The Blood and Bone Marrow in Yellow Cross Gas (Mustard Gas) Poisoning.

Changes produced in the bone marrow of fatal cases.*

E. B. Krumbhaar, M.D., Ph.D., Recently Major, M.C., U.S.A., and Helen D. Krumbhaar.

(*From the John Herr Musser Department of Research Medicine, University of Pennsylvania.*)

The remarkable changes in the peripheral blood caused by yellow cross gas (mustard gas or yperite, with dichlorethyl-sulphide as the chief constituent) have been described in a previous paper.[1] The chief of these changes was a more or less extreme leucopenia, which followed the initial leucocytosis and in severe cases frequently fell below one thousand

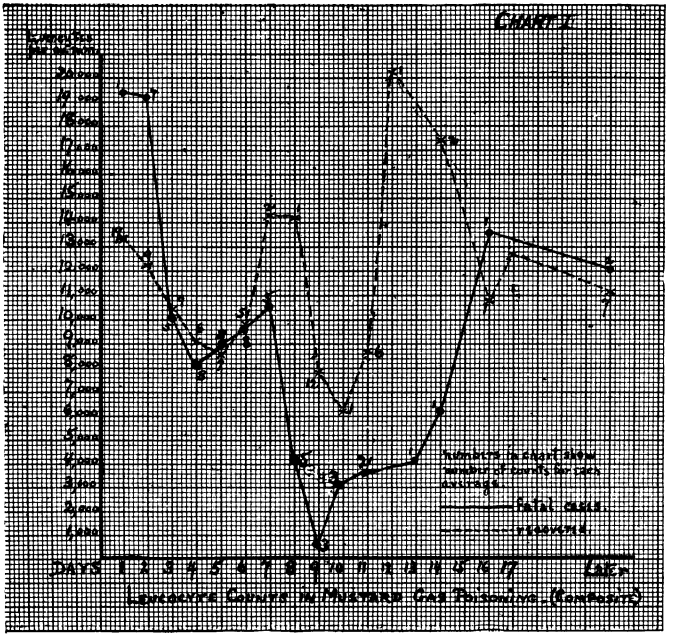

Chart I. — Average leucocytic counts of fatal and non-fatal cases of yellow cross gas poisoning.

* Received for publication July 10, 1919.

cells per cubic millimeter. A leucopenia has been found to exist in twenty-three of the one hundred and eight cases examined, but owing to conditions of service most of these could only be examined once, shortly after being gassed. Of those cases in which more than one examination was made, nineteen out of thirty-one had a definite leucopenia, and six of the remaining twelve showed a falling count. When the leucocytic count fell below five thousand, recovery followed in only three cases, so that severe leucopenia came to indicate a very bad prognosis, and persisting leucocytosis a good prognosis. The changes in the leucocytic count proved to be chiefly due to variations in the polymorphonuclear elements, with disappearance of eosinophiles in the acute stages and temporary appearance of myelocytes. It was suggested that the leucopenia might have an important bearing on the lack of resistance that is shown by such cases. A comparison of Charts I. and II. strengthens this view, in that the period of

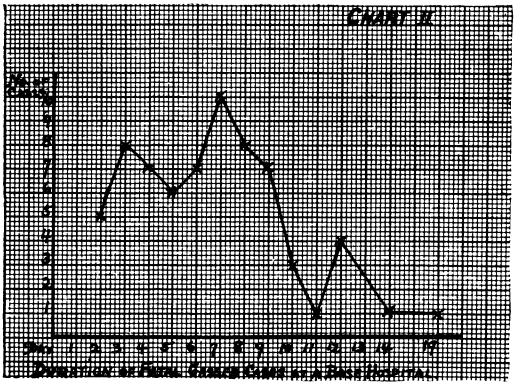

CHART II. — Duration of fatal cases of yellow cross gas poisoning.

highest mortality coincides with the most severe leucopenia. That the leucopenia was chiefly due to lessened blood formation was indicated by the accompanying anemia without the appearance of blast cells, or increase in the number of reticulated erythrocytes, and by a diminution in the number of platelets. These findings have since been supported by Pappenheimer's[2] experimental work on rabbits, and further confirmed by our later blood counts on "gassed" soldiers.

