

STUDIES IN THE PATHOGENESIS OF LUPUS ERYTHEMATOSUS
EXPERIMENTAL PRODUCTION OF HEMATOXYPHIL BODIES IN THE KIDNEY

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PLATES 12 TO 15

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The pathogenesis of systemic lupus erythematosus (L.E.) is not understood though valuable observations regarding the clinical state, pathology, and immunologic mechanisms have been made. During recent years much attention has been given the *in vitro* L.E. cell phenomenon (3, 5) which occurs when injured cells as well as active phagocytes are in the presence of a factor from gamma globulin of the L. E. patient. Furthermore, careful pathologic studies (4, 6, 10-13) have revealed the unique position held by hematoxyphil, or hematoxylin, bodies in tissues from persons with L.E. These bodies, found most frequently in the heart and kidney, have been demonstrated in tissues of other structures including lymph node, spleen, liver, bone marrow, pancreas, ovary, blood vessel wall, and serosal, synovial, and mucous membranes. Besides the frequency with which hematoxyphil bodies are found in the kidney, the fact has had emphasis that they have not been found in this organ in any condition other than L.E. Hematoxyphil bodies and the inclusion bodies of L.E. cells are of approximately the same size, and both have been shown by histochemical techniques (9, 10) to have nuclear origins.

By phase contrast microscopy (8) the L.E. cell in the living state is seen to be larger than the normally circulating formed elements of the peripheral blood (Figs. 1 and 2). At times its diameter is more than twice that of the erythrocyte. These observations suggest that such a cell, if it should enter the circulating blood stream, could lodge in and obstruct a small blood vessel. The following preliminary studies into the pathogenesis of L.E. demonstrate that this actually occurs.

Experimental Methods

A liquid suspension of L.E. cells was injected into the abdominal aorta of guinea pigs and rabbits following which the kidneys were examined for pathological changes. The method of preparation of the L.E. cell suspension was that described by German and Huber (8).

In this method freezing is the means of injuring cells. Leukocyte nuclei, if in the presence of the L.E. factor at the time of freezing to minus 50°C., will, upon being thawed, have be-

come characteristic swollen, homogeneous, chemotactic nuclear remnants known as L.E. bodies. Then, if to this suspension of L.E. bodies is added a suspension of fresh active normal leukocytes, the final phase of the L.E. phenomenon, phagocytosis, rapidly occurs.

The addition of 5 per cent dextran (one part) to heparinized fresh whole blood (6 parts) results in rouleau formation and rapid sedimentation of the erythrocytes. The supernatant plasma, containing most of the leukocytes, is removed by pipette to another tube which is placed in a minus 50°C. deep freeze. The L.E. factor is already present in this preparation if the source of the blood was a patient with L.E. However, stored serum from such a patient may be used; in this case, leukocytes can be separated as above from the blood of either a normal person or a guinea pig. The L.E. patient's serum is then added to the leukocyte suspension (a 1 to 5 proportion of serum to cell suspension is adequate if the serum is potent), and after a 30 minute incubation period at 37° the mixture is frozen to minus 50°C.

The cell suspension can be kept at least many months in the frozen state. When thawed, the leukocyte nuclei will have become characteristic chemotactic bodies with or without attached fragments of cytoplasm. To convert these bodies to L.E. cells fresh normal phagocytic cells are added. These are obtained from the heparinized blood of a normal individual by use of dextran as described above. After obtaining the leukocyte-containing plasma from the blood, the leukocytes are separated by centrifugation (800 r.p.m. for 10 minutes), the supernatant plasma is poured off, and the leukocytes are resuspended in 0.5 ml. of the plasma. These normal active leukocytes are then added to the thawed suspension of L.E. bodies, and incubation of the mixture at 37°C. for a few minutes follows.

Examination of a drop of the preparation through the phase-contrast microscope reveals a surprisingly great number of L.E. bodies, L.E. cells, and L.E. rosettes, freely suspended in the fluid medium with only minimal cellular aggregation (Figs. 1 and 2). The L.E. bodies,—which usually are chemotactic,—are quite rigid when compared to the flexible erythrocytes, the latter easily and readily bending, elongating, flattening, or stretching to pass through narrow passages. This relative rigidity of the L.E. bodies is transferred to the L.E. cell when the bodies are engulfed. This lack of pliability of L.E. cells is most apparent when rivulets of fluid are present on the slide immediately after the coverglass is applied to a drop of the preparation. The erythrocytes, and to a less extent the leukocytes, rush along with the current and easily pass through even very tortuous and narrow channels of a rivulet. However, bulky L.E. cells, because of their large size and their rigidity, tend to move along the stream very sluggishly, if at all. They seem sticky, in that they promptly attach themselves to the glass or neighboring cells or platelet masses.

