THE CYTOLOGY OF THE EQUINE ENCEPHALOMYELITIS VACCINE RESPONSE IN EQUINE SKIN WINDOWS

By

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INTRODUCTION

In 1955 Rebuck and Crowley described a technique which allowed the in vivo serial study of the cytologic changes occurring in a single lesion over a specific time interval. This simple technique is known as the "skin window technique".

The skin window technique has enhanced the study of inflammation and has demonstrated itself as a possible diagnostic aid in disease, for there can occur a variation of cellular exudate in normal individuals, diseased individuals, and when using different antigenic stimulants in experimental skin lesions. This technique has primarily been applied to human experimentation with little work done on the domestic animals.

An advantage of this technique is that it requires only slight, superficial skin abrasions to the experimental animals. Since negligible harm to the animal occurs by this technique, studies of the cellular response in normal and diseased animals can be investigated in species previously considered uneconomical and impractical for experimentation. A careful review of the literature revealed no reports available on skin window investigations in the equine species. The purpose of this study is to observe and record the cellular exudate on skin windows in the equine using inactivated encephalomyelitis virus as the antigenic stimulus.

REVIEW OF THE LITERATURE

In 1955 Rebuck and Crowley described a method of studying leukocytic functions in vivo which allowed the serial study of changes in the cellular exudate in a specific lesion for an extended period of time. This technique is commonly called the skin window technique.

Rebuck and Crowley (1955), using the skin window technique, studied the transformation of lymphocytes into macrophages in man. Functional and structural changes in the lymphocytes of the cellular exudate were observed. The transformation changes included increases in the amount of cytoplasm, in the phagocytic ability for cellular debris and vital dyes, in the division of coarse chromatin masses into fine angular pieces and in the irregularity of the nuclear membrane. Rebuck and Crowley concluded that macrophages of cellular exudate were derived from lymphocytes present in the exudate.

Conversely, Volkman and Gowans (1965a), in skin window studies in cellular exudate in rats, suggested a monocytogenous origin of exudate macrophages. They observed exudate macrophages labeled with tritiated thymidine one day after an intravenous injection of tritiated thymidine, suggesting that macrophages originate from rapidly dividing precursors. Small lymphocytes could not be

macrophage antecedents, since the proportion of labeled small lymphocytes in the blood never rose above 2%. A high labeling of monocytes was observed.

In further experiments, Volkman and Gowans (1965b) applied the skin window technique to rats to identify the tissues in which the precursors of macrophages proliferate. Lymphocyte-depletion by either chronic drainage from the thoracic duct or x-irradiation with 400 rads failed to suppress the emigration of macrophages or to reduce the proportion of them which became labeled after an injection of tritiated thymidine. The emigration and the labeling of the exudate macrophages were suppressed following x-irradiation with 750 rads, but were restored to normal when the tibial marrow was shielded during irradiation. In recipients transfused with radioactive-labeled cell suspensions obtained from thoracic duct lymph, lymph nodes, thymus, spleen and bone marrow, only recipients of labeled bone marrow and spleen cells demonstrated emigration of labeled macrophages onto coverslips. Labeled monocytes were also found in the blood of rats which had received injections of labeled bone marrow.

Trepel and Begemann (1966) studied the origin of skin window macrophages in rats by labeling the leukocytes with H^3-thymidine or India ink. By comparing the characteristic labeling pattern of the peripheral leukocytes with the labeling of the cells of the inflammatory exudate on the skin windows, a transformation of monocytes of the

blood into macrophages was observed. Histochemical studies by Wulff and Sparrevohn (1966) on the origin of mononuclear cells in human skin windows revealed a strong resemblance between skin window mononuclear cells and blood monocytes with regard to sudanophilia, peroxidase activity and esterase activity. A monocytogenous origin of emigrating mononuclear cells was suggested.

By means of inflammatory lesions in skin windows in man, Rebuck et al. (1960) demonstrated the phagocytic properties of lymphocytes, macrophages and monocytes in storing vital dyes (pyrrol blue, lithium carmine, trypan blue and trypan red).

Rebuck et al. (1951) and Rebuck and Mellinger (1953) using adrenalcorticotrophic hormone and cortisone, respectively, under skin windows, observed a modification of the cellular exudate. A depletion of the leukocytic and phagocytic response was reported.

A marked suppression of cellular migration at the inflammatory site in patients with a neutropenia was observed in skin windows by Page and Good (1958). They concluded that the inflammatory exudate is dependent upon, and in part, a function of, the circulating neutrophils.

Rebuck and LoGrippo (1960), in a study of the leukocytic response to poliomyelitis vaccines in human skin windows, demonstrated a poor lymphocyte response in the cellular exudate of non-vaccinated individuals, and a high lymphocyte response in individuals vaccinated with Salk polio vaccine.

Perillie et al. (1960) suggested using the skin window technique as a simple diagnostic aid in systemic lupus erythematosus. Lupus erythematosus cell bodies and rosettes were found on coverslip preparations in patients afflicted with systemic lupus erythematosus.

In studies of the inflammatory exudate produced by diphtheria toxoid under the skin window, Hu et al. (1961) noted an early migration of phagocytic granulocytes which were later supplemented by migrating lymphocytes and blood mononuclears. Application of Rhus oleoresin under the skin windows resulted in no difference in inflammatory response in positive and negative reactors to Rhus prior to the 33-hour stage. After this time, the Rhus-positive individuals demonstrated an eosinophilia, a larger number of lymphocytes, and large multinucleated giant cells.

Wolf-Jurgensen (1962) demonstrated an increased number of eosinophils and especially basophils in the cellular exudate in experimental contact allergy, using 2-4 dinitrochlorobenzene as the sensitizing agent. Priest and Rebuck (1962) observed a high migration of polymorphonuclear basophilic leukocytes in patients with acute ulcerative colitis (3 weeks) and in patients with chronic disease (over 20 years).

Hu et al. (1963), utilizing the skin window technique in patients with furunculosis and "open" skin lesions, reported a high incidence of basophilic and eosinophilic response. The response was elicited only when suspensions of the whole staphylococci, live or killed,

were used as the inoculum under skin windows. When a purified polysaccharide antigen prepared from the same staphylococci strain was applied to the skin lesions, a basophilic and eosinophilic response did not occur. The basophilic and eosinophilic response possibly represents a hypersensitivity reaction to the staphylococci organisms.

A skin window technique was employed by Wisseman and Tabor (1964) to study the early cellular response of man to typhus rickettsiae. In the first 24 hours the reactions observed were essentially those of an acute inflammatory process. In non-immune subjects, polymorphonuclear leukocytes appeared in 1.5 to 3 hours, increased rapidly in numbers and persisted as the dominant cell type during the 24 hour period. Mononuclear cells appeared in increasing numbers by 6 hours and underwent development into large mononuclear cells and macrophages. The inflammatory process was intensified in immune subjects.

Harman (1965), using skin windows in normal control patients, also showed an initial, almost exclusively neutrophil migration which slowly gave way to an influx of mononuclear cells. In normal skin of psoriatics, the same qualitative pattern of migration occurred but at a faster rate. Mononuclear cells predominated 9 hours after trauma instead of 24 hours as in the normal control patients.

According to Drees (1966), Eidinger in 1960 observed that the cellular exudate in patients allergic to fish extract, grasses or ragweed was composed of over 50% eosinophils. Eidinger et al. (1964), in a study of cellular responses utilizing the skin window

technique, demonstrated a tissue eosinophilia following application of an allergen in the skin window. The eosinophilia was greatest when an allergen was used that was clinically responsible for the patient's allergic symptoms. In a similar study by Weiss (1967) the number of eosinophils in the cellular exudate increased in a degree proportional to the individual's sensitivity to the allergen. The cytoplasm and nuclei of the eosinophils contained many vacuoles, and numerous degranulated eosinophils were present. Jurgensen and Zachariae (1965) observed an emigration of eosinophils following injections of polymyxin and histamine.

Riddle and Barnhart (1965) and Drees (1966), using the skin window technique, studied the cellular response in the dog and concluded that it was very similar to the cellular response in man. The stage of peak cellular migration occurred at 10 hours as opposed to 12 hours in the human species. Lymphocytes, hypertrophied lymphocytes and macrophages appeared in increasing numbers from about 6 to 14 hours. Small numbers of eosinophils were observed between 3 and 7 hours and seldom comprised more than 5% of the cellular exudate. The dog was capable of mobilizing its greatest cellular defense mechanism to an irritant more rapidly than man. In the same study Riddle and Barnhart suggested that specific granules in the eosinophil may function as a source for profibrinolysin in acute inflammation. Eosinophils were attracted into the skin window area of inflammation by fibrinogen or fibrin.

Wilkinson et al. (1966), using the skin window technique, made a comparison of the cellular exudate between 10 rheumatoid arthritic patients and 6 normal patients. A simple irritant type of response was observed in each case. No significant differences between arthritic and control subjects were observed.

In a skin window study in patients with chronic uremia exhibiting delayed homograft rejection, Lang et al. (1966) observed a lower percent of lymphocytes and macrophages at 8 and 24 hours in uremic patients. The lymphocytes and macrophages showed morphologic evidence of necrobiosis. Lang and coworkers feel that the reduced number may reflect an increased rate of destruction, and/or a reduced production; and, that this phenomena may be a factor in the delayed homograft rejection in uremic patients.

MATERIALS AND METHODS

The skin window technique of Rebuck and Crowley (1955) and Drees (1966) was used in this investigation. Modifications were made by the author to adapt the technique to the horse. Five horses from the Michigan State University Veterinary Clinic were used in this study. The animals consisted of 1 Quarter Horse, 2 Standardbreds, and 2 Thoroughbreds, all geldings. Age ranged from 5 to 10 years, and weight from 800 to 1200 pounds. A total white blood cell count, differential count, and hemoglobin and hematocrit determinations were made on each animal to insure use of hematologically normal subjects.

The site of this test was an 8 by 12 inch area at the region of the transverse processes of the lumbar vertebra on the dorsum of each horse. Only one side of each horse was used. Hair was removed from the horse's back with an electric animal clipper. In several cases it was necessary to use a rope twitch to prevent the horse from becoming too excited. The clipped area and surrounding area were cleaned with warm water and germicidal soap. Excess water was removed with sterile gauze.

The experimental 3 by 5 mm lesions were created by scraping the epidermis with a sterile No. 22 Bard-Parker blade. The depth

of each lesion was considered adequate when the papillary layer of the corium was reached. Evidence of the correct depth was indicated when fine bleeding points were observed in the lesion, and when a small amount of blood accumulated on the cutting edge of the scalpel blade.

