A CLINICAL AND EXPERIMENTAL STUDY OF THE FUNCTION OF NEUTROPHILS IN THE INFLAMMATORY RESPONSE *

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Much has been written about the role of the lymphocyte in the cellular response to inflammation. The progression from lymphocyte to macrophage has been well documented by Metchinikoff,[1] Maximow,[2] Downey,[3] Bloom,[4] Kolouch,[5] and Rebuck.[6,7] The factors responsible for initiation of this cellular response were studied extensively by Menkin.[8] On the basis of his observations, he concluded that specific chemical substances were released in the inflammatory area, and one of these, leukotaxine, was responsible for attracting leukocytes to the site. He also presented evidence indicating the existence of a separate leukocytosis promoting factor. On the other hand, Moon[9] concluded that the production of leukocytosis was a nonspecific response to the introduction of protein breakdown products into the circulation. He also believed that the local events in inflammation were due to the presence of polypeptides. Polypeptides with 8 to 14 amino acids were thought to produce increased capillary permeability and leukocyte migration, while those with 5 amino acids were said to provoke leukocyte migration without increasing capillary permeability. To date, although neutrophil infiltration regularly represents an important morphologic event in acute inflammation, no one has made a systematic study of the role of the neutrophil in the inflammatory cycle. Those who have studied inflammation have noted that neutrophils are the first blood cells to appear in the inflammatory site, and their participation in the body defense has been attributed to their role in the inflammatory process. However, thus far only Rebuck[6] has postulated that the neutrophil is responsible for some of the subsequent morphologic mechanisms of the inflammatory cycle. For example, he presented evidence which he interpreted to indicate that the transformation of lymphocytes into mononuclear polyblasts and macrophages was a function of their ingestion of polymorphonuclear leukocytic debris.

One of the most striking features of the inflammatory cycle is the regular sequence of morphologic events which characterize it. Regard-

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less of the nature of the irritant used to elicit inflammation, the initial event is activation of histogenous wandering cells which appear in abundance during the first hour following introduction of the irritant. Subsequently polymorphonuclear leukocytes invade the area from the peripheral blood and dominate the inflammatory process in the early hours. The exudation of neutrophils is followed in turn by infiltration with small mononuclear cells indistinguishable from the circulating lymphocytes. These cells first appear along the blood vessels and later extend throughout the inflamed tissue. If serial studies are made of the inflammatory process, the lymphocytes can be seen to become transformed into hematogenous polyblasts and macrophages. Subsequently, fibroblasts begin to proliferate and macrophages seem to be transformed into fibroblasts or resting wandering cells as the process subsides. Although the absolute time relationships may vary with the irritant used, the fundamental morphologic sequence remains constant. The constancy of the sequence of events in the acute inflammatory cycle makes it attractive to postulate that in some way each event is conditioned and induced by the one preceding it. Menkin's[10] attempt to explain the inflammatory cycle on the basis of relative resistance of the cell types to low pH, although providing a possible explanation of the sequential predominance of neutrophils, lymphocytes and macrophages during the different stages of inflammation, does not account for the sequential appearance of these cells in the exudate.

The availability of a patient with cyclic neutropenia who at times possessed no circulating neutrophils and at other times had an adequate supply of these cells provided a unique opportunity to study the validity of our hypothesis, that each morphologic event conditions and effects the subsequent inflammatory process. This patient, observed over a period of 11 years, suffered from a disease featured by regular fluctuations in the number of circulating neutrophils at 21 day intervals. During the episodes of neutropenia the circulating neutrophils fell to imperceptible levels for several days and then returned to nearly normal values. It was also established in our study of this case that during the period of neutropenia, neutrophils and their precursors were absent from the blood forming tissues as well as the peripheral blood.

In initial studies the patient received intradermal injections of typhoid-paratyphoid vaccine, Piromen®* and streptokinase-streptodornase in order that the effect of neutropenia on the development of delayed type hypersensitivity might be investigated. These stimuli were given once when the peripheral blood was devoid of neutrophils

* Piromen is a commercially available polysaccharide endotoxin derived from cultures of *Pseudomonas aeruginosa* and generously supplied for our studies by Dr. Harry Fevold of the Baxter Laboratories.

and again when the differential count revealed 42 per cent neutrophils. The patient developed erythema and induration after the administration of each of the antigens, and there was no gross difference in the appearance of the cutaneous lesions which developed during the two periods. However, punch biopsy specimens procured from the inflammatory sites 24 hours after the introduction of typhoid-paratyphoid antigen revealed a remarkable difference in the inflammatory cellularity of the exudate in the two specimens. When the patient was in neutropenic state, examination of the section showed connective tissue with only minimal perivascular round cell infiltration. Examination of the specimen taken when the patient had adequate numbers of neutrophils revealed edematous connective tissue with a profuse cellular exudate. The difference in the inflammatory process during the neutropenic and neutrophilic phases of the cycle is illustrated in Figures 1 and 2.

The interesting observation suggesting the profound effect of neutropenia on the inflammatory cycle in this patient led to attempts to study systematically the early phases of the inflammatory response in cyclic neutropenia.

Clinical Study

Method

A simple atraumatic experimental method for investigating the inflammatory cycle in man has been described by Rebuck.[1] The forearm is prepared by the application of an antiseptic. Then an area approximately 0.5 cm. square is denuded of its cornified epithelium by scraping with a sterile scalpel. Care must be taken not to cause bleeding by scraping too deeply. A drop of antigen is placed on the denuded area which is covered by a chemically clean and sterile cover slip. A small cardboard square is taped tightly over the cover slip to fix it in position. The inflammatory cells migrate onto the under surface of the cover slip and may be sampled at any time by its removal. The cover slips are replaced at frequent intervals. The exudate is fixed by quickly drying the cover slip specimens in air, and staining is accomplished with Romanowsky polychrome stains. An excellent demonstration of the progress of cellular migration and metamorphoses occurring in the inflammatory cycle may be obtained in this way.

This method was employed in the study of the patient with cyclic neutropenia. Two sites were prepared on her forearm so that 2 different antigens could be used. A drop of streptokinase-streptodornase (SK-SD) containing 100 units per cc. was applied to one site (the patient was known to be sensitive to this antigen), and a drop of 1:1,000 old tuberculin (O.T.) to which she was not sensitive was applied to the

other. The cover slips were replaced every 2 hours for the first 16 hours and at 4 hour intervals thereafter for 28 to 32 hours. At the time of the first trial the white blood cell count was 5,000 per mm.^{3}, and the differential count revealed 54 per cent lymphocytes, 35 per cent monocytes, 10 per cent eosinophils, 1 per cent basophils and no neutrophils.