This technique provided the L.E. material for animal injections (Figs. 3 and 4). Identical control material for injection was prepared except blood or serum from a normal individual was used instead of that from the L.E. patient (Fig. 5). Study of the control injection material revealed no evidence of the L.E. cell phenomenon; non-chemotactic, shadowy, amorphous remnants of nuclei were the result of freezing cells in the absence of the L.E. factor.

The rabbit and the guinea pig, anesthetized by intravenous or intraperitoneal pentobarbital sodium plus light inhalation of ether, served as test animals. The abdominal aorta of the supine animal was exposed and ligated just proximal to the iliac bifurcation. Proximal to this ligature but distal to the renal arteries an incision was made into the lumen of the aorta, and through this incision a small polyethylene tube was inserted so that its open tip rested near the point from which the renal arteries leave the aorta. Just above the incision a ligature was placed about the aorta and its indwelling catheter to prevent escape of blood. Next, the right kidney was removed and fixed (as a control for the individual animal) and the mesenteric artery was temporarily clamped, the clamp removed only at the completion of injection.

Into the polyethylene tube of such a preparation was injected over a 12 to 30 minute period the dextran-plasma medium containing either L.E. bodies and L.E. cells or control cellular remnants and cells (Table I). Gentle thumb pressure on the piston of an ordinary ground glass syringe was adequate to introduce the material into the aorta. During the

TABLE I
Injection Data

Experiment No.	Material injected (8)	L. E. cells injected	Animal	Duration of injection	Volume injected	Time before autopsy	Hematoxyphil bodies
				min.	ml.		
570409	GP WBC + L.E. serum, + normal WBC	+	Rabbit	30	30	15 min.	+
570425.2	GP WBC + L.E. serum, + normal WBC	+	Rabbit	15	30	4 hrs.	+
570425.4	GP WBC + L.E. serum, + normal WBC	+	Rabbit	15	20	1 hr.	-
570429	GP WBC + L.E. serum, + normal WBC	+	Guinea pig	23	20	15 min.	+
570501	GP WBC + L.E. serum, + normal WBC	+	Rabbit	12	20	30 min.	+
570502.B11	GP WBC + L.E. serum, + normal WBC	+	Rabbit	15	20	30 min.	+
570524.2	L.E. pt's. plasma and WBC, + normal WBC	+	Rabbit	15	25	20 min.	+
570507.B1	GP WBC + normal serum, + normal WBC	-	Rabbit	15	15	15 min.	-
570515	GP WBC + normal serum, + normal WBC	-	Rabbit	12	20	1 hr.	-
570516	GP WBC + normal serum, + normal WBC	-	Rabbit	15	17	20 min.	-
570517.B	GP WBC + normal serum, + normal WBC	-	Rabbit	30	30	15 min.	-
570520A	GP WBC + normal serum, + normal WBC	-	Guinea pig	20	20	15 min.	-
570522	GP WBC + normal serum, + normal WBC	-	Rabbit	15	25	4 hrs.	-
570523B	GP WBC + normal serum, + normal WBC	-	Rabbit	15	22	30 min.	-
570627	Normal human plasma and WBC, + normal WBC	-	Rabbit	15	27	20 min.	-

injection the kidney developed small pale areas in the gross, most of which tended to disappear after a few minutes. Some of these areas remained pale or became hyperemic, suggesting minute infarctions. After varying periods (15 minutes up to 4 hours) the injected left kidneys were removed, fixed in neutral formalin, and examined for pathological changes. Sections of kidney were made at thicknesses of 3 and 6 microns. They were stained with hematoxylin and eosin as well as by Feulgen's method.

Pathologic Findings¹

The architecture of the kidney was preserved in all instances. The tubules in experiment No. 570425.2 contained laked blood. The arterioles and arteries were intact with the exception of isolated arteries that were occluded by coagulated blood; these thrombi appeared in equal frequency in the experimental and control animals.