Six experimental lesions were created on the back of each horse in two rows of three each. A total of 30 lesions were prepared. The trauma of the scalpel blade creating the lesion served as an inflammatory stimulant, so the experiment was timed from this point.

One drop of an antigen, Encephalomyelitis Vaccine-Eastern and Western Avian Tissue Culture Origin[1], from a sterile syringe was placed on the surface of the lesion to act as an additional inflammatory stimulant and enhance leukocytic participation. This antigen was applied to 21 lesions. No antigen was applied to 9 of the lesions, so that a comparison could be made between the antigenic stimulated and the nonantigenic stimulated cellular exudates.

The phagocytic properties of the cellular exudate were studied by applying one drop of Pelikan[2] ink from a sterile syringe to the surface of 6 of the experimental lesions, previously inoculated seconds earlier with the inflammatory stimulant.

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[1]Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

[2]John Henschel and Co., Inc., 425 Park Avenue, South New York 16, New York.

After each lesion was prepared, it was immediately covered with a sterile No. 2 - 15 mm square glass coverslip. The coverslips had previously been attached to a 20 mm cardboard square by means of a 2 by 8 mm strip of masking tape folded upon itself. Half of the masking tape was attached to the coverslip and half to the cardboard square. The cardboard facilitated handling of the coverslip, and reduced chance of contamination of the coverslip and the lesion (Drees, 1966). The coverslip-cardboard unit was wrapped in aluminum foil and placed in a metal container. Four hundred units were prepared in this manner and sterilized with dry heat. Each coverslip-cardboard unit could then be unwrapped from the aluminum foil and used as needed, continually maintaining sterile technique.

After application of the coverslip-cardboard unit to the lesion, it was covered by a 1 by 3 inch strip of adhesive tape to maintain correct position and contact with the lesion.

To reduce chance of contamination of the lesions, the tail of each horse was wrapped with gauze, and the horse was tied with a rope halter to the front of the stall to maintain a standing position.

The cells of the inflammatory exudate in the lesion migrate to the undersurface of the coverslips. Every 2 hours for 16 hours the coverslips were removed from the lesions, air dried, and mounted, cell-side-up, on microscope slides with a drop of Permount[1]. Upon removal of the coverslip from the lesion, it was immediately replaced

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[1]Fisher Scientific Co., Fair Lawn, New Jersey.

with another sterile coverslip. A serial study could then be made of the cytologic content of each lesion at short intervals throughout the first 16 hours of acute inflammation. Exposure of the lesion to the air for any length of time will allow the cellular exudate to dry, and will prevent continuation of that lesion.

An individual serial study consisted of 8 slides covering 16 hours. Thirty such serial studies were performed. The cells on the coverslips mounted on microscope slides were stained like blood smears. Ten series were stained using the May-Grunwald-Giemsa method (Pappenheim, 1912); 10 series were stained using Wright's-Leishman stain (Coles, 1967), modified by increasing the fixation time to 3 minutes and the staining time to 8 minutes; 6 series were stained for peroxidase according to Sato and Sekiya (Darmady and Davenport, 1958); and, 4 series were stained using Sudan black B according to Bayliff and Kimbrough (Darmady and Davenport, 1958). The photomicrographs (Figures 1-28) illustrate the cells which have migrated to the undersurfaces of the coverslips.

RESULTS AND DISCUSSION

Since encephalomyelitis vaccine antigen was applied to some skin lesions and not to others, a comparison could be made between the antigenic stimulated and the nonantigenic stimulated cellular exudates. This comparison revealed an essentially identical qualitative cellular response between those lesions containing the vaccine antigen and those lesions containing no vaccine antigen. However, a more intense quantitative response was observed in the vaccine stimulated lesion. Due to the similarity of the response between the two types of lesions, and since the majority of the skin lesions contained the vaccine antigen, the following description will be concerned with the cytology of the encephalomyelitis vaccine reaction in the equine skin windows.

Since there still exists a controversy in the literature concerning a lymphocytogenous or monocytogenous origin of the mononuclear cells in skin window preparations (Rebuck and Crowley, 1955, Volkman and Gowans, 1965b, Drees, 1966, Trepel and Begemann, 1966, Wulff and Sparrevohn, 1966), all such cells in this discussion will be referred to as mononuclear cells. Most of their work would seem to indicate a monocytogenous origin of mononuclear cells. Wulff and Sparrevohn (1966) indicated that lymphocytes were devoid of peroxidase and sudanophila material, whereas, most blood

monocytes contain these substances. In view of the work of Wulff And Sparrevohn (1966), several serial studies were performed on the horse utilizing a peroxidase stain and a Sudan black B stain. The peroxidase stain revealed peroxidase activity and the Sudan black B stained numerous sudanophilic granules in all mononuclear cells from the 2-hour stage to the 16-hour stage of the experiment (Figures 26-28). Since Wulff and Sparrevohn (1966) demonstrated that blood monocytes and not blood lymphocytes showed these staining characteristics, this histochemical study would seem to indicate that the mononuclear cells in skin windows are emigrated monocytes.

The first coverslip from each series of preparations was somewhat difficult to interpret. As a result of the initial traumatization of the skin and application of additives, serum, dye and antigen accumulated on the coverslip. Subsequent coverslips improved in quality and detail.

Two-Hour Stage

Neutrophilic leukocytes were the predominant cell type in the exudate, accounting for approximately 95% of the total cells (Figure 1). The nuclear lobes appeared swollen and rounded, giving a generalized edematous appearance to the entire neutrophil. The neutrophils measured 12-14 microns in diameter. The cytoplasm was usually colorless, but occasionally stained a pale blue.

Several mononuclear cells were observed at this 2-hour

stage, but were an infrequent finding. Cytoplasm was sparse in these mononuclear cells, with the dense chromatin nucleus almost filling the entire cell (Figure 2). These cells measured 10-11 microns in diameter.

Four-Hour Stage

At four hours, the primary cell present was still the neutrophilic leukocyte (Figure 3). The neutrophils measured 12-14 microns, and closely resembled the 2-hour neutrophil. The nuclear lobes still appeared swollen, often causing the cell membrane to be stretched (Figure 4).

The mononuclear cells made up only a very small percentage of the cellular exudate and resembled the 2-hour mononuclear cell.

Fragments of leukocytic cytoplasm were frequently observed dispersed throughout the cellular exudate, a probable result of degenerating and injured cells (Figure 5). These fragments varied in size from as small as 2 microns in diameter to as large as 10 microns in diameter.

Six-Hour Stage

At 6 hours neutrophils still dominated the cellular exudate (Figure 6), but many of the neutrophils had lost their cytoplasm, leaving only their nuclei floating in the exudate. The first indication of phagocytic activity was observed in this 6-hour stage as small accumulations of Pelikan ink were occasionally present in the cytoplasm of intact neutrophils (Figure 7).

Mononuclear cells (12-14 microns) had increased in number in the total cellular exudate. The nucleus was frequently irregular, round or oval in shape. The chromatin was deeply stained and coarse. Cytoplasm was very abundant and stained a pale blue with few vacuoles being present. The cellular outline was elongated and oval, often irregular in shape (Figure 8).

An occasional eosinophilic leukocyte was observed in areas associated with fibrin. This agrees with the findings of Riddle and Barnhart (1965) and Drees (1966). The cytoplasmic granules were few in number, and smaller than the peripheral blood eosinophil (Figure 9).

Eight-Hour Stage

At the 8-hour stage the percentage of neutrophils in the cellular exudate had decreased (Figure 10). The majority of these neutrophils had also decreased in size, now measuring 11-12 microns in diameter. Many of the nuclear lobes were clumped and pyknotic. The chromatin masses stained darker than normal. Cytoplasm was sparse (Figure 11).

The mononuclear cells in this stage were larger measuring 14-20 microns. The nucleus was usually indented and contained large clumps of chromatin (Figure 12). Cytoplasm was distinct and stained basophilic. In a few cells vacuoles were present in the cytoplasm.

A few consolidated eosinophil granules were observed in areas

associated with fibrin. These granules measured 1-3 microns in diameter, and appeared as free entities in the cellular exudate.

Ten-Hour Stage

The morphology of the neutrophils of the 10-hour stage was similar to the neutrophils of the 8-hour stage, except that the percentage of neutrophils in the cellular exudate had decreased. The small size of the degenerating neutrophils persisted, measuring 7-9 microns in their greatest diameter. The size of the intact neutrophils was somewhat larger, averaging 12 microns. A few neutrophils contained cytoplasmic vacuoles. Phagocytic activity of the neutrophil continued in this stage as evidenced by the presence of Pelikan ink within the neutrophilic cytoplasm (Figure 13).

An increasing number of the exudative cells were mononuclear cells (16 by 22 microns), and were somewhat larger than those of the earlier stages. The number of mononuclear cells possessing cytoplasmic vacuoles appeared to increase in this stage (Figure 14). The cytoplasm of these mononuclear cells also contained phagocytized Pelikan ink (Figure 15).

Several eosinophils with intact cellular membranes and small granules were observed in this stage. These cells average 12 microns.

Twelve-Hour Stage

At 12 hours, the majority of the neutrophilic leukocytes (Figure 17) were shrunken with clumped chromatin and pyknotic nuclei. Cytoplasm was very sparse, and frequently nonexistent (Figure 18).

These degenerating neutrophils averaged 7 microns in diameter. The remaining neutrophils were large (12 microns) and edematous. The nucleus was often quite well deliniated. Filaments connecting nuclear lobes could occasionally be observed (Figure 19). The cytoplasm of these cells was abundant, and sometimes vacuolated.

The mononuclear cells were readily apparent at this 12-hour stage (Figure 17). The majority of these mononuclear cells were fixed in ameboid motion, as characterized by their bizarre shapes (Figure 20). Consequently, the length of these cells was much greater than the width, the average dimensions being 12 by 26 microns. The cellular outline was very irregular. The nucleus was large, accounting for half to two-thirds of the total cell. The nuclear outline was irregular, but smooth. Cytoplasm was abundant, and usually well vacuolated.

The eosinophilic leukocyte response diminished to a point where only a few granules could be located in the cellular exudate.

The peak cellular response for the total 16-hour experiment occurred in both the lesions of the 12-hour stage and the 14-hour stage. This timed response in the equine species is similar to the reports of Rebuck and LoGrippo (1960) for the human species (12-hour stage), but 2 to 4 hours later than Drees (1966) reported for the dog (10-hour stage).