Results

The response at the SK-SD stimulated site consisted of a few large mononuclear cells, probably histogenous macrophages, which appeared first at 4 hours, reached a numerical peak at 12 hours and remained until 20 hours. After 20 hours no cells were found. The response at the site stimulated with old tuberculin was similar except even fewer cells were present on the cover slip. At the end of the study the peripheral white blood cell count was 5,200 with a differential count of 17 per cent monocytes, 58 per cent lymphocytes, 24 per cent eosinophils, and fewer than 1 per cent neutrophils. Another trial was run as a check on these results 3 weeks later when the white blood cell count was 3,500 with a differential count of 1 per cent neutrophils, 69 per cent lymphocytes, 23 per cent monocytes and 11 per cent eosinophils. This time again only a few mononuclear cells were found and these only at the 12 hour point following the application of SK-SD. The other slides were completely blank. Following the application of the O.T., the 6, 8 and 10 hour samples showed a few lymphocytes, and the 12 hour specimen had a few large mononuclear cells.

The control tests were run when the patient had a white blood cell count of 3,900 with a differential count of 38 per cent neutrophils, 46 per cent lymphocytes, 6 per cent monocytes, 9 per cent eosinophils and 1 per cent basophils. The SK-SD stimulated lesion showed neutrophil migration beginning at 2 hours and reaching a peak at 4 hours. At this time lymphocytes were just beginning to migrate onto the cover slip while at 6 hours an extensive migration had taken place, and small lymphocytes constituted the predominant cell type observed. At the 10 hour stage large mononuclear cells predominated and fewer neutrophils and lymphocytes were seen. An extensive influx of neutrophils and small lymphocytes appeared again in the 12 hour sample. These were gradually replaced by large mononuclear cells which again predominated in the 20 hour specimen. In the 24, 28 and 32 hour specimens fibroblasts were present on the cover slip. The lesion to which old tuberculin had been applied showed an identical reaction for the first 10 hours. Here, however, there was no secondary influx of neutrophils and small lymphocytes at 12 hours. Large hematogenous mononuclear cells remained the predominant elements until they were

replaced by fibroblasts in samples taken during the later hours. These observations are illustrated in Figures 3 and 4. Here the inflammatory exudate which appeared during the neutropenic phase is contrasted with the inflammatory exudate occurring during the neutrophilic phase of the cycle.

Discussion

This experiment demonstrates a marked suppression of cellular migration at the inflammatory site in our patient during her neutropenic phase. The observation is compatible with the hypothesis that a lymphocytic exudation is dependent upon preliminary changes induced by an earlier polymorphonuclear leukocytic exudation. Lymphocytes, therefore, do not appear unless a polymorphonuclear leukocytic phase has taken place previously. The possibility that an antiphlogistic agent present in the patient's circulation coincidental with the neutropenia might account for this phenomenon seemed remote since studies of adrenal function revealed normal ACTH concentration, normal 17-hydroxycorticosteroid values and normal ACTH response test both when the patient's blood contained ample neutrophils and when it had none. Although the circulating blood contained approximately 10 per cent eosinophils during all 3 experiments, eosinophils were not found at the inflammatory site in significant numbers at any time. Monocytosis occurring during the neutropenic phase led to speculation as to whether or not the monocyte might assume the defensive function of the neutrophil. Our observations suggest that monocytes do not migrate into the field in the early phase of the inflammatory cycle as in the case of neutrophils, but rather infiltrate at a later time, as suggested by Rebuck.[6] During one trial in the neutropenic stage there was a rich infiltration of monocytes at 12 hours. However, during other trials only a few monocytes were found on the cover slips. This may have been due to variation in the intensity of the stimulus at the inflammatory site.

Finally, numerous studies of the inflammatory cycle were carried out in an identical manner in normal persons and in a group of patients with diseases not accompanied by neutropenia. In these instances the inflammatory process was featured by the same sequence of events as observed during the neutrophilic phase in the patient with cyclic neutropenia. We were forced to conclude that the availability of circulating neutrophils had a profound influence on the early events in the inflammatory cycle and that in their absence a markedly diminished lymphocytic invasion occurred. This, in turn, accounted for the dearth of hematogenous round cells and macrophages noted in the later stages of the inflammatory cycle.

Experimental Study

In an attempt to further evaluate the role of the neutrophil in the inflammatory response, studies were carried out upon experimental animals. An experiment was planned to investigate the time relationship of the cellular events in experimental inflammation, to study lymphocyte infiltration in normal animals and in those with neutropenia, and to determine, in addition, the effect of the local introduction of inflammatory neutrophils or aqueous homogenates of neutrophils upon the inflammatory response.

Methods

Method 1. The inflammatory cycle was studied in rabbits, utilizing the method of Kolouch.[5] In this procedure egg white labeled with carbon particles was used as an irritant. Multiple injections of the egg white were made into the subcutaneous tissue of the back, and serial surgical biopsy specimens of the connective tissue were obtained 2, 4, 8, 12, 16, 20 and 24 hours after introduction of the antigen. The connective tissue obtained by biopsy was spread in a single cell layer on a clean glass slide, fixed by rapid drying in air, and stained by the Wright-Giemsa method of Downey. By using this technique, all phases of the inflammatory cycle may be studied and the participating cells readily identified.

Method 2. For this portion of the study neutropenia was induced in rabbits by a single intravenous administration of 1.2 mg. per kg. of nitrogen mustard (HN_2). To insure an adequate supply of lymphocytes, the lymphoid tissue of the intestine, liver and spleen was protected from the effects of nitrogen mustard by clamping the celiac axis and the superior and inferior mesenteric arteries just prior to and for 5 minutes after the administration of HN_2. This method takes advantage of the fact that HN_2 is quickly hydrolyzed or fixed in the tissues following injection and is distributed by the circulation for only a very short period of time (less than 5 minutes).[11] By this means neutropenia became manifest 72 hours after the treatment and continued for 24 to 36 hours. In contradistinction, the lymphocyte count remained at normal or near normal levels throughout. We chose for experimental study only rabbits with an absolute lymphocyte count greater than 2,000 per mm.^{3} and with fewer than 2 per cent neutrophils. In some animals sham operation was performed by opening the abdomen and clamping the arteries; nitrogen mustard was not introduced. These, as well as normal unoperated and untreated animals, were used as controls.

Method 3. White blood cells were obtained by the intraperitoneal

introduction of 100 cc. of brain-heart infusion broth containing 5 per cent glucose into two 3-pound rabbits. Sixteen hours later 200 cc. of normal saline solution was administered intraperitoneally into each animal. One hour later this fluid was removed with a 15 gauge needle and centrifuged at 1,000 r.p.m. for 15 minutes. The cells obtained were pooled and resuspended in 8 cc. of a 10 per cent solution of egg white in normal saline. The white blood cell count in the final preparation was 70,000 per mm.^{3} with 85 per cent neutrophils and 15 per cent lymphocytes. Examination in a wet chamber indicated that approximately 40 per cent of the cells were motile. Four cubic centimeters of this suspension was thoroughly homogenated in a Virtis homogenizer in order to destroy all of the cells; the remaining 4 cc. was used as an injection source of living cells.

In this experiment 3 groups of rabbits were utilized. *Group A:* Three rabbits with HN_2 induced neutropenia received egg white alone at 8 subcutaneous sites, and 3 sham-operated control rabbits were similarly treated with egg white alone at 8 subcutaneous sites. *Group B:* Two animals with neutropenia and 2 sham-operated control rabbits were injected with white cell homogenate and egg white. *Group C:* Two animals with neutropenia and 2 sham-operated control rabbits were given viable white blood cells and egg white. In addition 4 unoperated control rabbits were similarly treated. Since no differences were observed between these rabbits and the sham-operated controls, this group will not be further considered.