In six of the seven kidneys receiving L.E. material prepared in the way just described and injected by way of the aorta, glomerular capillaries contained varying numbers of hematoxyphil bodies (Figs. 6 to 8). These bodies were generally spherical, although at times they were elongated or curved, conforming to the contour of the capillary. Their diameter was estimated to be 10 to 14 microns. They had fairly sharply delimited borders. In some cases, a clear, unfilled space existed, apparently separating the body from the capillary wall. The bodies varied in their tinctorial properties. Most often they appeared purple in the hematoxylin and eosin-stained slides, not deep blue like the nuclei of viable leukocytes and renal tissue. Some of them had a pale, gray-blue color. In the Feulgen preparations, they were red-purple. Occasionally darkly stained nuclei were seen lying against the bodies (Figs. 6 and 7); presumably they were those of L.E. cells. The capillary walls were not thickened. Rarely glomerular capillaries in the experimental and control animals contained hyaline occlusive material.

The hematoxyphil bodies were seen with varying ease in the injected kidney tissues of different animals. In some, virtually all glomeruli in a section contained at least one, and sometimes as many as ten bodies in a section of a single glomerulus were to be seen. In the kidney removed 4 hours after the injection, they were less frequent. They were not present in the lumina of renal arteries and for this reason are believed to have reached the glomerular capillaries where they lodged.

Hematoxyphil bodies were not seen in the control animals.

DISCUSSION

The pathologic lesions found in the kidney of lupus erythematosus have been well described by Klemperer (1, 12, 13), Smith (4), Muehrcke (11), and others. In the glomeruli hyalin thickening of capillary loops, hematoxyphil bodies, a peculiar focal necrosis, and focal proliferative glomerulitis are, according to Smith, the most important histologic changes. The hematoxyphil bodies, purple in sections stained with hematoxylin and eosin, give a positive Feulgen reaction. They appear at times as isolated bodies within the lumina of capillaries, more rarely as larger masses. They are usually found in conjunc-

¹ The pathologic examination and interpretation of these tissues were possible through the kind cooperation of Dr. Leon Sokoloff, Laboratory of Pathology and Histochemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

tion with other pathologic changes in the capillaries, but the presence of hematoxyphil bodies together with the peculiar hyalin thickening of capillary loops seem to be the fundamental changes. They may combine to result in "focal necroses" or capillary loop thickenings, or they may remain separate as individual lesions.

Klemperer has repeatedly emphasized the pathologic uniqueness and consistent presence of hematoxyphil bodies in autopsy material from lupus patients, having observed them in the kidney of 44 such cases. Since they have never been found in the kidney in other conditions, he describes them as "the pathognomonic criterion for the postmortem diagnosis of systemic lupus erythematosus."

In renal biopsies of L.E. patients in various degrees of remission, relapse, and treatment Muehrcke and associates (11) found these bodies in 10 per cent of cases and confirmed their specificity for this disease. They constructed a logical theoretical sequence of events in the development of the pathologic and clinical manifestations of "lupus nephritis."

Klemperer and coworkers (13) and Gueft and Laufer (10), utilizing histochemical and optical techniques, presented evidence that the hematoxyphil bodies, hyalin thrombi, and fibrinoid material of the L.E. tissues are of nuclear origin.

The histogenesis of the hematoxyphil bodies in the kidney is uncertain. In the original descriptions of the renal lesions in this disorder, hyaline "thrombi" were observed in the glomerular capillaries in a proportion of cases. These were continuous with the thickened glomerular walls and were regarded as intraluminal excrescences of fibrinoid material that infiltrated the walls of the capillaries. Thus, they were not considered to have the character of a true thrombus in the sense that a thrombus is a coagulum of hematic material arising within the circulating blood.

Subsequently, however, it was suggested that the occlusive hyaline masses arise from hematoxyphil bodies located within the lumen of the glomerular capillaries; and that the mural changes might be the result of impregnation of the tissues with fibrinoid material arising from this source (10). In support of this concept has been the demonstration of discrete intraluminal hematoxyphil bodies and the recognition of sharply delimited margins of the hyaline masses (4).