Fourteen-Hour Stage

At 14 hours, the percentage of neutrophilic leukocytes in the

cellular exudate had decreased (Figure 21). The majority of these neutrophils were shrunken with clumped and pyknotic nuclei. Most of the cytoplasm was missing. These cells measured 7-9 microns in diameter. Structurally intact neutrophilic leukocytes, 12-14 microns in diameter (Figure 22), were also present in moderate numbers. The nuclear chromatin was heavily plaqued which caused the nuclear membrane to assume a jagged appearance, giving a multilobulation impression. Filaments were occasionally observed. Cytoplasm was quite abundant in these cells and contained evenly dispersed, fine granules. Occasionally, small, clear intracytoplasmic vacuoles were observed.

The mononuclear cells had increased in number, and they were now a predominant cell type in the exudate. Cell size measured 14 by 26 microns. Cell characteristics closely resembled the 12-hour mononuclear cells.

Sixteen-Hour Stage

The cell morphology and characteristics of the neutrophilic leukocytes in the 16-hour stage (Figure 23) were consistent with the degenerating and structurally intact neutrophils of the 14-hour neutrophil. A slight degree of phagocytosis of Pelikan ink occurred in this stage, but its importance appeared negligible.

The mononuclear cells in the skin windows had increased in number (Figure 23). Cell size averaged 22 by 27 microns. As in the preceding several stages, the cellular outline assumed an irregular

shape. The shape of the nucleus was also very irregular. Generally, one side of the nucleus was indented to various degrees. Any similarity or conformity between the nuclei of different mononuclear cells was a rare occurrence. The basophilic cytoplasm contained numerous vacuoles and azurophilic granules (Figure 24). A marked degree of phagocytosis of cellular debris and Pelikan ink had occurred (Figure 25).

This experiment was concluded at the 16-hour stage.

SUMMARY AND CONCLUSIONS

The cytology and emigration sequence of inflammation produced by a combined Eastern and Western Encephalomyelitis Vaccine was studied in the equine species using a skin window procedure over a period of 16 hours. A vital dye was used to study phagocytosis; and, a histochemical study was employed to examine the origin of emigrating mononuclear cells.

Neutrophilic leukocytes were the first cells to migrate into the skin lesions and onto the coverslips. These cells were the primary constituent of the cellular exudate for the entire 16 hours after inoculation of the vaccine stimulant. The neutrophils were able to phagocytize the vital dye. After functioning in phagocytosis, the neutrophils underwent degeneration with nuclear clumping and a loss of cytoplasmic fragments to the fluid exudate with subsequent decrease in cell size. Emigration of new neutrophils persisted, in decreasing amounts, throughout the 16 hours of the experiment.

A slow influx of mononuclear cells occurred early in the experiment. After 6 hours, the number of mononuclear cells increased in rate of emigration becoming a predominant cell type in the exudate at 12 to 14 hours. It appeared that the mononuclear cells were still increasing in number, size and phagocytic activity at the

termination of the experiment at the 16-hour stage.

The results of the histochemical study revealed peroxidase activity and sudanophilia in the mononuclear cells. According to Wulff and Sparrevohn (1966), only blood monocytes show these staining characteristics. Therefore, a monocytogenous origin of emigrating mononuclear cells is probable.

The cellular exudate contained only about 1% eosinophilic leukocytes. Very few of these cells were observed with intact cellular membranes. Most of the eosinophilic granules were free in the fluid exudate.

The control lesions with no vaccine stimulation revealed a similar qualitative cellular response, but a diminished quantitative cellular response at each stage in the experiment.

The peak cellular response in the equine species occurred at 12 to 14 hours when the greatest number of cells appeared in the exudate. This response coincides closely with that reported for man (Rebuck and LoGrippo, 1960), but is 2 to 4 hours later than the peak cellular response in the dog (Drees, 1966).

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FIGURES

FIGURE 1

Two-hour stage. Note predominance of neutrophilic leukocytes. X 1126.

FIGURE 2

Two-hour stage. Five neutrophils and one small mononuclear cell. X 2816.

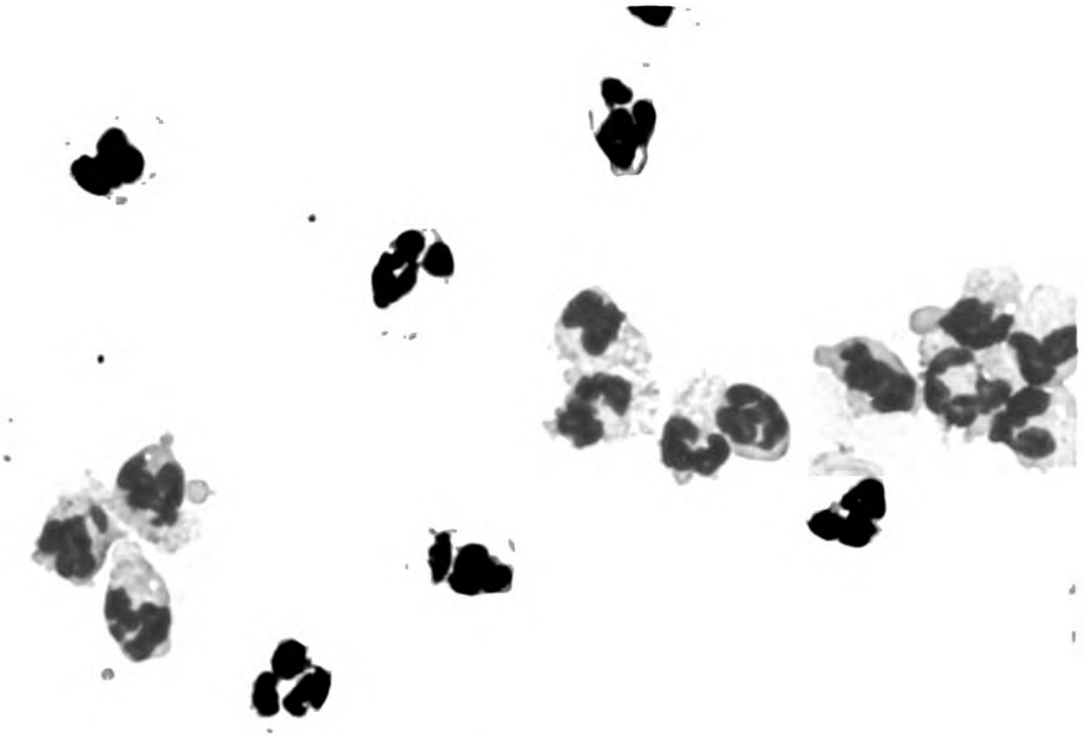

FIGURE 1

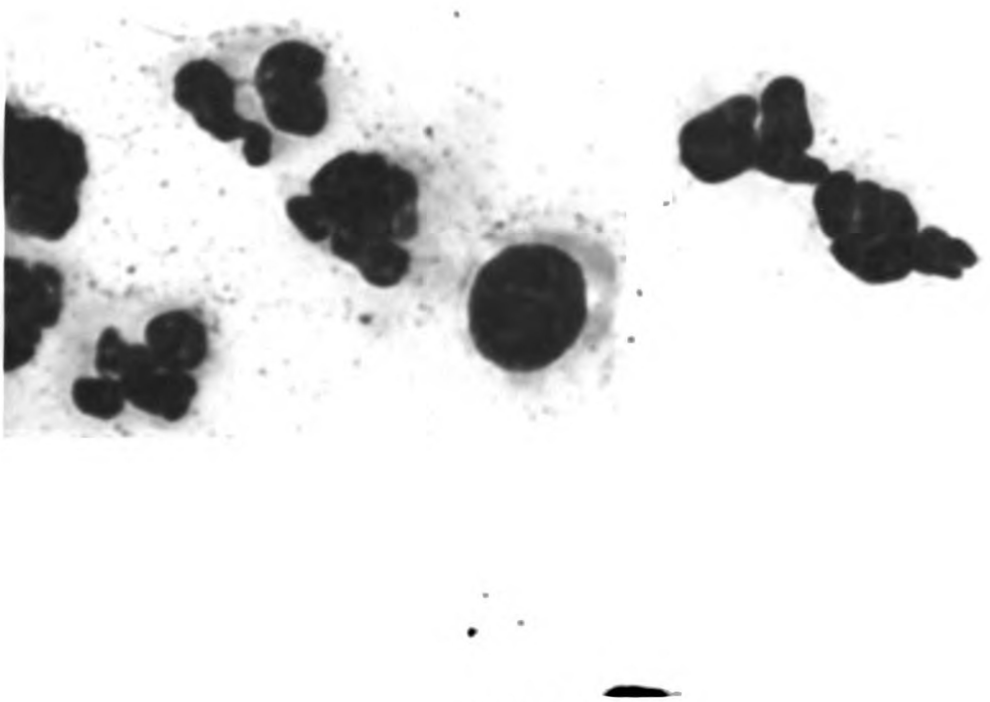

FIGURE 2

FIGURE 3

Four-hour stage. Cell population primarily neutrophils. X 1126.

FIGURE 4

Four-hour stage. A swollen neutrophil. X 2816.