In each instance the inoculum marked with carbon particles was introduced into 8 sites in the subcutaneous tissues. Biopsy specimens were taken at 2, 4, 8, 12, 16, 20 and 24 hours.

Results

It will be seen in Table I that in these experiments the absence of neutrophils from the circulation (group A) interfered not only with the infiltration of neutrophils in the inflammatory sites, as would be expected, but also resulted in a marked delay in infiltration of lymphocytes as well. For example, in the control sham-operated rabbits without neutropenia in group A, lymphocytes were present in the inflammatory exudate 4 hours after injection of egg white and constituted the dominant inflammatory cell at 8 and 12 hours. To the contrary, in the rabbits with neutropenia, mobilization of the histogenous macrophages proceeded as in the controls, but neither neutrophils nor lymphocytes were present at the inflammatory site during the first 12 hours. Approximately 16 hours after the administration of the antigen in the animals with neutropenia, lymphocytes appeared in the inflam-

TABLE I

Inflammation in Normal and Neutropenic Rabbits
Effect of Leukocytes and Leukocyte Homogenates on Inflammatory Response

Time after antigen	Inflammatory response				
	Histogenous macrophages	Neutrophils	Small lymphocytes	Hematogenous macrophages	Edema
2 hrs.					
*Group A**					
Control rabbits	+	+	o	o	+
Experimental rabbits§	+	o	o	o	o
Group B†					
Control rabbits	+	+++	o	o	++
Experimental rabbits	+	o	o	o	o
Group C‡					
Control rabbits	+	++	o	o	+
Experimental rabbits	+	+	o	o	+
4 hrs.					
Group A					
Control rabbits	++	+++	+	o	+++
Experimental rabbits	++	o	o	o	o
Group B					
Control rabbits	++	+++	+	o	+++
Experimental rabbits	++	o	o	o	o
Group C					
Control rabbits	++	+++	+	o	+++
Experimental rabbits	++	+	+	o	+++
8 hrs.					
Group A					
Control rabbits	+++	++	+++	+	+++
Experimental rabbits	+++	o	o	o	o
Group B					
Control rabbits	+++	+++	++	+	+++
Experimental rabbits	+++	o	o	o	o
Group C					
Control rabbits	+++	++	++	+	+++
Experimental rabbits	+++	+	++	+	+++
12 hrs.					
Group A					
Control rabbits	+++	++	++	++	+++
Experimental rabbits	+++	o	o	o	o
Group B					
Control rabbits	+++	+++	++	++	+++
Experimental rabbits	+++	o	o	o	o
Group C					
Control rabbits	+++	++	+++	++	+++
Experimental rabbits	+++	+	+++	++	+++
16 hrs.					
Group A					
Control rabbits	+++	++	++	+++	+++
Experimental rabbits	+++	o	++	++	+++
Group B					
Control rabbits	+++	+++	+++	++	+++
Experimental rabbits	+++	o	++	++	+++
Group C					
Control rabbits	+++	++	++	+++	+++
Experimental rabbits	+++	o	++	+++	+++
20 hrs.					
Group A					
Control rabbits	+++	++	++	+++	+++
Experimental rabbits	+++	o	++	+++	+++
Group B					
Control rabbits	+++	++	++	+++	+++
Experimental rabbits	+++	o	++	+++	+++
Group C					
Control rabbits	+++	++	++	+++	+++
Experimental rabbits	+++	o	++	+++	+++

matory exudate and became the predominant cell type. Except for the absence of neutrophils, the subsequent biopsy specimens were similar to those in the normal animal (Figs. 5 to 13). The rabbits in group B which received the homogenate of white blood cells showed inflammatory responses similar to those observed in animals with neutropenia which had received injections of egg white alone. The lymphocytic exudation was again markedly delayed in its appearance as compared to the controls. One difference between the rabbits in group B and those in group A was noted. The inflammatory process appeared to be more severe in the control rabbits of group B than was the case in those of group A. It seemed clear from these observations that a homogenate of leukocytes administered to rabbits with neutropenia did not result in a restoration of the early lymphocytic infiltration regularly observed in the controls. The results of this study are illustrated in Figures 14 to 20.

The control rabbits and those with neutropenia in group C received egg white combined with viable inflammatory leukocytes. The results of this study were in striking contrast to those of the previous groups. Within 4 hours after the administration of antigen-leukocyte mixture into the subcutaneous tissue of rabbits with neutropenia, exudation of lymphocytes was present, and at the 8 and 12 hour stage (Figs. 21 to 24) lymphocytes and hematogenous polyblasts dominated the inflammatory exudate. In the group C control rabbits an inflammatory cycle similar to that observed in the control animals of groups A and B was observed. These results demonstrated that intact, viable, polymorphonuclear leukocytes at the site of inflammation were followed by the early infiltration of lymphocytes and the early appearance of hematogenous polyblasts and macrophages.

Another interesting observation made in the course of this study bears mention. As will be seen in Table I, an absence of local edema characterized the inflammatory process in the animals with neutropenia who received egg white alone or egg white combined with white cell homogenate. In contradistinction, the inflamed connective

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* *Group A:* Sham-operated normal control rabbits and rabbits with neutropenia which received subcutaneous injections of egg white only.

† *Group B:* Sham-operated normal control rabbits and rabbits with neutropenia which received subcutaneous injections of egg white plus homogenates of exudate leukocytes.

‡ *Group C:* Sham-operated normal control rabbits and rabbits with neutropenia which received injections of egg white plus viable exudate leukocytes.

§ Experimental rabbits: Neutropenia but not lymphopenia produced by the injection of HN_3; protection against the latter was effected by clamping vessels supplying intestine, mesenteric lymph nodes and spleen.

tissue of the control rabbits with normal numbers of circulating neutrophils and the rabbits with neutropenia who had received intact leukocytes was markedly edematous even within the first few hours.

Discussion

These observations appear to demonstrate that the absence of neutrophils from the circulating blood results in a dramatic inhibition of the early stages of the acute inflammatory cycle in both man and experimental animals. Not only is there a deficit in the exudation of neutrophils, as would be expected since these cells are absent from the circulating blood, but the lymphocyte (small mononuclear cell) infiltration which in the normal animal is well under way 4 hours after the administration of the inflammation provoking irritant is also significantly delayed. Indeed, in some instances lymphocytes do not appear at all at the inflammatory site in the absence of circulating neutrophils. This results in a local deficit of hematogenous macrophages in the early hours of acute inflammation. The latter cells appear to be derived from the lymphocytes or small mononuclears by a process of differentiation which begins almost as soon as these cells have gained access to the connective tissue spaces. The studies carried out in the rabbit indicate that homogenates of exudate polymorphonuclear leukocytes introduced along with the antigenic irritant are provocative of a more intensive inflammatory response in normal rabbits than occurs after injection of foreign antigenic irritant alone. On the other hand, this does not provoke the early appearance of lymphocytes (small mononuclears) in the inflammatory exudate. To the contrary, the administration of viable exudate leukocytes, mostly intact polymorphonuclear neutrophils, with the antigenic irritant, results in the prompt appearance of exudate lymphocytes which proceed rapidly in their maturation toward hematogenous macrophages. It would thus appear from these studies that whatever is contributed to the acute inflammatory cycle by the exudative polymorphonuclear leukocyte and results in the subsequent early appearance of lymphocytes and hematogenous macrophages requires viable polymorphonuclear leukocytes and not just leukocytic debris.