The histochemical identification of hematoxyphil bodies of lupus tissues with the L.E. bodies and L.E. cells prepared *in vitro*, coupled with the interesting suggestion obtained from phase-contrast observations of supravital preparations, stimulated the above described animal experiments. Though the degree to which the L.E. cell phenomenon occurs in the body of the lupus patient has not been clarified (2, 7), the circulating blood is a source of "injured" cells (either through senescence or from physical or chemical agents) as well as phagocytes. Cell injury and death occur also at varying rates in other tissues, perhaps more rapidly in tissues subject to continual trauma (*e.g.*, synovia, pleura, pericardium, heart valves). To speculate further, small vessels in body areas other than the glomeruli conceivably could be obstructed if such sizeable masses should enter the circulating blood.

In the present experiments, hematoxyphil bodies, morphologically compara-

ble to the discrete type observed in the glomerular capillaries in systemic L.E., have been produced (14). The observations lend support to the hypothesis that circulating hematophyl inclusions of L.E. cells and possibly L.E. cells may become impacted in glomerular capillaries. They do not establish whether they may persist for a long period of time or whether they give rise to the so called wireloop or nephritic changes in the kidney. Further studies are to be undertaken to clarify these problems.

SUMMARY

Hematophyl bodies, both in appearance and staining characteristics resembling those seen in the kidney of the patient with systemic lupus erythematosus, have been produced in experimental animals. Kidneys examined from 15 minutes to 4 hours following intra-aortic injection of L.E. cells and L.E. bodies have exhibited these Feulgen-positive bodies in the glomerular capillaries. Control injections have given completely negative results.

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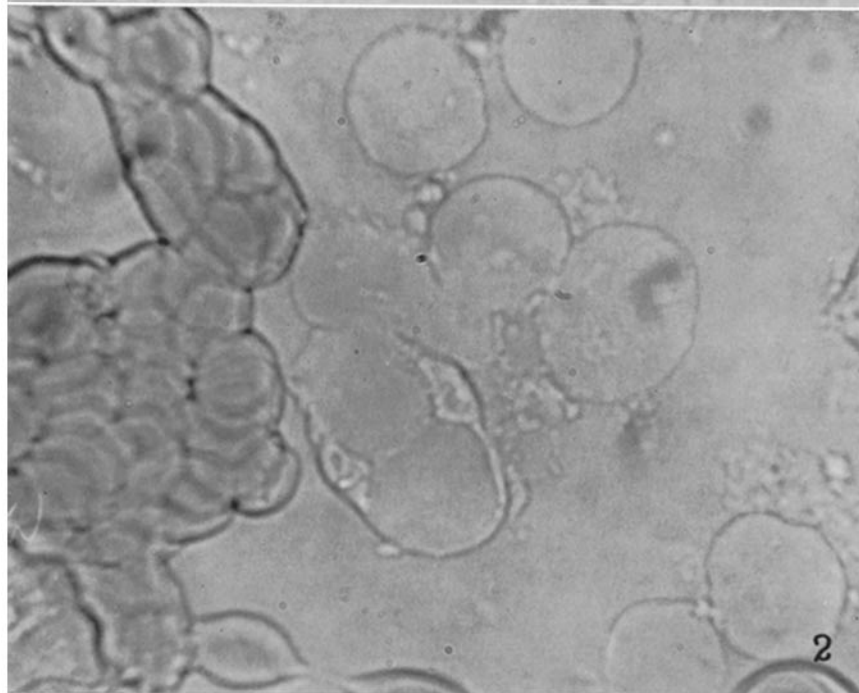
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EXPLANATION OF PLATES

PLATE 12

FIG. 1. L.E. bodies being engulfed by granulocytes; free L.E. bodies and erythrocytes. Supravital preparation, phase contrast microscopy. $\times 1600$.

FIG. 2. One L.E. cell containing two engulfed L.E. bodies; free L.E. bodies and erythrocytes also present. Phase contrast. $\times 1600$.