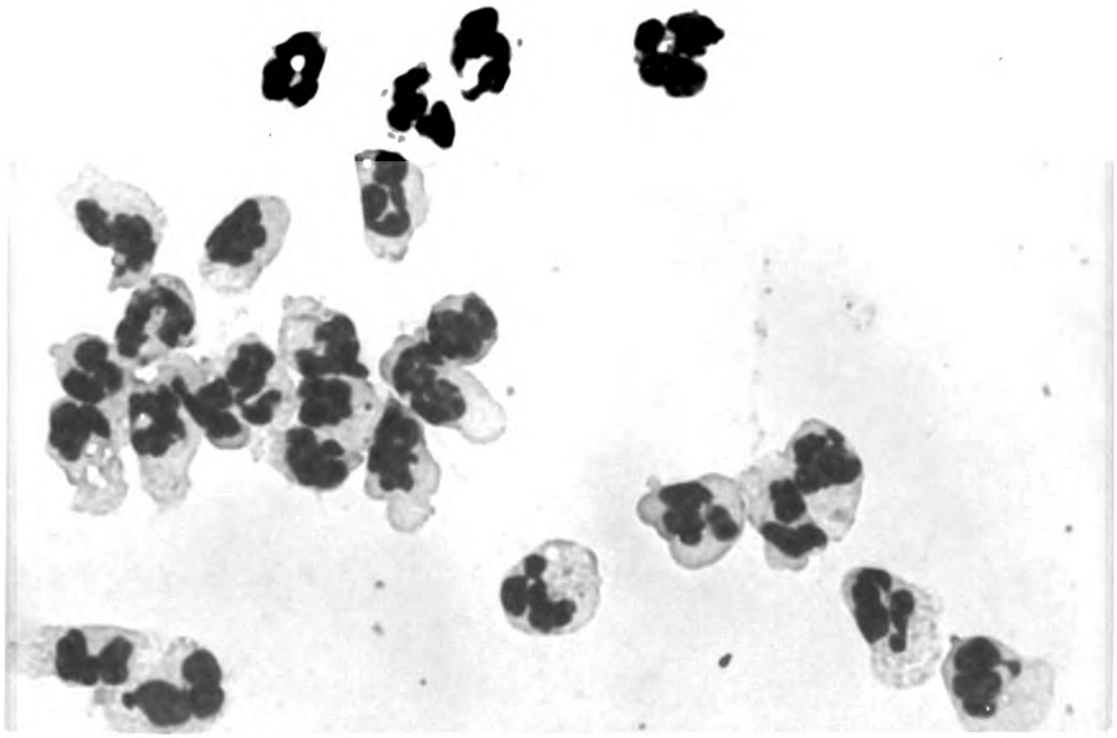

FIGURE 3

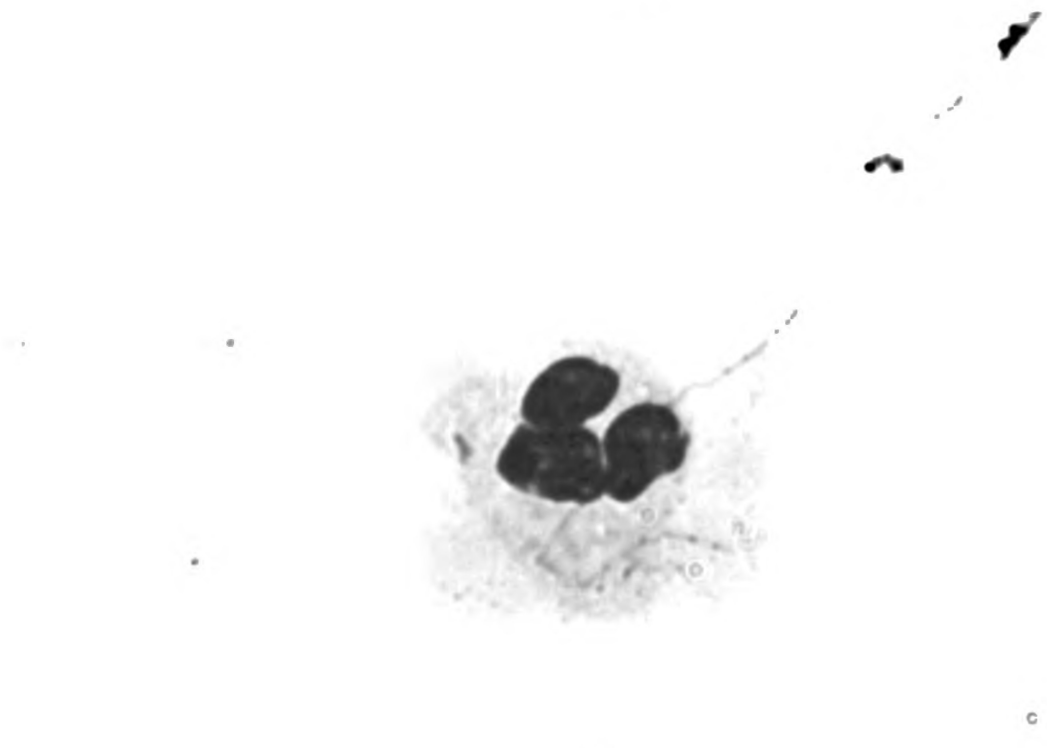

FIGURE 4

FIGURE 5

Four-hour stage. Note cytoplasmic fragment adjacent to degenerating neutrophil. This cell was identified as a neutrophil because of the small amount of cytoplasm and the nuclear lobulation which was obvious on the coverslip, but difficult to photograph because of limited depth of focus. X 1760.

FIGURE 6

Six-hour stage. Predominance of neutrophils. X 704.

FIGURE 5

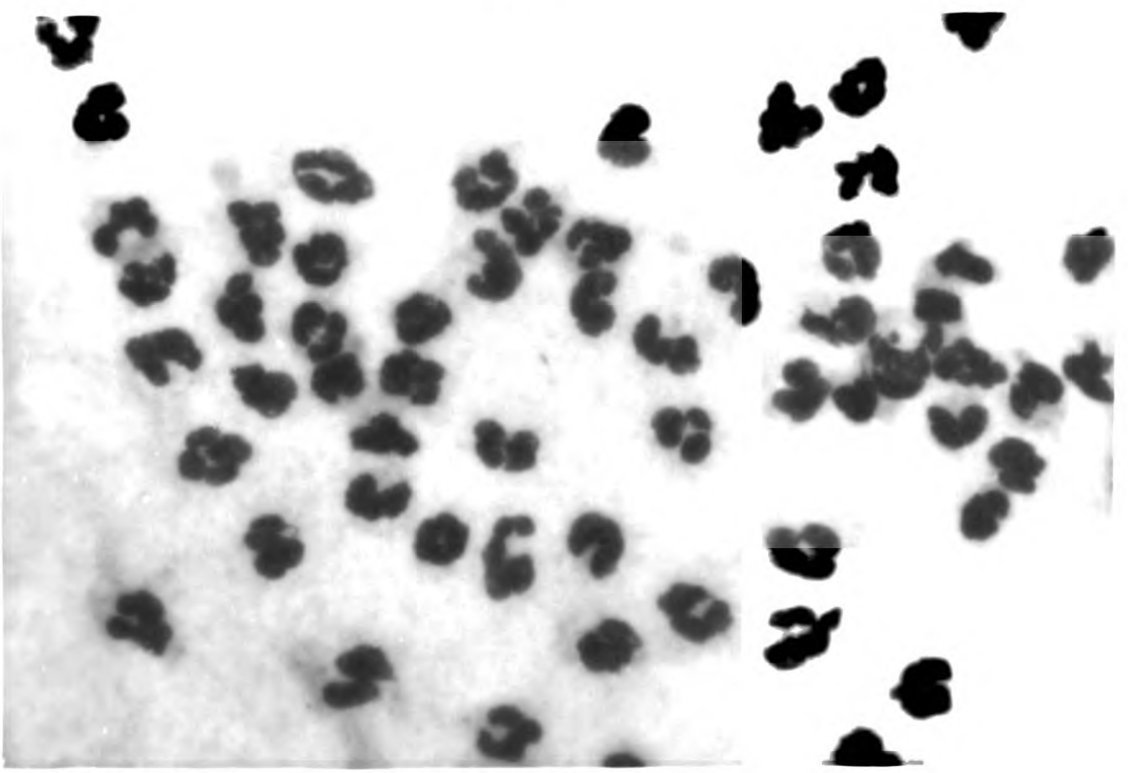

FIGURE 6

FIGURE 7

Six-hour stage. Three neutrophils surrounding one neutrophil containing phagocytized Pelikan ink in cytoplasm. X 2816.

FIGURE 8

Six-hour stage. Small mononuclear cells. X 2816.

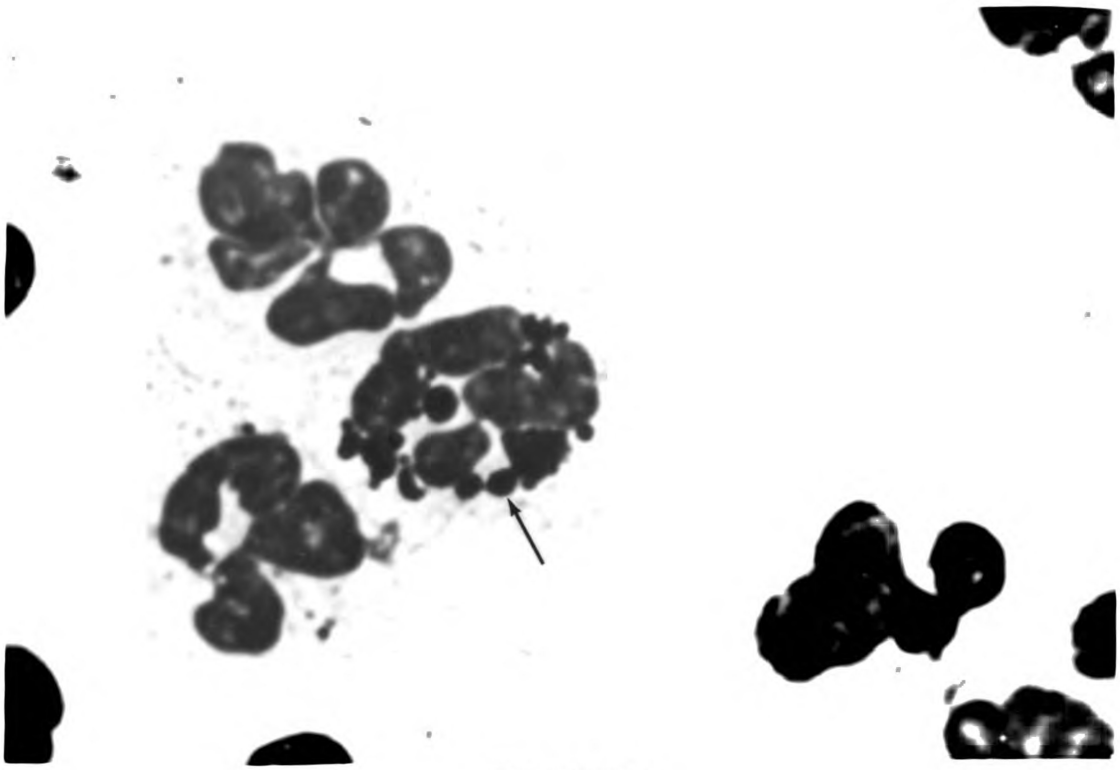

FIGURE 7

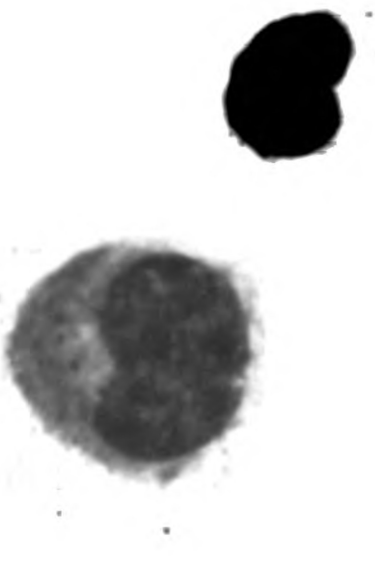

FIGURE 8

FIGURE 9

Six-hour stage. Eosinophil with small granules. Note fusion of several granules into larger granules. X 2816.