The key question remains; namely, how does the neutrophil in the early stages of inflammation attract lymphocytes into the loose connective tissue? The neutrophil itself might conceivably produce a specific substance or act on the connective tissue to induce production of substances chemotactic to lymphocytes. It would seem from our studies that the latter hypothesis fits better with the observations. For example, approximately 16 hours after administration of the antigen

to the rabbits, lymphocytes accumulated at the inflammatory site even in the animals with neutropenia. Perhaps in this circumstance the histogenous macrophages accumulating at the site of injection of the irritant caused an alteration in the tissue, resulting in a release of products necessary for the attraction of lymphocytes. However, the histogenous macrophages seem to produce these tissue changes at a slower rate than is the case when the early inflammation is dominated by polymorphonuclear leukocytes.

The absence of edema in the early stages of inflammation leads one to consider the relationship of cellular infiltration to the capillary permeability in inflammation. Most authors[8,9] consider altered capillary permeability as the initial feature of inflammation. Although no data are presented here which conclusively contradict this observation, these experiments seem to suggest that the sequence begins with the attraction of actively motile neutrophils to the site of irritation. Since little edema is present at the time of the earliest infiltration by these cells, it seems possible that the initial cells, at least, gain access to the injured site through capillaries that are relatively intact. It is proposed that these cells then act on the connective tissue, possibly through an enzymatic mechanism, to produce substances which increase capillary permeability. It could well be that an alteration in the capillary integrity which would permit the occurrence of local edema might be the mechanism by which the lymphocytes (less actively mobile than the polymorphonuclear leukocytes) gain access to the inflammatory area and participate in the defense reaction. The sudden simultaneous appearance of both edema of the connective tissue and lymphocyte exudation in the rabbits with neutropenia 16 hours after the introduction of antigen (Table I) lends support to this hypothesis.

Summary

1. In an extensive study of inflammation in a patient with cyclic neutropenia and in rabbits with experimentally induced neutropenia we have shown that the absence of neutrophils from the circulation profoundly alters the early events of the inflammatory cycle.

2. The early neutrophil infiltration does not occur since none of these cells are present in the peripheral blood.

3. Even when a normal number of lymphocytes are present in the circulation, the early lymphocytic infiltration and development of hematogenous macrophages from them either is markedly delayed or fails to occur entirely when the inflammatory process takes place in the absence of circulating neutrophils.

4. The development of local edema of the connective tissues which,

in the normal inflammatory cycle, is temporally related to infiltration with lymphocytes, fails to take place when neutrophils are absent from the circulation.

5. These observations are interpreted as evidence that the sequence and time relationship of events in acute inflammation in normal man and animals is a function, in part at least, of the circulating neutrophils. They also support the hypothesis that each step in the morphologic sequence in inflammation is dependent upon the preceding event.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

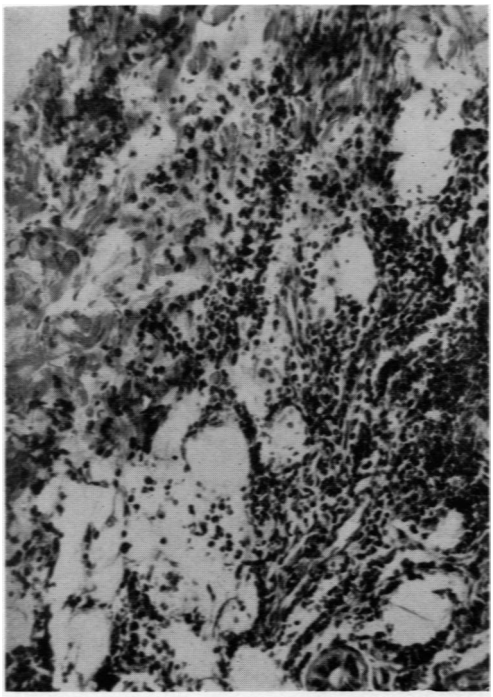
Photomicrographs comparing the intensity of the inflammatory response in a patient with cyclic neutropenia during phases of neutropenia and neutrophilia.

FIG. 1. Biopsy made 24 hours following the intradermal injection of typhoid-paratyphoid antigen, when the patient had normal numbers of circulating neutrophils, showing dense cellular infiltration of the subcutaneous tissue. Hematoxylin and eosin stain. × 85.

FIG. 2. Biopsy made 24 hours following the intradermal injection of typhoid-paratyphoid antigen, when neutrophils were absent from the patient's blood, showing only minimal perivascular cellular infiltration. Hematoxylin and eosin stain. × 85.

FIG. 3. Sampling of the inflammatory exudate at 12 hours, obtained by Rebuck's cover slip technique, when the patient had normal numbers of circulating neutrophils, showing the abundant neutrophil and mononuclear cell exudation. Wright's and Giemsa stains. × 150.

FIG. 4. Sampling of the inflammatory exudate at 12 hours, obtained by Rebuck's cover slip technique, when neutrophils were absent from the patient's circulation, showing only a few mononuclear cells. Wright's and Giemsa stains. × 150.



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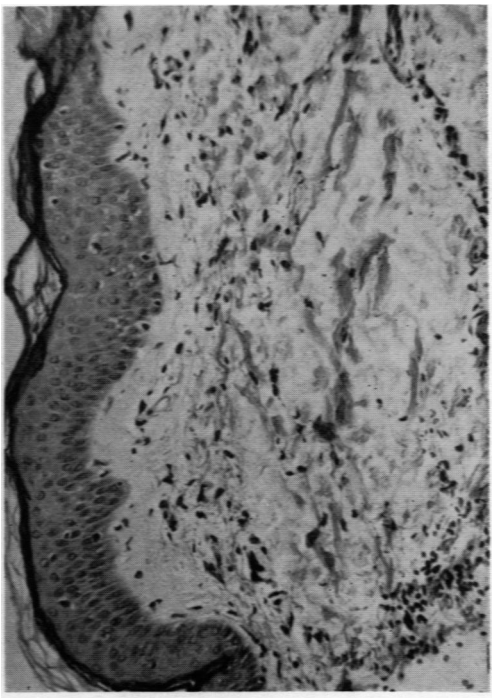