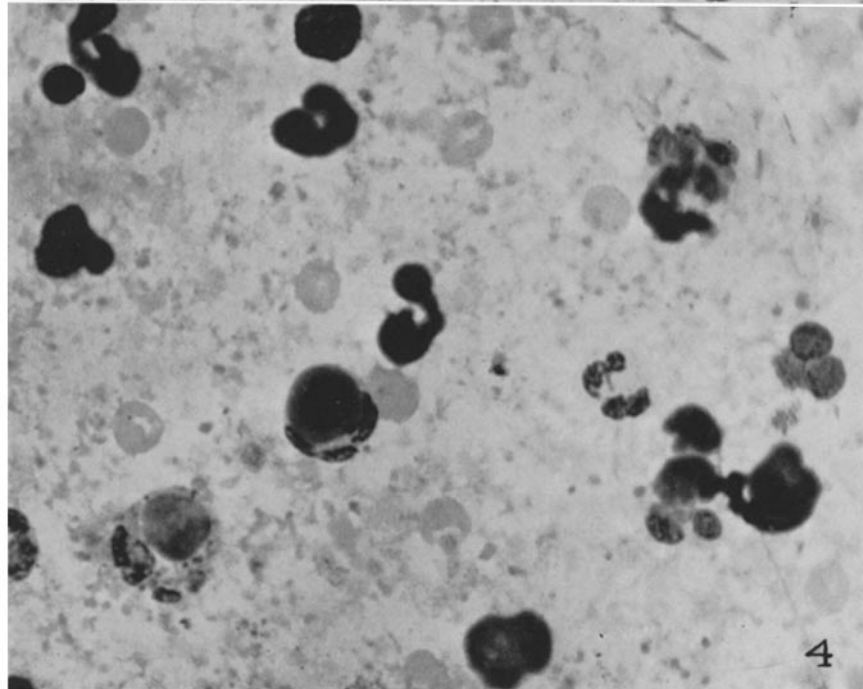
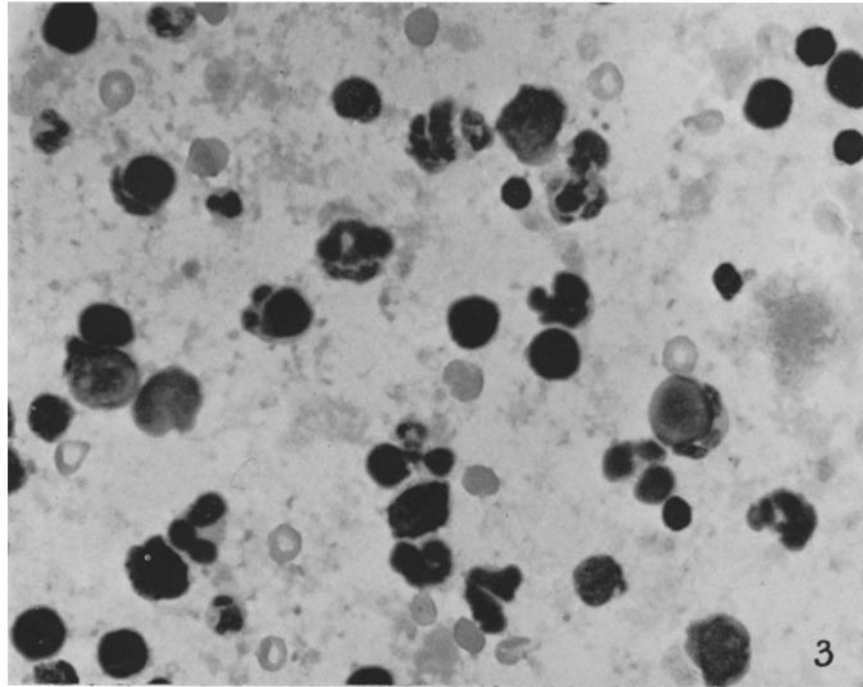


(German: Pathogenesis of lupus erythematosus)

PLATE 13

FIG. 3. L.E. cells and L.E. bodies prepared by the freezing technique for intra-aortic injection. Giemsa stain. $\times 940$, enlarged to approximately 1880.

FIG. 4. L.E. cells and L.E. bodies prepared by the freezing technique for intra-aortic injection. Giemsa stain. $\times 940$, enlarged to approximately 1880.