Two other studies of the blood of gassed soldiers have not revealed this striking leucopenia. Miller[3] in cases of gas poisoning with persistent symptoms found an absolute increase of small lymphocytes, and " in some cases a diminution in the number of polymorphonuclear leucocytes, which will of course accentuate the sign," but states that it appears in the second to fourth months. Hermann[4] found in nine severe cases, two of which were fatal, " a slight secondary anemia with a well-marked polymorphonuclear leucocytosis, a definite eosinophilia, and the appearance of myelocytes and young forms of leucocytes. The blood platelets were usually increased." He says that " unfortunately our cases were not accessible until the tenth day after the gassing," which is just the period at which, according to our findings, regeneration of the blood is apt to become evident, and his data suggests that in most of his cases blood regeneration was active. It is therefore easily possible that his cases would have shown a leucopenia if they could have been examined earlier. Dr. Warthin, on the other hand, tells me that in experiments with pure dichlorethyl-sulphide he found no such leucopenia, so that the possibility of its being due to other elements of the yellow cross gas must be considered. The benzine solvents would of course tend to have this effect, but they were hardly present in sufficiently large amounts, particularly in 1917, when typical leucopenia was found in our cases. It must also be recognized that some of the cases studied must have been exposed to other gases as well; but the clinical manifestations of yellow cross gas poisoning are sufficiently characteristic to allow its action to be detected, even if combined with other gases.

The study of the bone marrow indicated by such changes in the blood was next undertaken. This may be said in a few words to have shown that attempts at blood regeneration were occasionally entirely absent, nearly always slight or moderate, and never to be compared with the amount of hyperplasia found in acute infections. Even in two cases surviving for longer periods (twenty-seven and thirty-one days)

evidences of disturbed bone-marrow function were still obvious (increased amount of pigment and phagocytosis, moderate hyperplasia). It is believed that the blood and bone-marrow changes are due to direct action of the poison and not to secondary infection, (1) because they have been found well marked in cases where infection was slight or absent; (2) because influenza, typhoid, malaria and such "leucopenic" infections played no part in these cases; and (3) because the kind of infection found to be present (pyogenic) does not lead to leucopenia or impaired bone-marrow function.

Material. — The material for this study came from seventy-five autopsies on mustard-gas cases, in fifty-five of which the bone marrow was examined. In all cases a cylinder of about three inches was removed from the mid portion of the right femur, placed on filter paper, put at once into Zenker's fluid and then through the routine paraffin technic. The most satisfactory staining results were obtained with hematoxylin and eosin, though Ehrlich's triacid, Wright's, Pappenheim's and Unna's polychrome methylene blue were also used in some cases. Occasional sections from a rib, and smears from rib and femur were also made.

Comments. — It was soon recognized that the gross appearance of the marrow, while of great importance in estimating the amount of regeneration, had always to be controlled by the subsequent histological examination, as cellular hyperplasia could not be distinguished macroscopically from congestion. In the moderate degrees of hyperplasia observed in this series, the marrow was usually mottled with indefinite spots of varying size. Though the most hyperplastic areas were always taken for section, it was always possible that a given section might give a false impression of the state of that marrow by falling too much in or between the plane of such spots. This was minimized by taking more than one block from each marrow and two slides from each block, but nevertheless the impression was gained that more consistent results would have been obtained if this uncertainty had not existed.

Another difficulty in interpretation lies in the fact that evidence of the vigor of the regenerative process at the moment of death is afforded by the type of cell present in the cellular areas as well as by the number. For instance, in the marrows of some of the most leucopenic cases, in which a moderate hyperplasia was found, the cells were found to be of the most immature varieties, and the polymorphonuclears and normoblasts almost or entirely absent. For vital purposes such a marrow was of less use than a less hyperplastic marrow, such as some of the "leucocytic" cases presented, which contained relatively more mature forms. This, coupled with the fact that for unexplained reasons some hyperplasias occurred diffusely and others in localized areas, renders a true estimate of the degree of the regenerative process a very difficult one. Thus in one case that reached the low leucocytic count of five hundred and twenty per cubic millimeter two days before death, an almost aplastic marrow was found. A few faintly mottled areas were taken for study, however, and these showed a fair amount of cellular tissue (almost thirty per cent) in localized areas. Further study showed the probable slight value to the economy of even these areas, in that they were composed almost entirely of immature cell forms. The possibility of an agonal sweeping out of adult forms (polymorphonuclears), such as described by Longcope[5], must here be taken into consideration, although they were much scarcer than in the control marrows of infectious cases. In passing, the tendency of the hyperplasia to be most marked on the periphery (that is, next to the compact bone) should be noted. While clumps of myelocytes or of normoblasts and megaloblasts were not infrequent in the cellular areas (leucogenetic and erythrogenetic centers), the nests of cells as described by Bunting,[6] with immature cells in the center and the more mature forms about to be "peeled off" the periphery of the nest, could not be identified. When megaloblasts did occur in conjunction with normoblasts, they were as apt as not to be on the extreme periphery of the clump. The most common arrangement of the hemopoietic cells was apparently an indiscriminate mixture.