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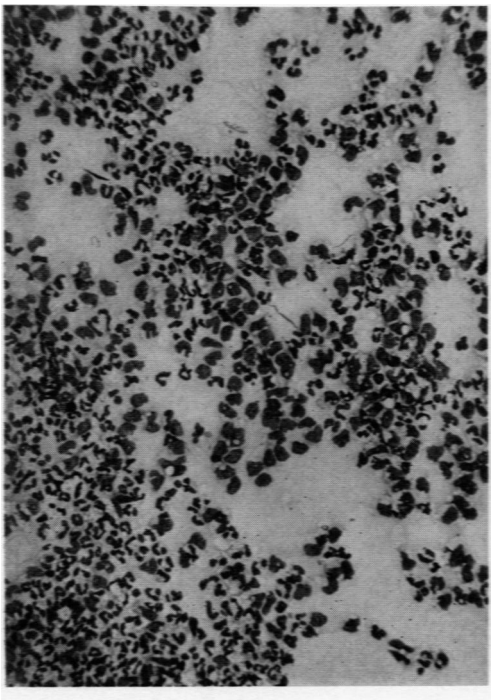

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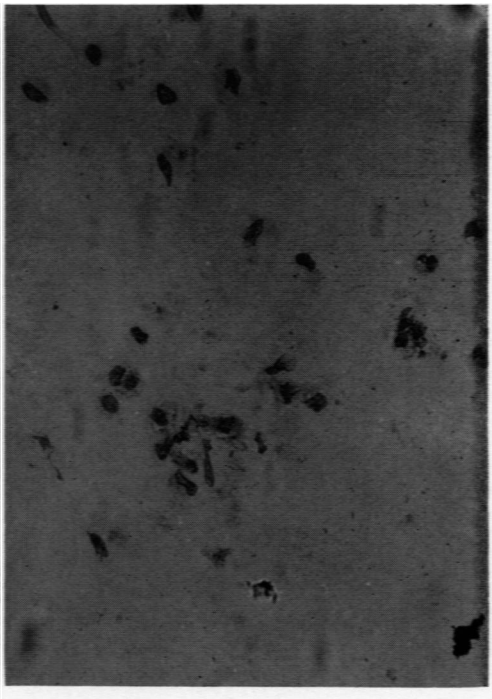

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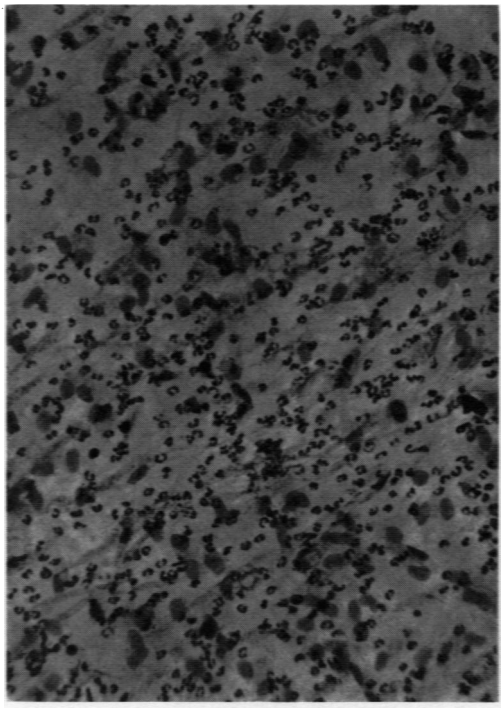
Connective tissue biopsy specimens, showing inflammation induced in normal rabbits by introduction of egg white.

FIG. 5. Inflammation at 2 hours, showing intensive neutrophil invasion. Wright's and Giemsa stains. × 150.

FIG. 6. Inflammation at 8 hours, showing lymphocyte to be the predominant cell type. Wright's and Giemsa stains. × 150.

FIG. 7. Inflammation at 12 hours, showing neutrophils, lymphocytes and macrophages. Wright's and Giemsa stains. × 150.

FIG. 8. Inflammation at 16 hours, showing intensive infiltration of lymphocytes and macrophages, accompanied by many degenerating neutrophils. Wright's and Giemsa stains. × 150.



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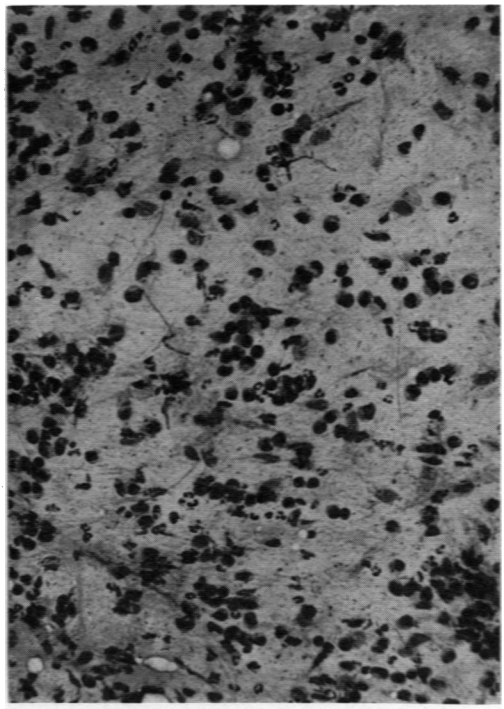

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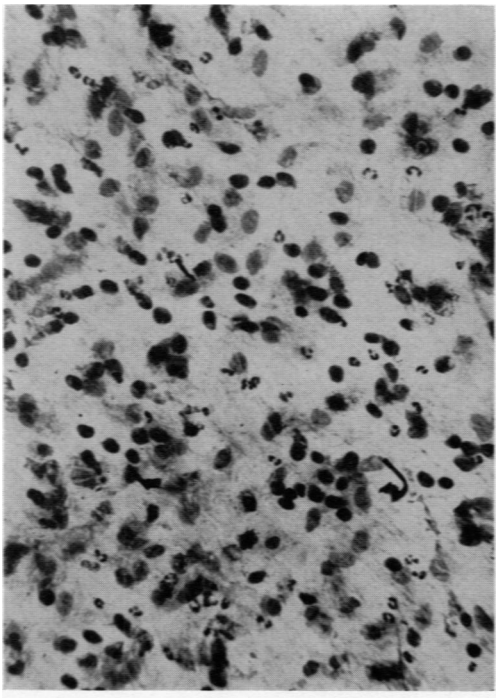

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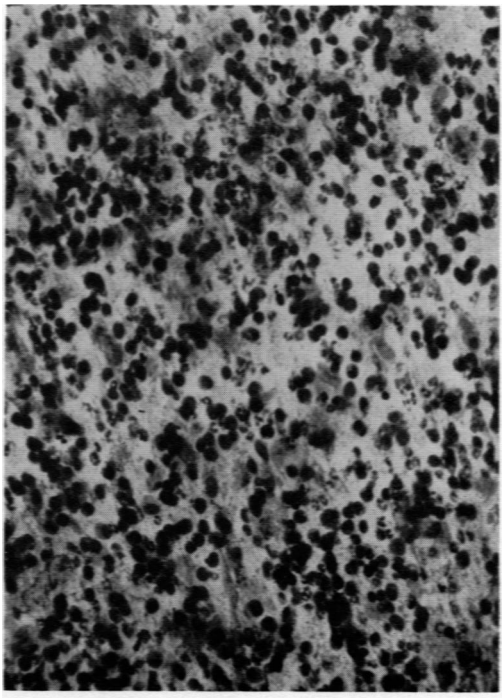

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Biopsy specimens of connective tissue with inflammation induced by egg white in rabbits with neutropenia.