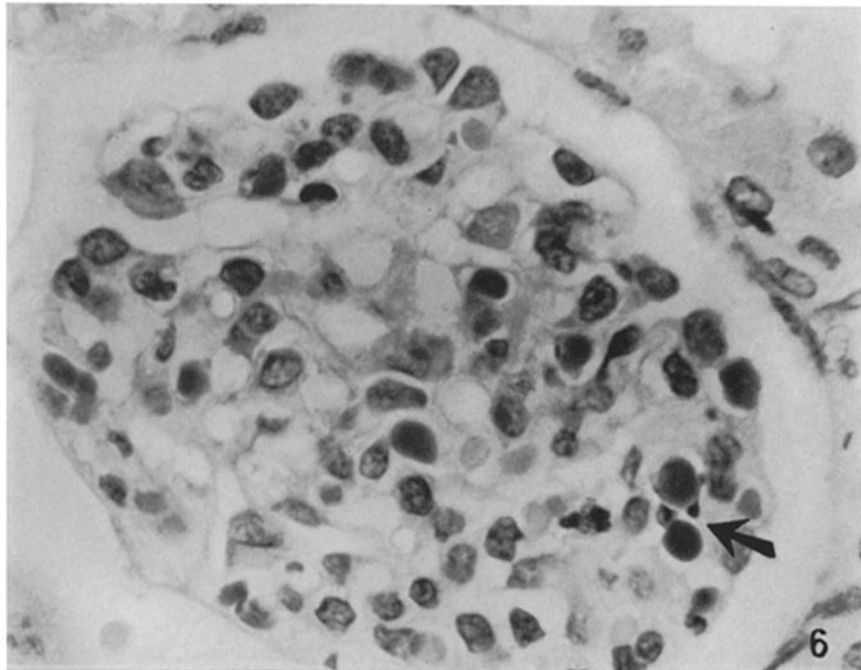
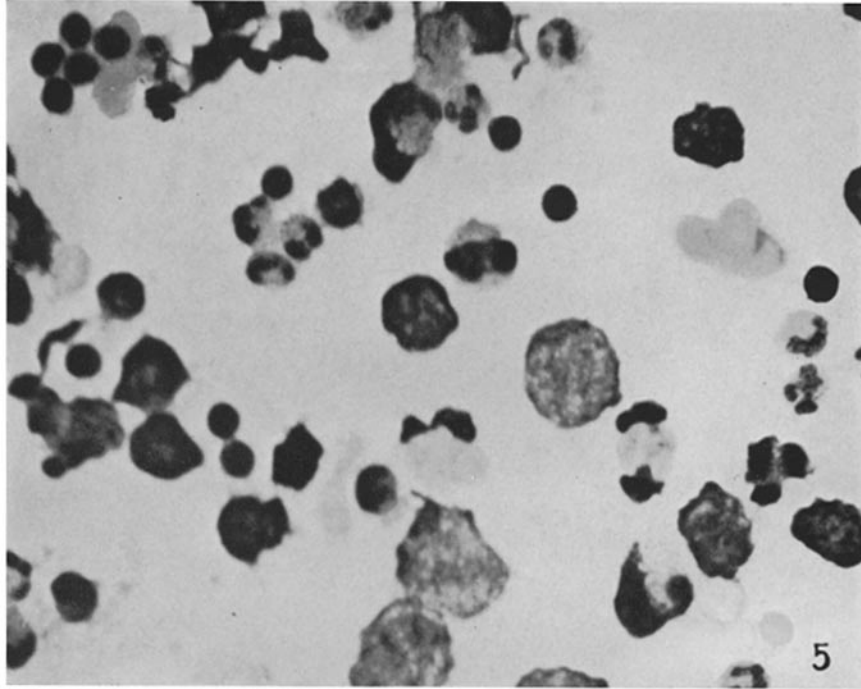


(German: Pathogenesis of lupus erythematosus)

PLATE 14

FIG. 5. Amorphous nuclear remnants resulting from freezing in absence of the L.E. factor. Preparation used for control injection. Giemsa stain. $\times 940$, enlarged to approximately 1880.

FIG. 6. Rabbit glomerulus showing numerous hematoxyphil bodies in capillaries. (Experiment 570524.2.) Some bodies are spherical; some take the contour of the capillary lumen. Note the two bodies with an intervening dark nucleus (arrow) resembling the L.E. cell shown in Fig. 2. Hematoxylin and eosin stain. $\times 1480$.