The bone marrows studied have been subdivided, according to their gross and microscopic appearance, into three groups, (1) those in which almost no reaction of the blood-forming tissues existed at the moment of death; (2) those with a slight reaction; and (3) those with a moderate reaction. The hyperplasia in these last two groups was either diffuse or in localized areas, as shown by gross mottling. Fourteen marrows were considered to be in the first group, twenty-eight in the second and thirteen in the third. Let it be emphasized that in no cases were the marrows as hyperplastic as in cases of ordinary lobar pneumonia, meningitis, dysentery or even of actue infections accompanied by leucopenia, such as typhoid and influenza.

Besides the fixed tissue cells, the following cells of the bone marrow have been considered: (1) The so-called primordial cell (large lymphocyte); (2) myeloblast, neutrophile myelocyte and (3) leucocyte; (4) eosinophile myelocyte and (5) leucocyte; (6) basophilic myelocyte; (7) small lymphocyte; (8) mononuclear and (9) multinuclear giant cells (megalocytes, often with orange-colored protoplasm); (10) phagocytes; (11) normoblasts and (12) megaloblasts. Other forms could not be classified; possibly they were transition forms or more often cells so pyknotic or altered in preparation that they had become unrecognizable. By the primordial cells was meant a large round or oval cell, containing a large nucleus with an extremely rich chromatin network and a narrow rim of faintly basophilic non-granular protoplasm. It was not possible always to separate myeloblasts from neutrophilic myelocytes (in which granules were often poorly marked); and the differentiation of small lymphocytes from megaloblasts was at times impossible. Mitosis was rarely encountered.

Details of some typical cases (arranged according to duration of condition). — 1. Rfm. H. N., lived 3 days. Gassed August 10, 1918. Vomited. Eyes and face swollen. Cough and bloody sputum. Cyanosed. Delirious. August 13, leucocytes 3,200 (differential count: polys 16 per cent; lymphocytes 2 per cent; mononuclears and transitionals 12 per cent; metamyelocytes 70 per cent.)

At autopsy marked typical lesions of the skin, eyes and upper respiratory tract. Broncho-pneumonia. Red bone marrow. Histologically, a

diffuse congestion and hyperplasia. In small areas, hemopoietic cells cover 60 per cent of the space, in most, however, not above 20 per cent. There is distinctly more increase than in the average bone marrow of this series. Some edema and apparent necrosis of the fat and connective cell nuclei. Immature forms predominate, though in some islands normoblasts are in the majority and in definite clumps. In others, eosinophile myelocytes are strikingly numerous. Polymorphonuclear neutrophiles are easily found, eosinophiles with more difficulty. Lymphocytes or megaloblasts are promiscuously scattered about, but not in apparent relation to the normoblast clumps. Very few giant cells are found and these with pyknotic nuclei.

2. Pte. J. B., lived 4 days. Gassed August 22, 1918. Vomited. Eyes inflamed, but relatively little swelling. Cough. Sore throat. August 24, collapsed. Leucocytes 6,100. Fever. Delirium.

At autopsy, confluent broncho-pneumonia. Acute splenic tumor, fatty changes in liver. Bone marrow distinctly reddened, especially in posterior portions. Histologically, very slight hyperplasia, more or less congestion. Most areas normal, in some the cellular tissue comprises 20 per cent of the whole. Here various types of cells are mixed together, the primordial cell, myeloblast and myelocyte (immature forms) predominating, but polymorphonuclears more numerous than usual in this series. Intra- and extra-cellular golden pigment is common. Normoblasts are frequent and occasionally in clumps, but few found in capillaries. Eosinophilic myelocytes, but no eosinophilic leucocytes present. Occasional mono- and multi-nuclear giant cells, but no cellular phagocytes.