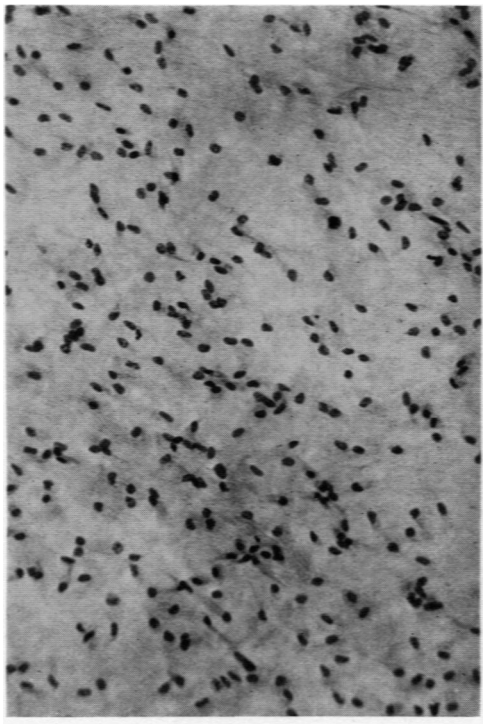
FIG. 9. Normal connective tissue prior to introduction of irritant. Wright's and Giemsa stains. × 80.

FIG. 10. Inflammation at 2 hours, showing mobilization of histogenous macrophages. Note absence of neutrophils. Wright's and Giemsa stains. × 150.

FIG. 11. Inflammation at 8 hours, showing histogenous macrophages. Note absence of both neutrophil and lymphocyte exudation. Wright's and Giemsa stains. × 150.

FIG. 12. Inflammation at 12 hours, showing histogenous macrophages. Note continued absence of neutrophils and lymphocytes from the inflammatory exudate. Wright's and Giemsa stains. × 150.

FIG. 13. Inflammation at 16 hours, showing earliest infiltration by lymphocytes. Neutrophils remain absent. Wright's and Giemsa stains. × 150.



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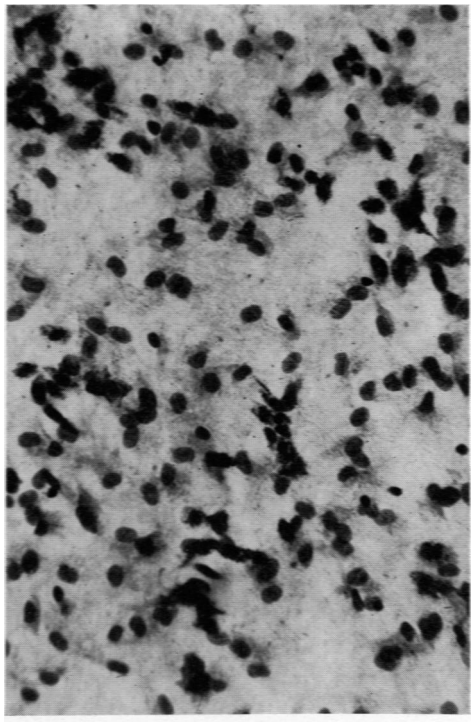

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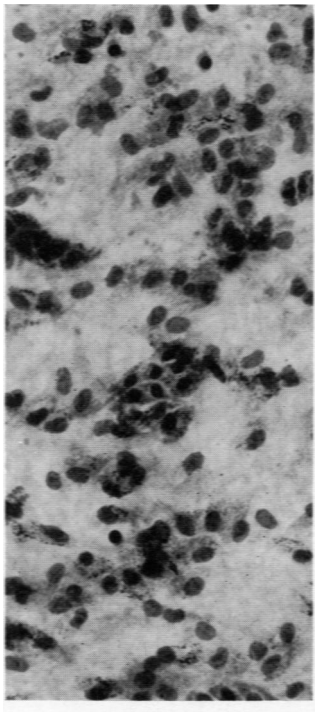

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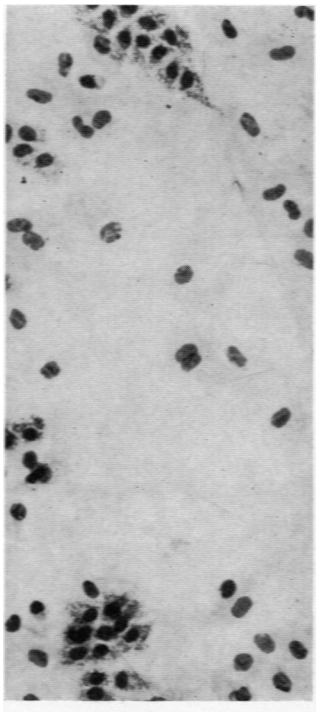

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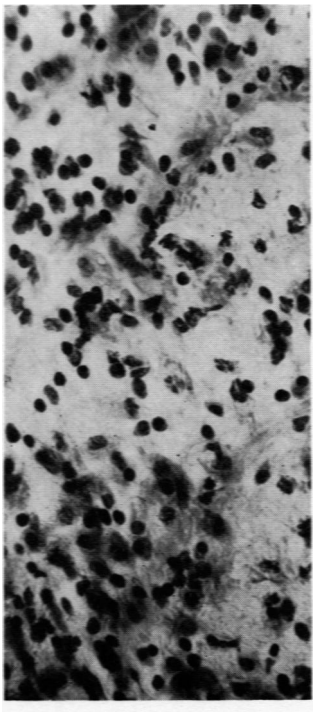


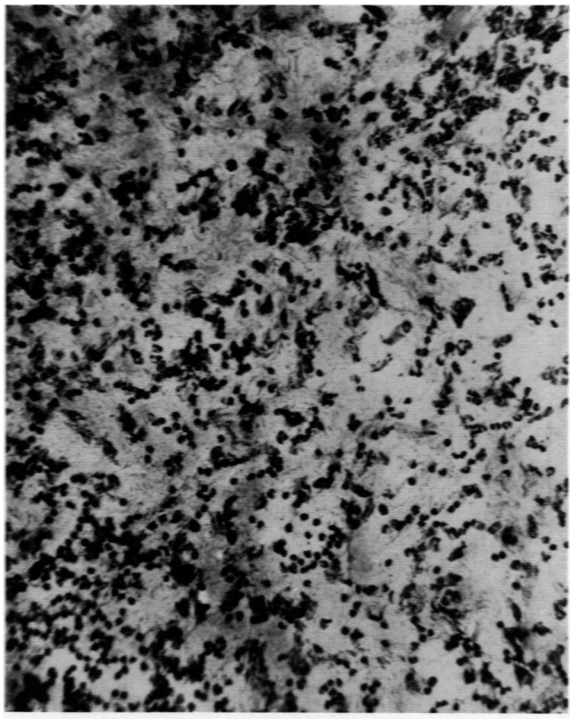
13

Biopsy specimens of connective tissue, demonstrating inflammation induced by homogenated leukocytes in normal rabbits.