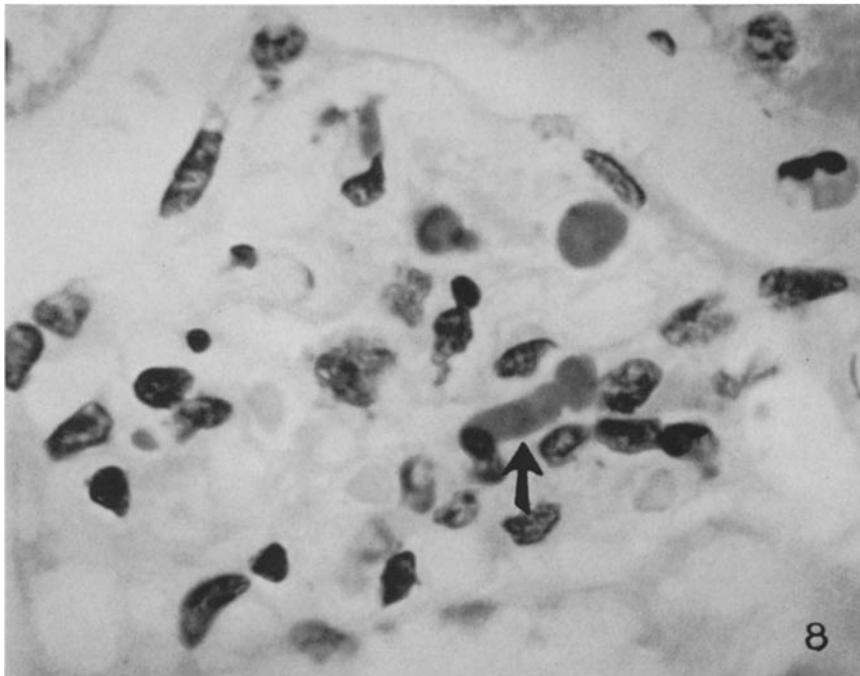
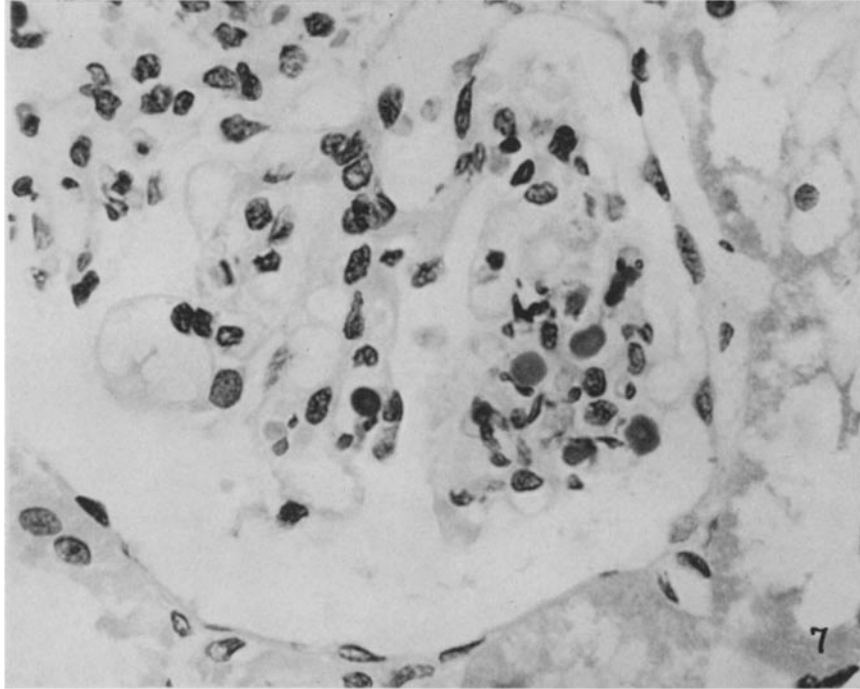


(German: Pathogenesis of lupus erythematosus)

PLATE 15

FIG. 7. Guinea pig glomerulus containing several hematoxyphil bodies. (Experiment 570429.) Feulgen preparation. $\times 1300$.

FIG. 8. Rabbit glomerulus showing hematoxyphil bodies, one round and one having the contour of the capillary lumen (see arrow). (Experiment 570524.2.) Feulgen preparation. $\times 1650$.



(German: Pathogenesis of lupus erythematosus)