3. Rm. L. W. L., lived 5 days. Gassed May 12, 1918. Very weak on admission. Eyes closed by swelling. Cough and pain in chest, moderate burns. Fever. Cyanosis. General condition got worse, though eyes and skin improved. Moderate cyanosis and lungs filled with râles on day of death.

At autopsy, in addition to eye and skin changes, early focal broncho-pneumonia in several lobes. Larynx and trachea inflamed, with necrotic gray spots and extensive exudate. Acute splenic tumor. Petechial hemorrhage of cardia. Bone marrow pink. Histologically, there is a diffuse, very slight cellular hyperplasia (never reaching 10 per cent). Myelocytes, myeloblasts, small lymphocytes, eosinophile myelocytes and leucocytes are most common. Normoblasts and megaloblasts much less so, and polymorphonuclear neutrophiles very rare. An occasional megakaryocyte and phagocyte is present, one with pyknotic nucleus.

4. Pte. C. M., lived 6 days. Gassed August 8, 1918. Vomited. Severe conjunctivitis, cough, pain in the chest, widespread burns. Leucocytes on the second day 16,200 (differential: polys 92 per cent, lymphocytes 3 per cent, mononuclears and transitionals 2 per cent, myelocytes 3 per cent). Arneth scale moderately shifted to the left. Urine: a trace of albumin. Grew steadily worse with dyspnea, cyanosis and weak, rapid pulse.

At autopsy, the usual appearance of eyes, skin and upper respiratory tract. Widespread focal broncho-pneumonia. Congestion of the viscera. Bone marrow mostly yellow; here and there slight reddish mottling.

Histologically, many fields normal, others contain small clumps of tightly packed cells. These approximate the description of Case 2, except that no pigment is found.

5. Pte. F. G., lived 7 days. Gassed March 12, 1918. Vomited. Cough. Admitted with usual signs of severe gassing. Leucocytic count, March 13, 17,800; March 14, 13,200; March 15, 11,400 (differential: polys 91 per cent, lymphocytes 5 per cent, mononuclears and transitionals, 4 per cent); March 17, 3,200 (differential: polys 84 per cent, lymphocytes 4 per cent, mononuclears and transitionals 10 per cent, myelocytes 2 per cent). The Arneth scale was much shifted to the right. Developed left apical pneumonia.

At autopsy, extensive confluent broncho-pneumonia, left apex in stage of resolution. Typical eye, skin, larynx and tracheal lesions. Esophagus milky white. Petechial hemorrhages of stomach (especially cardia and duodenum). Bone marrow mottled red (moderately hyperplastic?). Histologically, much congestion and fair amount of cell increase. Much golden pigment in the cellular areas. Immature forms most in evidence. No polys and few blasts. Eosinophile myelocytes most common, also occasional multinuclear giant cells.

6. Gun. W. S., lived 8 days. Gassed May 10, 1918. Condition very poor on admission. Eyes swollen, burns slight. Pulse weak and irregular. Systolic pressure, 99. Later, blood pressure rose but cyanosis increased. Bled 10 ounces. Said he felt better and was less blue, but got steadily worse and died in two hours. Leucocytic count two hours before death, 18,000.

At autopsy, acute fibrinous pleurisy, congestion and edema of lungs; no pneumonia. Dilatation of right side of heart. Bone marrow red. Histologically, extensive diffuse hyperplasia with a mixture of various cells and without definite islands. Eosinophiles and mono- and multi-nuclear giant cells especially prominent, polys, blasts and lymphocytes abundant. (This case is of unusual interest in that it presents a marrow in a relatively normal state of regeneration. Death was undoubtedly hastened by venesection [which was thereafter discontinued in this type of case] and occurred while there was still a leucocytosis).

7. Pte. H. K., lived 11 days. Gassed May 20, 1918. Usual sign of severe gassing, with bad burns of face, buttocks and legs. Fever, dyspnea, crusts on face, broncho-pneumonia.

Day.	Leucocytes.	Polys. %	Lymphocytes. %	Turck Cells. %	Mononuclear and Transitional. %	Myelocytes and Metamyelocytes. %
8th	640	10	35	50	5	..
9th	520	10	60	20	10	..
10th	1,200	4	6	2	4	84
11th	5,400	1	2	1	5	91

At autopsy, mild conjunctivitis, burns healing, widespread focal and confluent broncho-pneumonia. Oligemia of viscera. Spleen pale, flabby, weighs 60 grams. Bone marrow very pale, a few reddish splotches at proximal end only. Histologically, some edema and apparent necrosis.