FIGURE 10

Eight-hour stage. Cell population showing two-thirds neutrophils and one-third mononuclear cells. X 704.

FIGURE 9

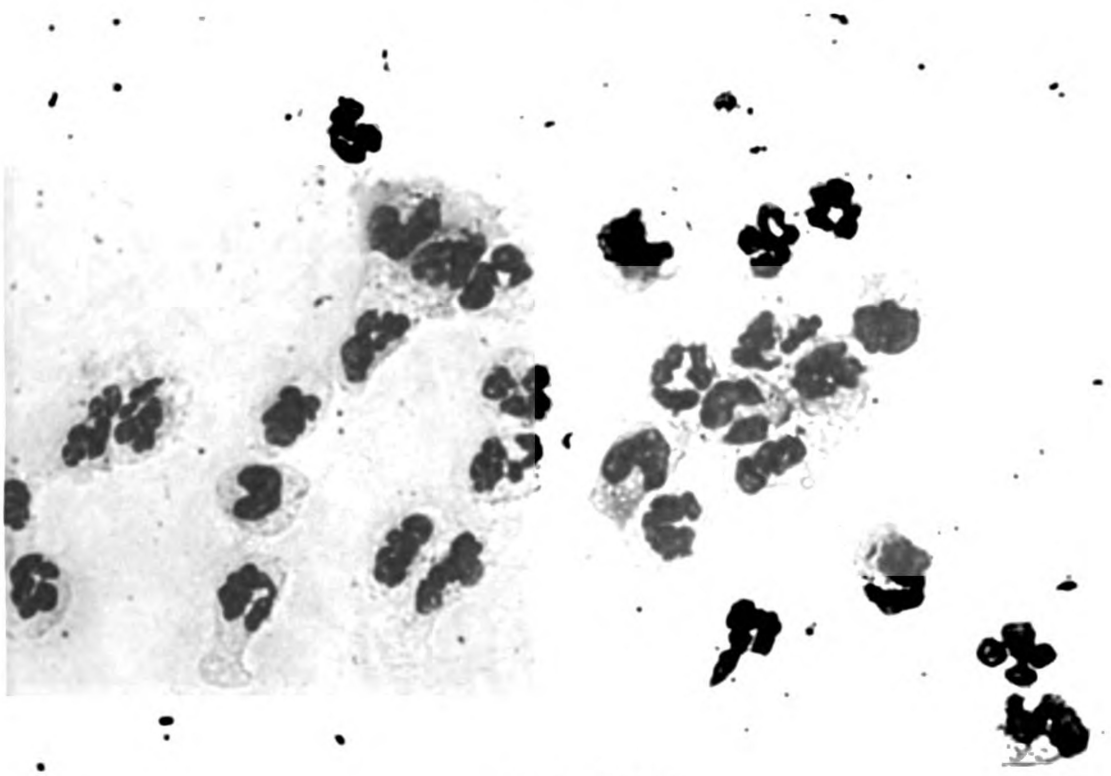

FIGURE 10

FIGURE 11

Eight-hour stage. Two degenerating neutrophils. Note sparsity of cytoplasm. X 2816.

FIGURE 12

Eight-hour stage. Mononuclear cell. Observe indented nucleus and chromatin clumps. X 2816.

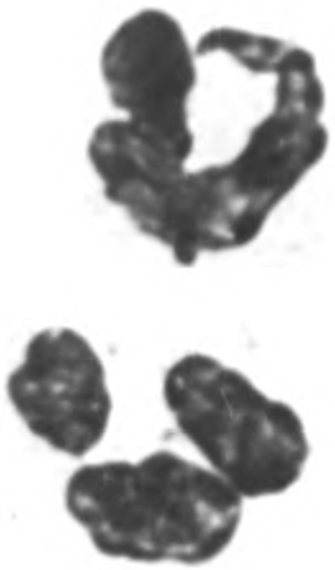

FIGURE 11

FIGURE 12

FIGURE 13

Ten-hour stage. Large neutrophils, several containing phagocytized Pelikan ink in cytoplasm. X 2816.

FIGURE 14

Ten-hour stage. Mononuclear cell with large vacuole. X 2816.

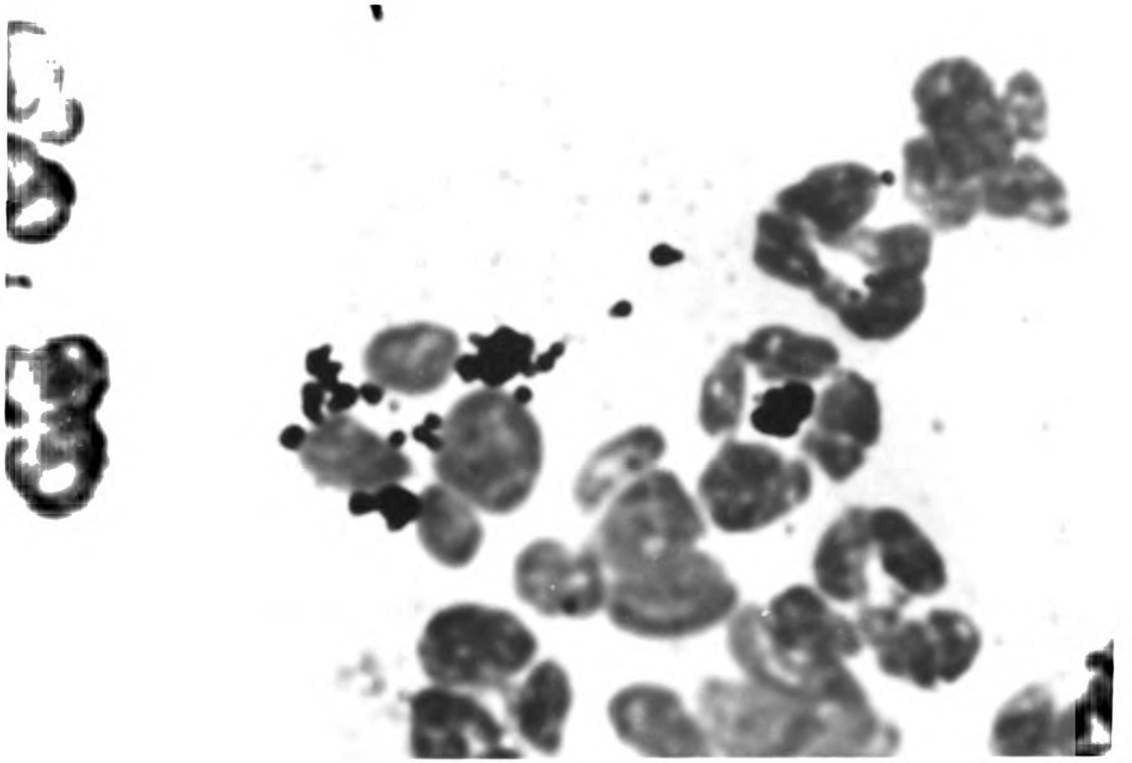

FIGURE 13

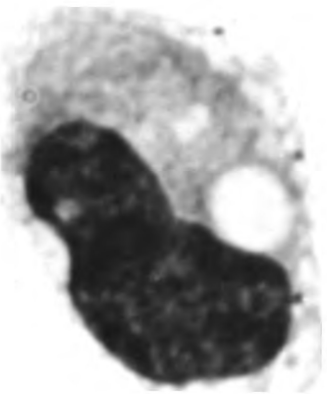

FIGURE 14

FIGURE 15

Ten-hour stage. Mononuclear cell containing phagocytized Pelikan ink in cytoplasm. X 2816.

FIGURE 16

Ten-hour stage. One eosinophil and two mononuclear cells surrounded by neutrophils. X 1760.