FIG. 14. Inflammation at 4 hours, showing neutrophil and lymphocyte infiltration. Wright's and Giemsa stains. × 85.

FIG. 15. Inflammation at 12 hours, showing neutrophils, lymphocytes and hematogenous polyblasts and macrophages. Wright's and Giemsa stains. × 85.

FIG. 16. Inflammation at 16 hours, showing extensive infiltration of neutrophils, lymphocytes and hematogenous polyblasts and macrophages. Wright's and Giemsa stains. × 85.



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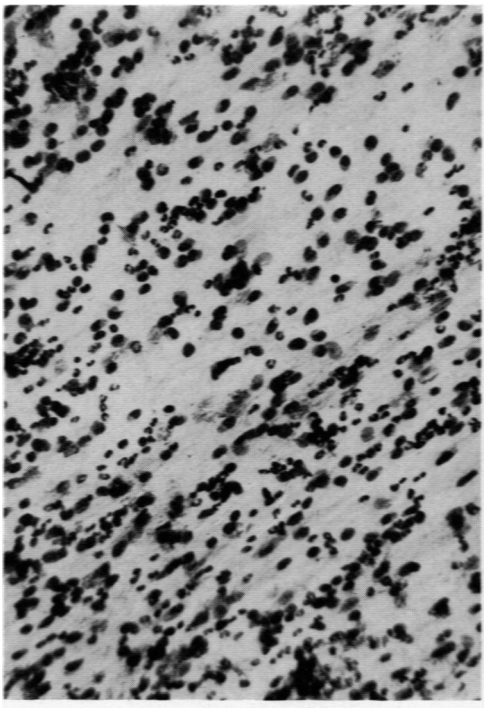

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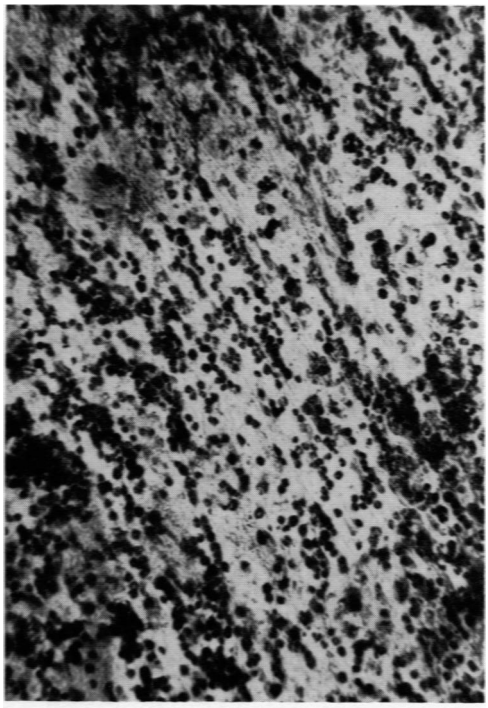

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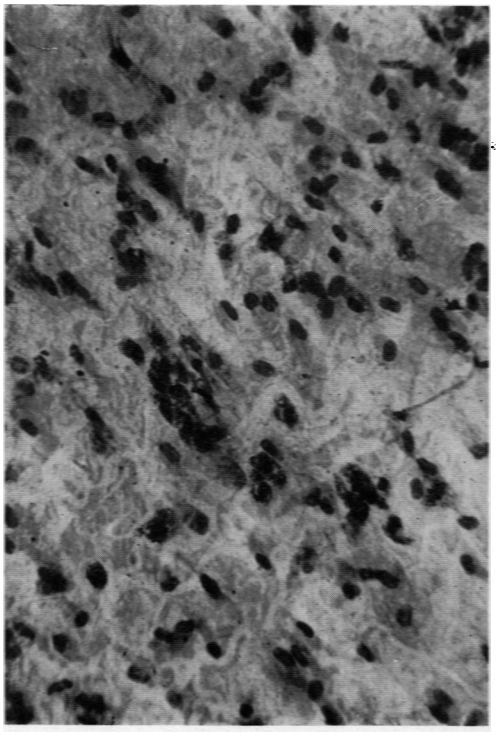
Biopsy specimens of connective tissue, demonstrating inflammation induced by homogenated leukocytes in rabbits with neutropenia.

FIG. 17. Inflammation at 4 hours, showing histogenous macrophages. Note absence of neutrophils and lymphocytes. Wright's and Giemsa stains. × 150.

FIG. 18. Inflammation at 12 hours. Note continued absence of neutrophils and lymphocytes. Wright's and Giemsa stains. × 150.

FIG. 19. Inflammation at 16 hours, showing infiltrate of lymphocytes. Note that neutrophils are still absent. Wright's and Giemsa stains. × 150.

FIG. 20. High power view of cells in Figure 19, showing hematogenous mononuclear cells. Wright's and Giemsa stains. × 800.



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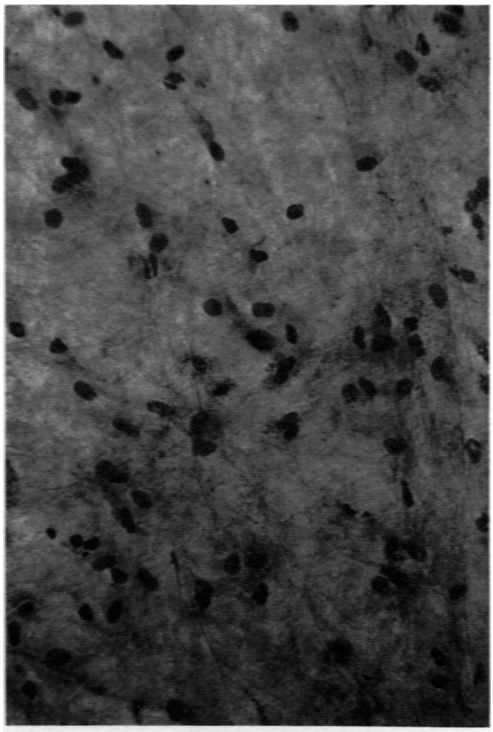

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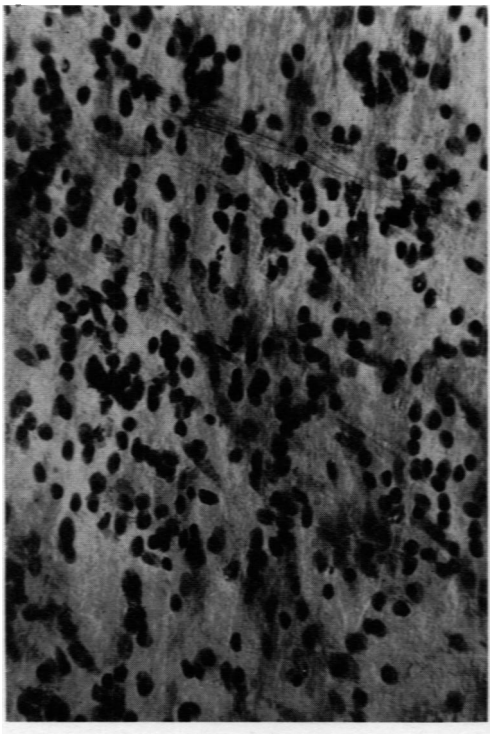

