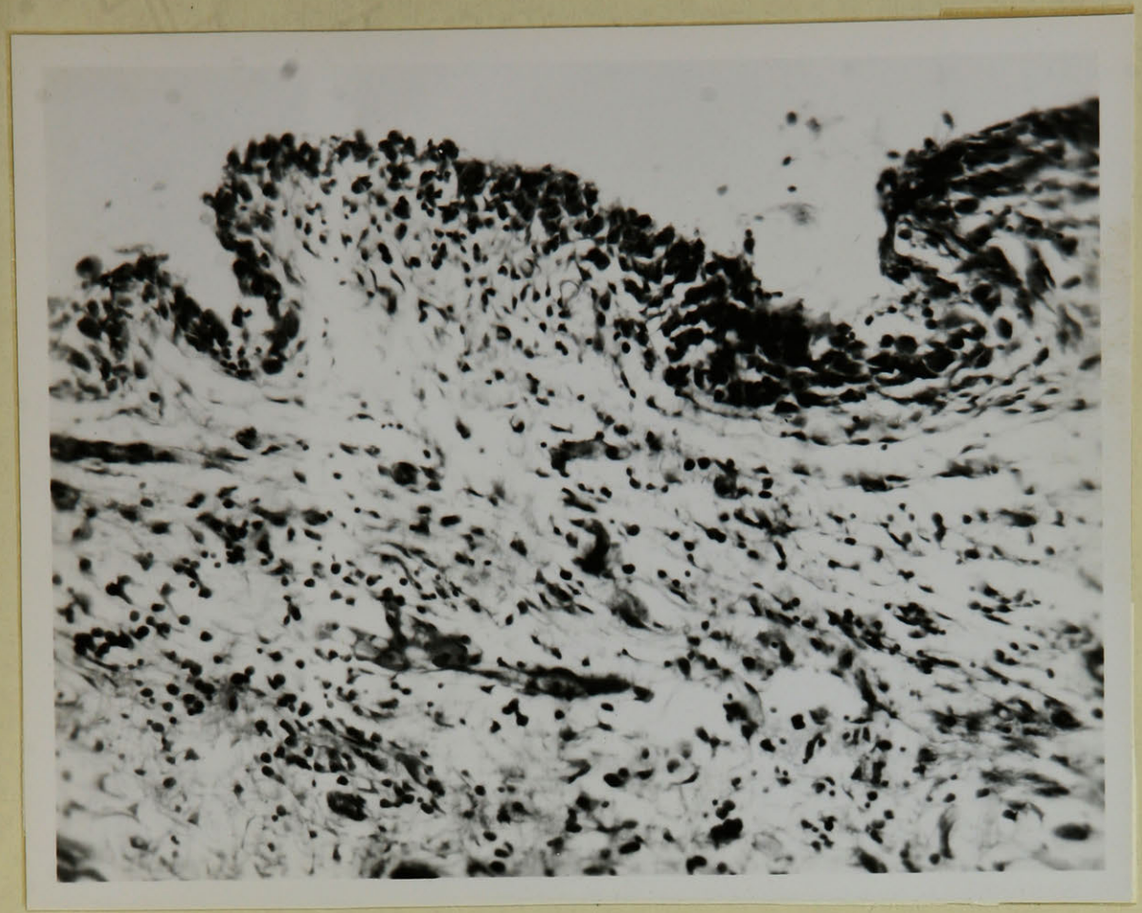
Fig. 68. Synovial membrane from joint of rabbit # 27. Vigorous proliferative activity of the synovial cells is present. The connective tissue is hyperemic and liberally sprinkled with a mixed leucocytic exudate. Seven injections. X 210.

Fig. 69. Synovial membrane from joint of rabbit # 32. A good deal of fibroblastic activity is seen, together with a mixed mononuclear leucocytic exudate. Two injections. X 210.



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69.