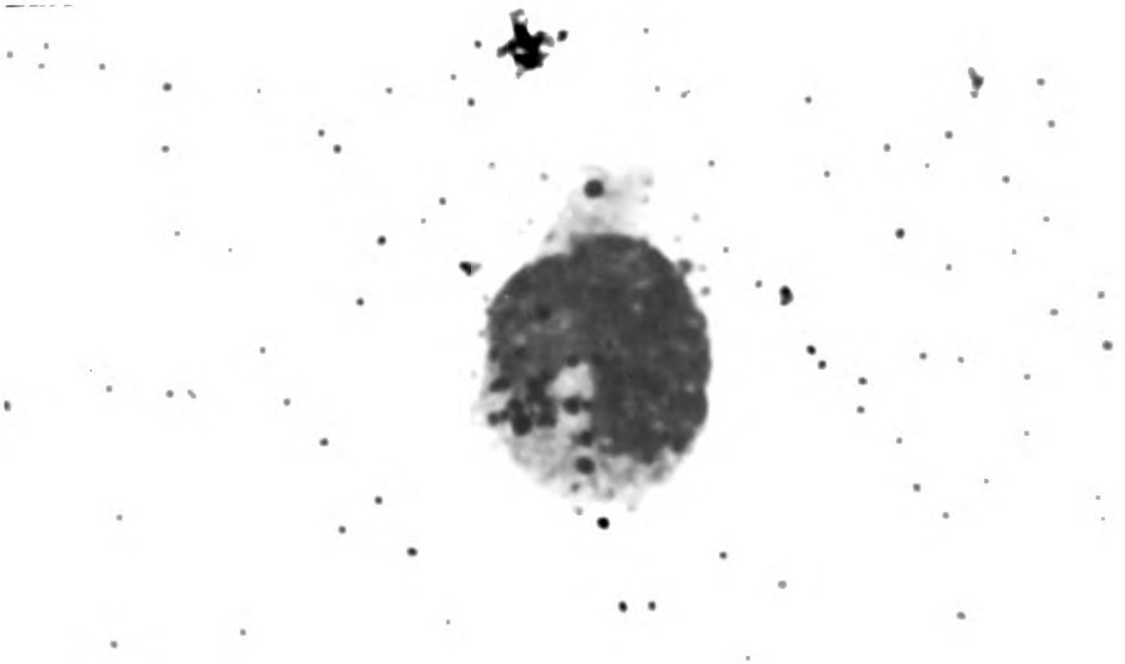

FIGURE 15

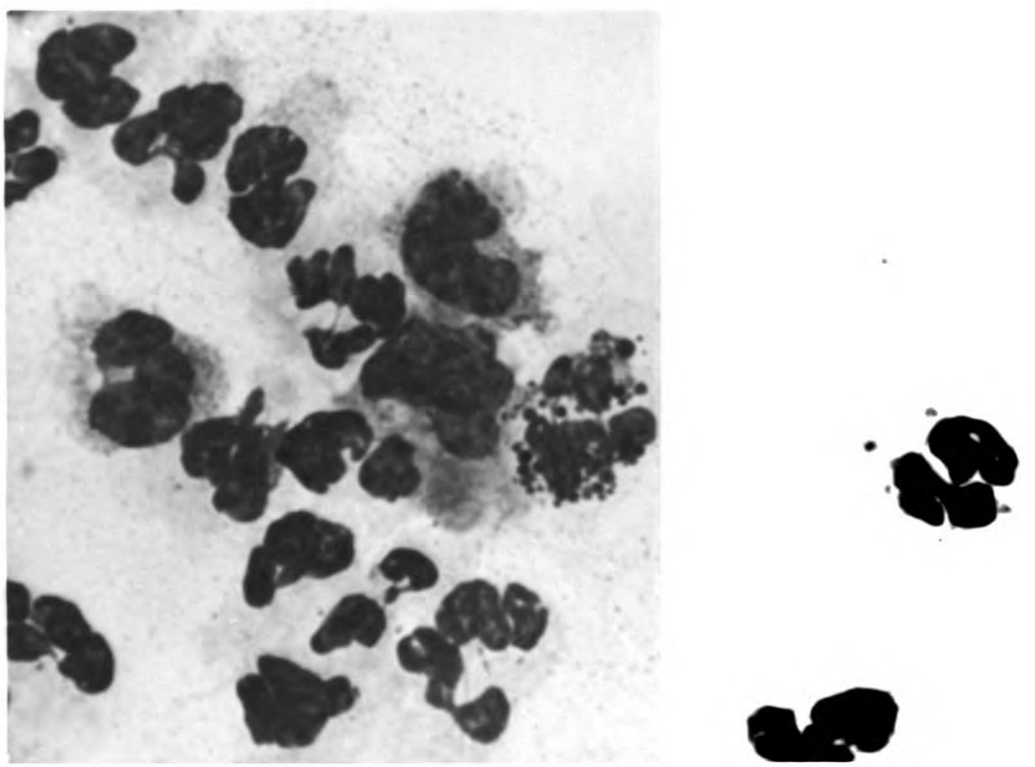

FIGURE 16

FIGURE 17

Twelve-hour stage. Note approximate equal numbers of neutrophilic leukocytes and mononuclear cells. X 640.

FIGURE 18

Twelve-hour stage. Three degenerating neutrophils. X 2816.

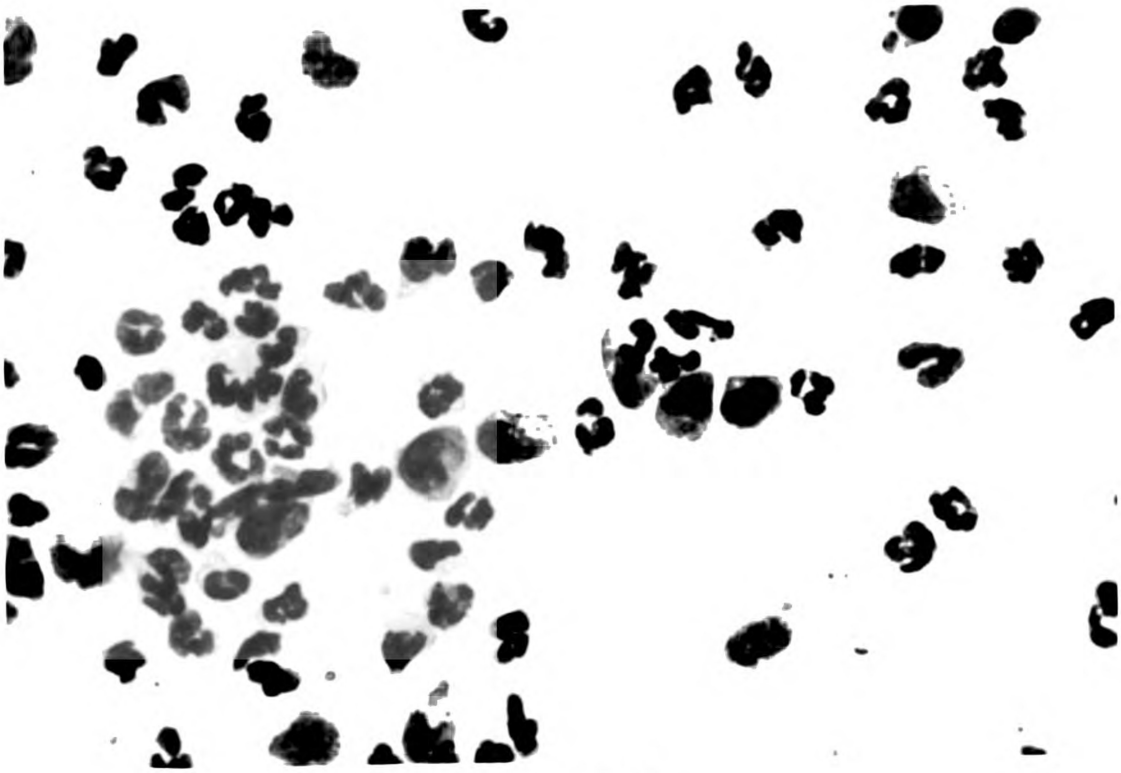

FIGURE 17

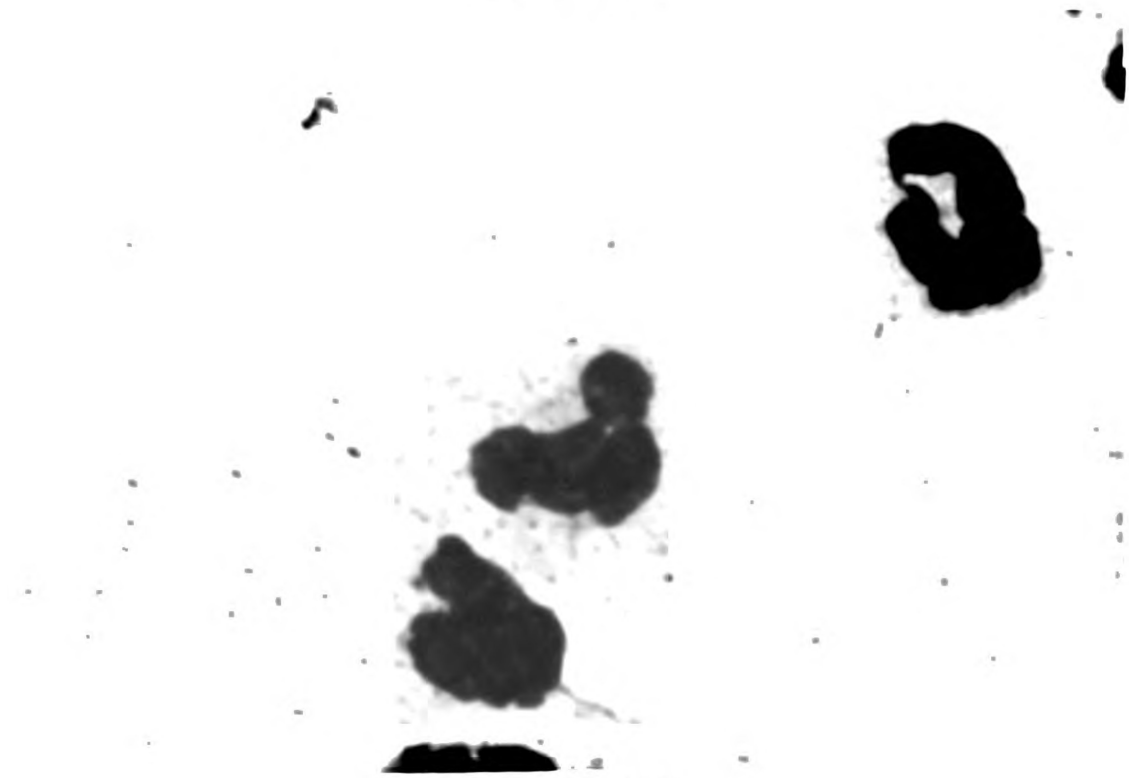

FIGURE 18

FIGURE 19

Twelve-hour stage. Neutrophil containing thin nuclear filament. X 2816.

FIGURE 20

Twelve-hour stage. Two mononuclear cells fixed in ameboid motion. X 2816.