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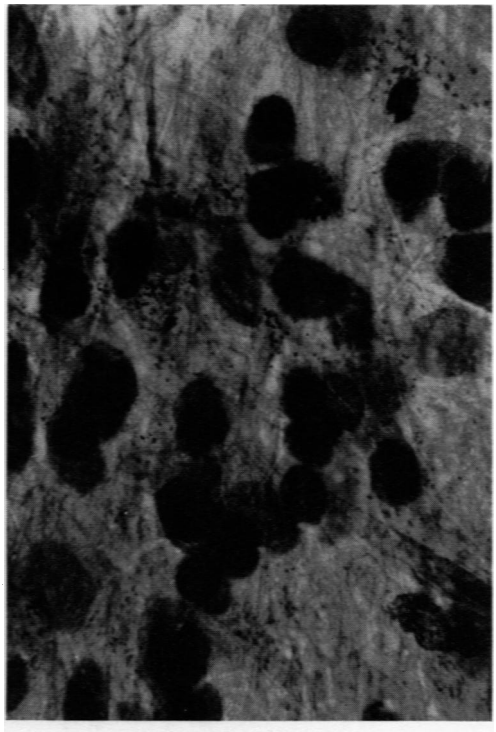

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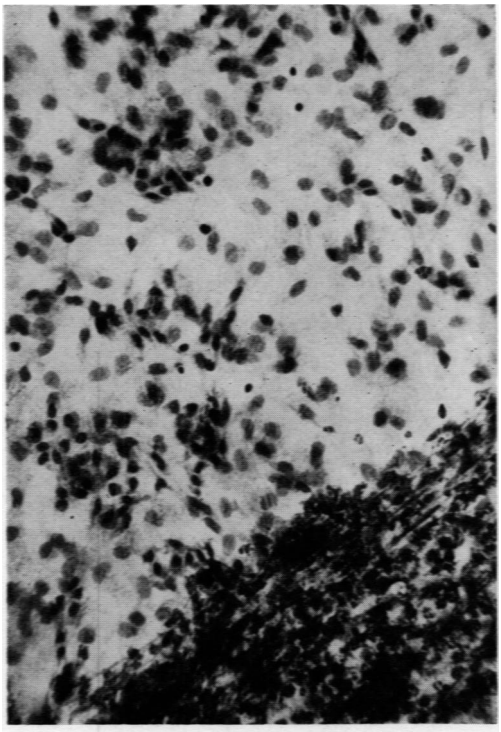
Biopsy specimens of connective tissue, demonstrating inflammation induced by viable leukocytes in rabbit with neutropenia.

FIG. 21. Inflammation at 4 hours, showing histogenous macrophages and a few lymphocytes. Wright's and Giemsa stains. × 150.

FIG. 22. Inflammation at 8 hours, showing intensive lymphocyte infiltration. Note an occasional neutrophil is present. Wright's and Giemsa stains. × 150.

FIG. 23. Inflammation at 12 hours, showing lymphocytes and hematogenous polyblasts and macrophages. Wright's and Giemsa stains. × 150.

FIG. 24. High power view of cells in Figure 23, showing phagocytosis of debris by hematogenous macrophages. Wright's and Giemsa stains. × 800.



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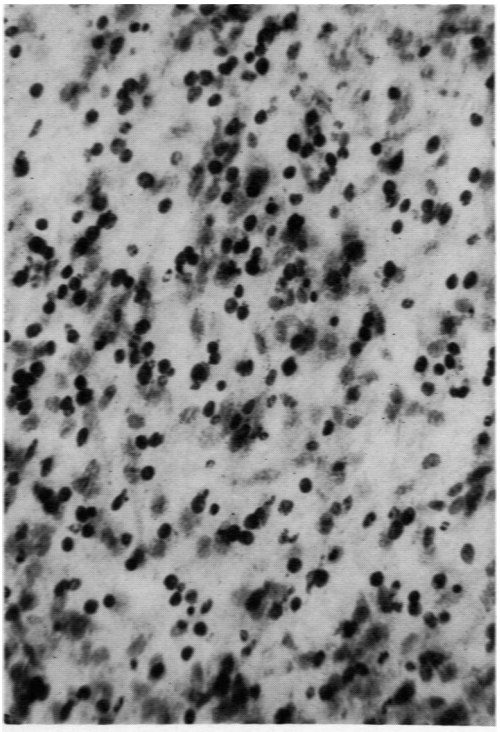

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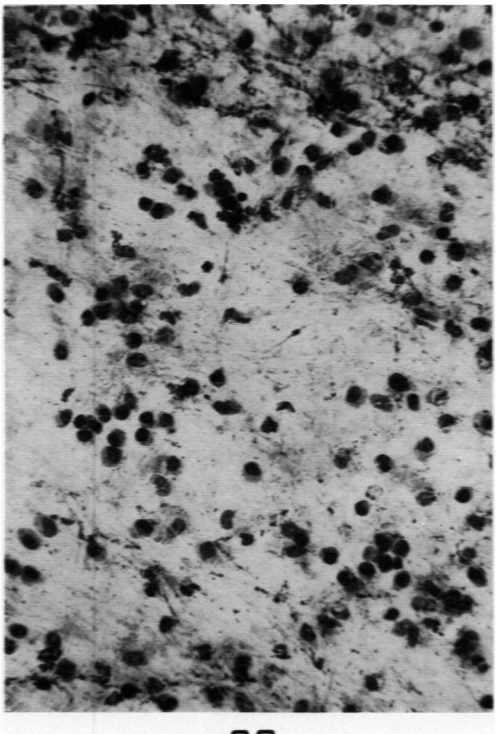

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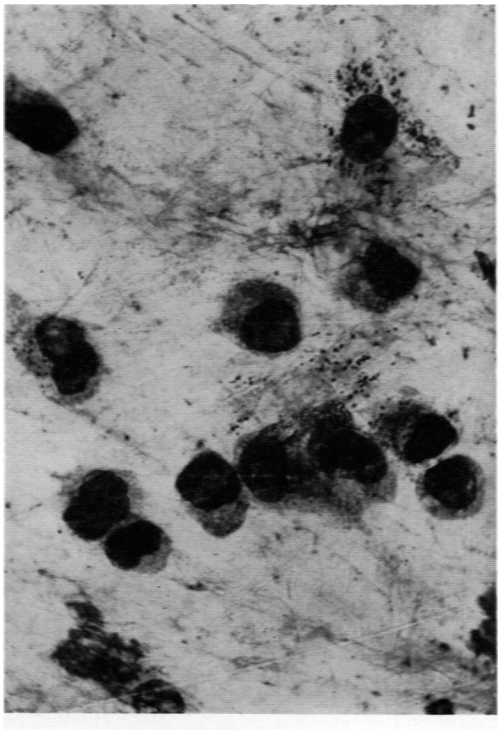

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