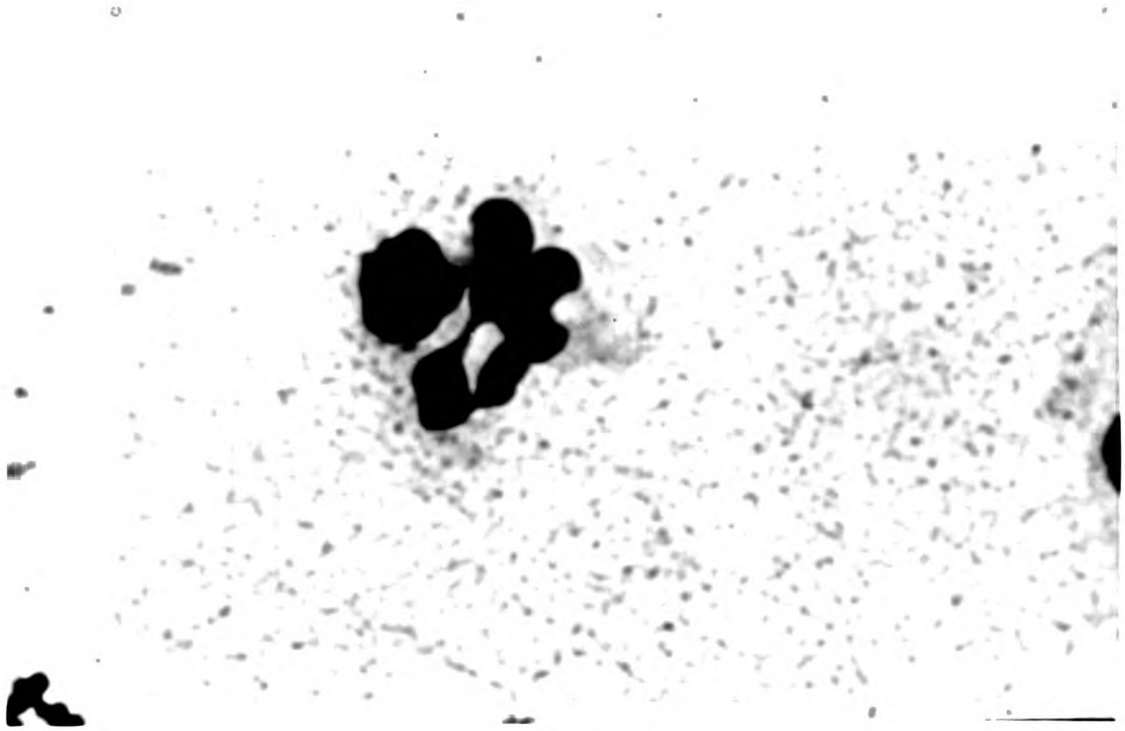

FIGURE 19

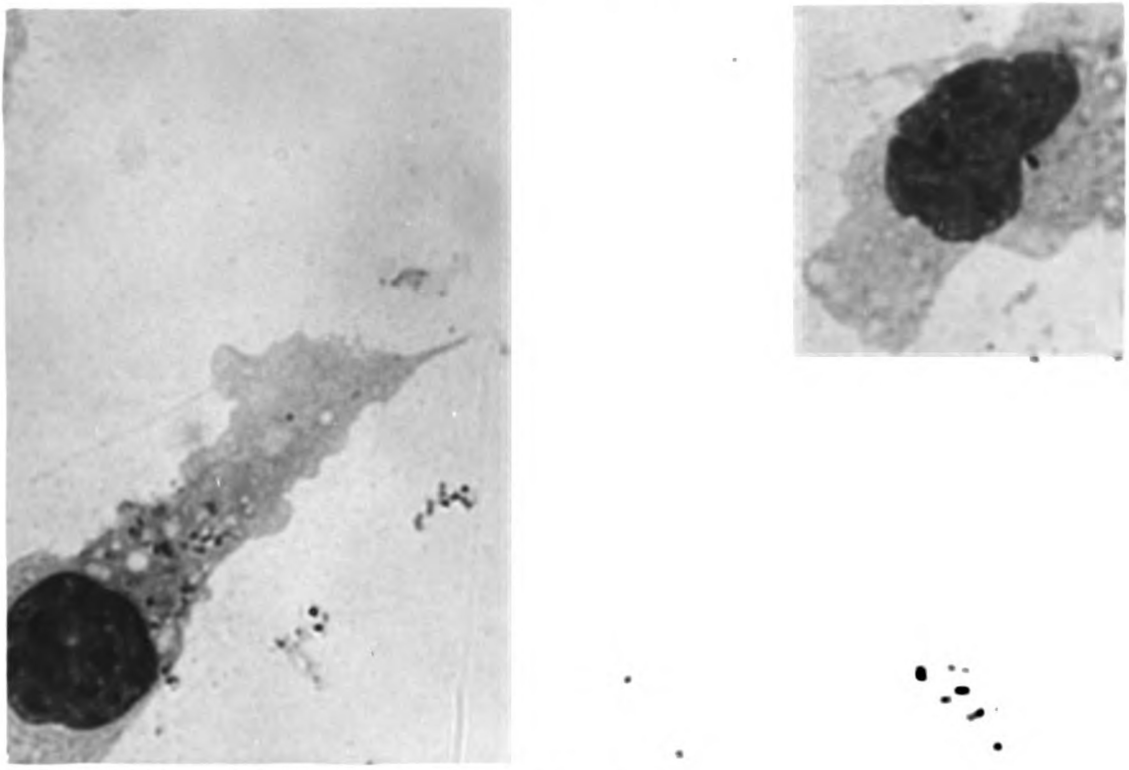

FIGURE 20

FIGURE 21

Fourteen-hour stage. Mononuclear cells predominating cellular exudate. X 704.

FIGURE 22

Fourteen-hour stage. Structurally intact neutrophil. X 2816.

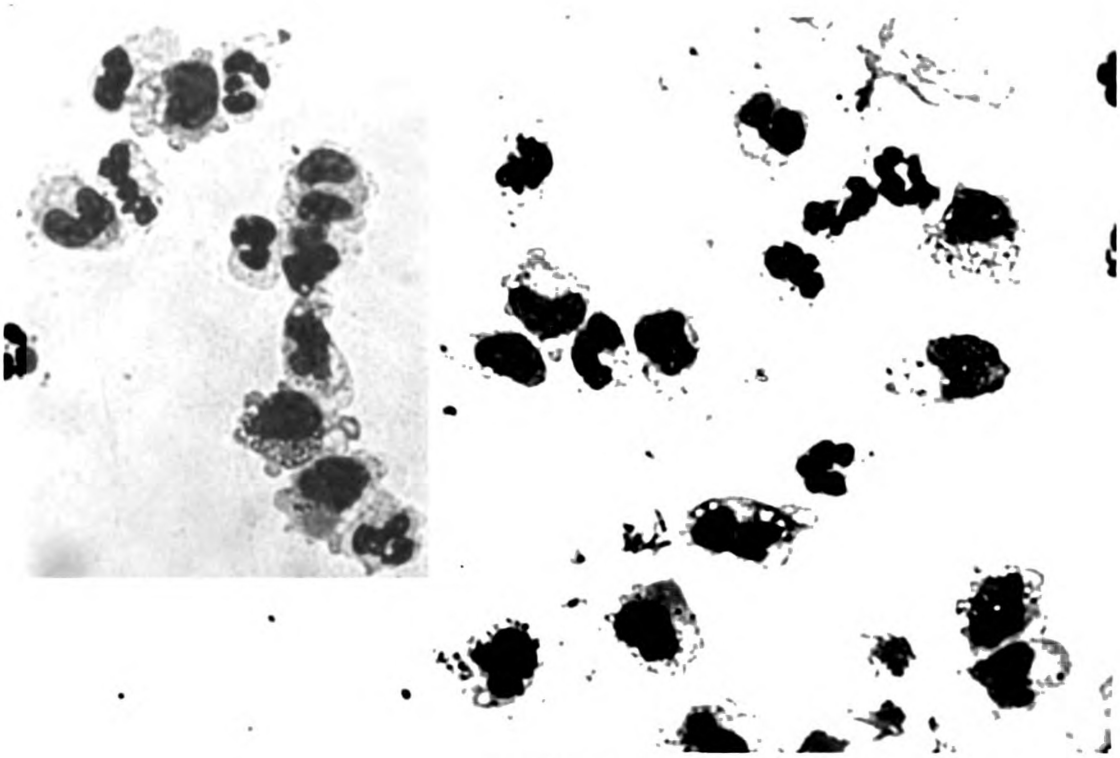

FIGURE 21

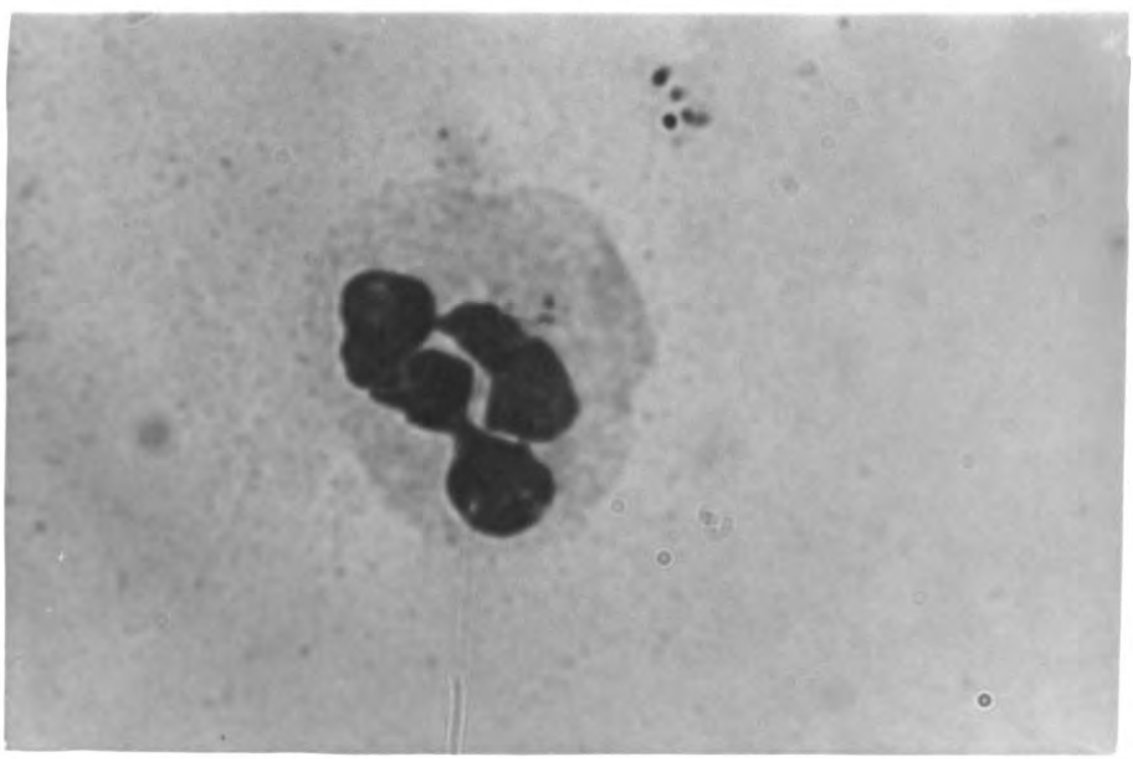

FIGURE 22

FIGURE 23

Sixteen-hour stage. Note predominance of mononuclear cells. X 1126.

FIGURE 24

Sixteen-hour stage. One mononuclear cell containing numerous cytoplasmic vacuoles and azurophilic granules, and one neutrophil. X 2816.

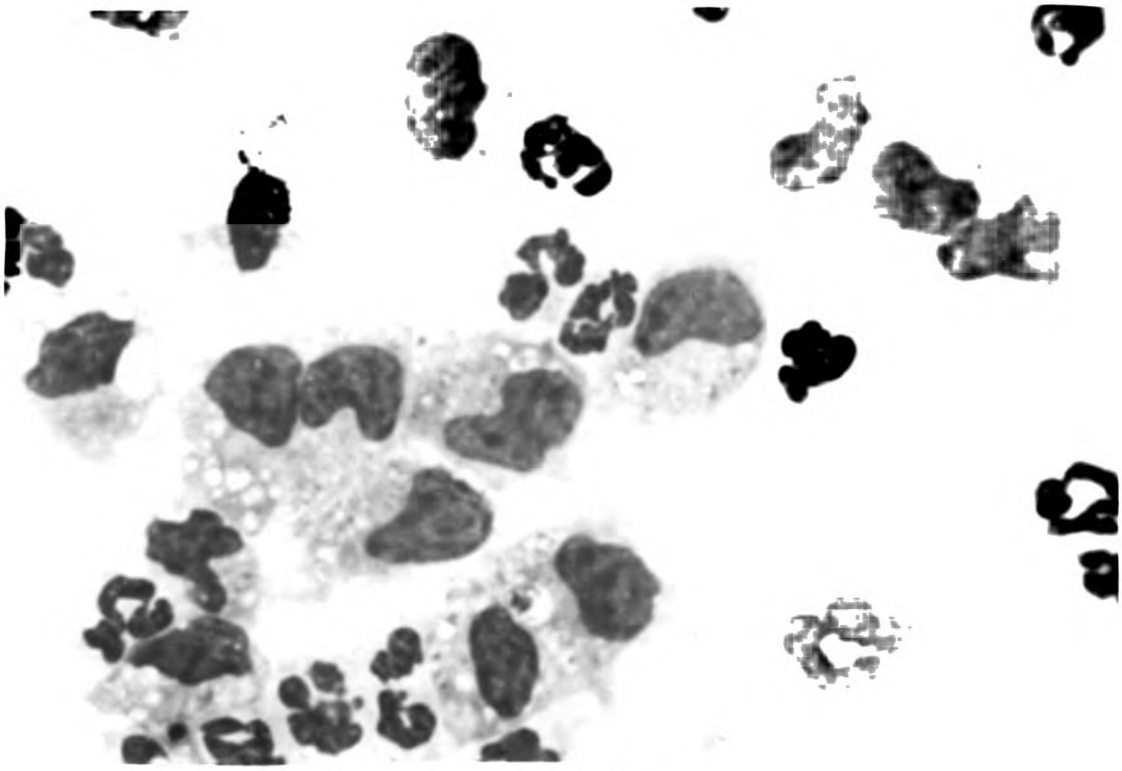

FIGURE 23

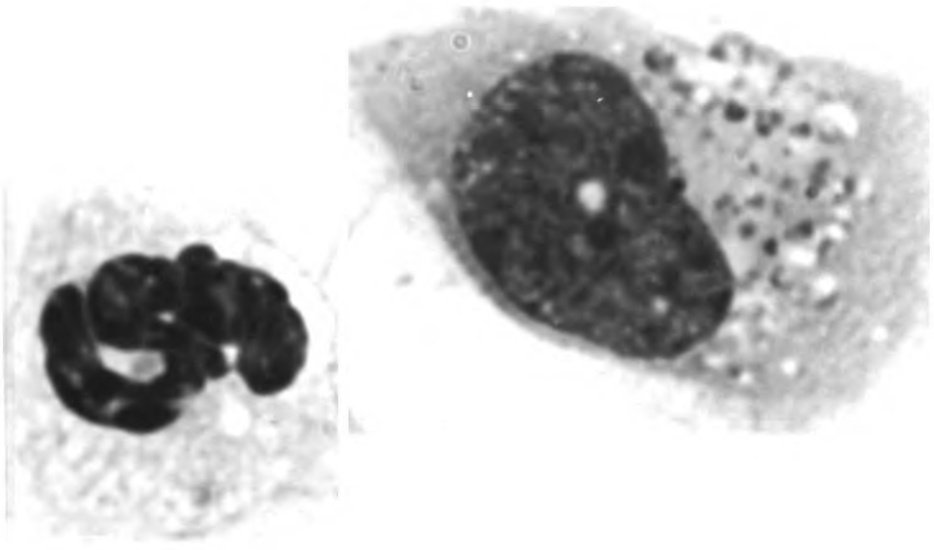

FIGURE 24

FIGURE 25

Sixteen-hour stage. Ingested Pelikan ink within the cytoplasm of a mononuclear cell. X 2816.

FIGURE 26

Peroxidase reaction. Mononuclear cell with positive peroxidase granules in cytoplasm. X 2816.

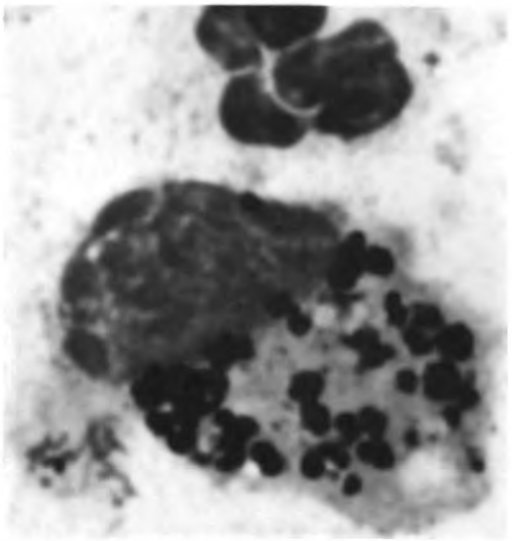

FIGURE 25

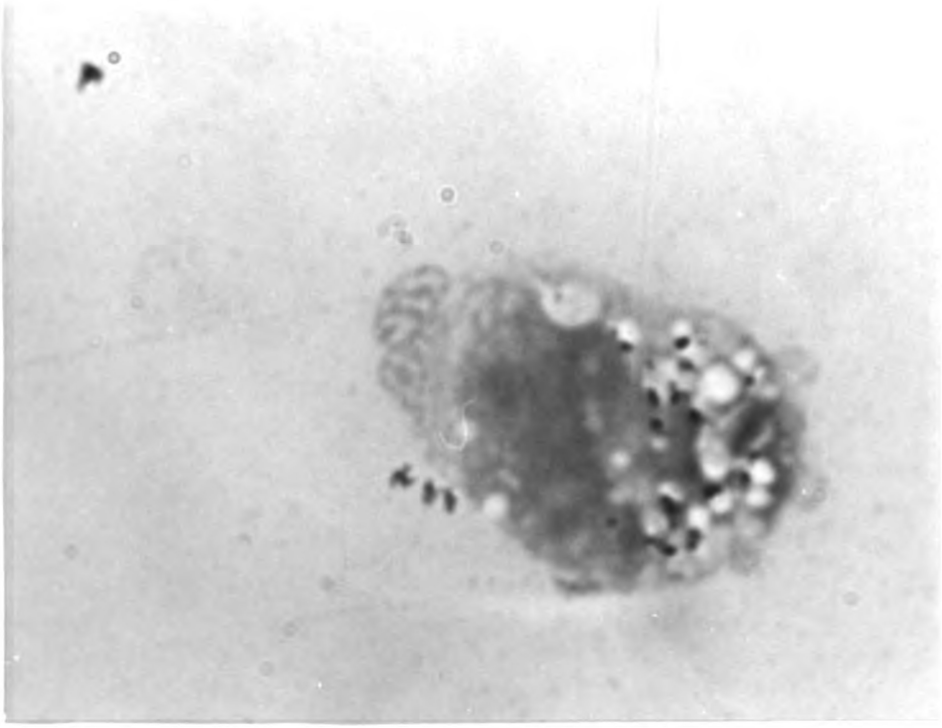

FIGURE 26

FIGURES 27-28

Sudan black B reaction. Polymorphonuclear cells and mononuclear cells containing numerous sudanophilic granules. X 2816.

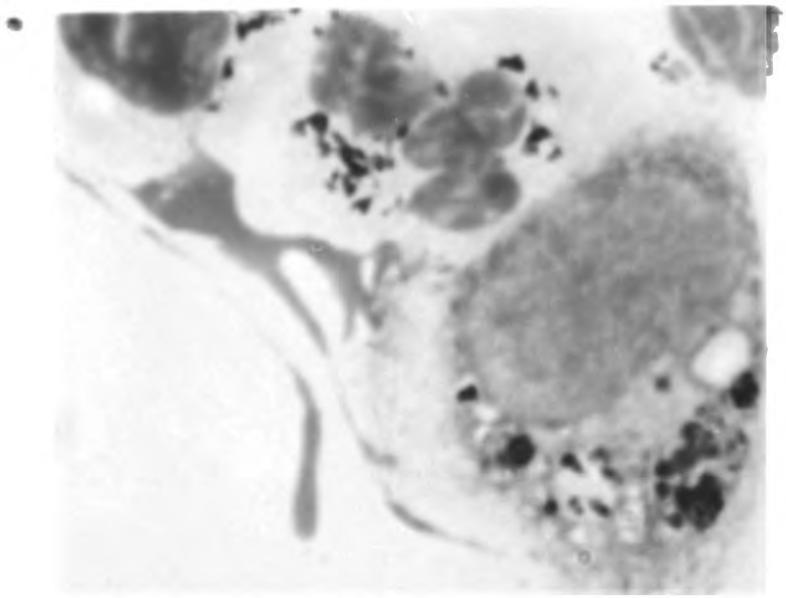

FIGURE 27

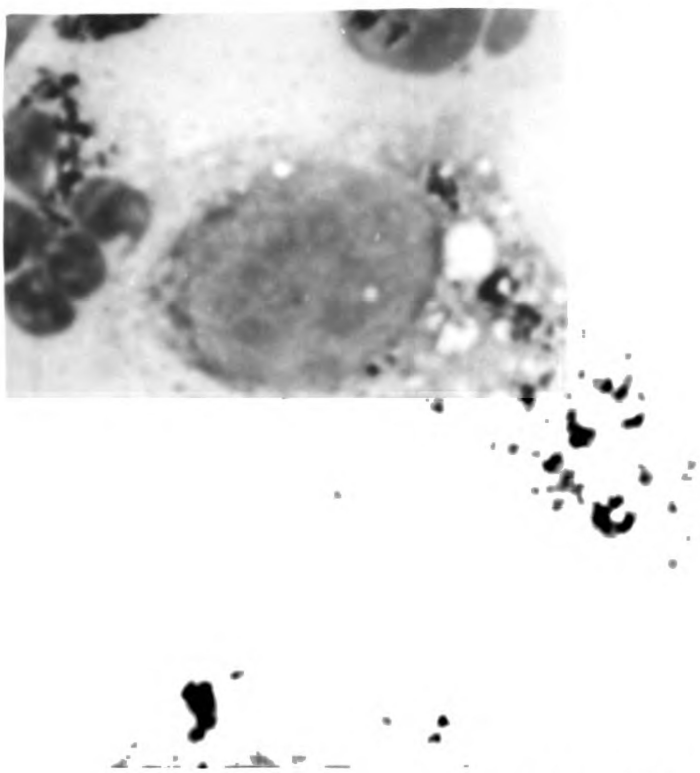

FIGURE 28