Hemopoietic cells mostly of immature types (primordial cells). A few normoblasts and eosinophile myelocytes. Occasional megakaryocytes and phagocytes (some of these pyknotic) and golden pigment, chiefly extracellular.

8. Sgt. J. H., lived 12 days. Gassed November 30, 1917. Severe conjunctivitis, laryngitis and burns of face. Fever and dyspnea. Nine days later general condition worse. Signs of pneumonia. Grew steadily worse.

Day.	Erythrocytes.	Leucocytes.	Polys. %	Lympho-cytes. %	Mononuclear and Transitional. %	Myelocytes and Metamyelocytes. %
9th	3,600,000	570	10	70	20	..
10th	.....	1,400	20	64	12	4
11th	3,500,000	2,075	52	23	12	13

At autopsy, mild conjunctivitis, dried scabs on face, pigmentation of inner side of thighs, and maceration of scrotum. Larynx and trachea slightly inflamed. Confluent broncho-pneumonia of lower lobes. Congestion of viscera. Spleen very little enlarged, weight 250 gms. Bone marrow yellow, except for posterior periphery, which is red. Histologically, normal bone marrow in several sections. In one, a considerable cellular increase. Primordial cells, myeloblasts, megakaryocytes (phagocytic or with orange-colored protoplasm) are most striking; but clumps of normo- and megalo-blasts, and many mitotic cells are also found. Occasional eosinophile and a few basophile myelocytes. No polymorphonuclears. In other words, though most of the marrow is aplastic, in a few areas active regeneration occurs.

9. Rfm. J. C. W., lived 15 days. Gassed September 30, 1918. Moderately severe burns and conjunctivitis. Apparently passed through broncho-pneumonia but continued to have much circulatory difficulty. Eyes and burns almost healed. Developed edema of the lungs in last forty-eight hours.

Day.	Leucocytes.	Polys. %	Lymphocytes. %	Mononuclear and Transitional. %	Myelocytes and Metamyelocytes. %
8th	900	34	34	6	26
12th	4,200	26	18	6	50
14th	6,200	2	16	2	80

At autopsy (partial). Face and skin as above. Bone marrow yellow throughout. Histologically, almost complete aplasia of bone marrow. Occasional hemopoietic cells, such as seen in normal adult marrow, but not enough to give an idea of the relative frequency of the different types. In the presence of an increasing leucocytic count, this state of the bone marrow illustrated the difficulty that has previously been referred to of comparing the two conditions.

10. Pte. J. M., lived 21 days. Gassed May 11, 1918. Father died of tuberculosis. Had been gassed once before. Was moderatedly gassed this time and seemed to be recovering from mild pneumonia, when signs

of abscess of the lung developed and patient died in three days. Leucocytes on 7th day, 14,000; on 8th day, 8,500; on 17th day, 13,600; on 18th day, 16,000; on 20th day, 8,000.

At autopsy, confluent broncho-pneumonia with multiple small abscesses and one large gangrenous cavity. Bone marrow shows marked red mottling — especially of the proximal end. Histologically, regeneration is extensive (up to 60 to 80 per cent of certain areas). All types of cells are represented, normoblasts, megakaryocytes, pigment-bearing phagocytes and polymorphonuclears being especially noticable. Extracellular pigment is also common. Mitosis is not uncommon. (This marrow resembles that of Case 6, except for the greater amount of pigment present [blood destruction?], and may be considered to have survived the toxic action of the gas.)

Conclusions. — 1. Yellow cross or mustard gas exerts on the bone marrow a direct toxic action, which, by depleting the leucocytes of the circulation, has an important bearing on the inability to resist secondary infection that is found in that form of gas poisoning.

2. This toxic action on the bone marrow is shown, not only by small areas of necrosis, but by an inhibition of the regeneration process (chiefly of the leucogenetic series.).

3. Not only is the amount of regenerative hyperplasia inadequate to the severity of the process (as compared with the marrow hyperplasia of various acute infections), but also the quality is inferior, that is, the great majority of the hemopoietic cells present are of immature types.

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DESCRIPTION OF PLATES XXI. AND XXII. CHARTS AND ILLUSTRATIONS.

PLATE XXI., FIG. 1. — Bone marrow from Case 1, dying on the third day, high power. Note the clumps of normoblasts in the middle of the figure (erythrogenetic center). This and subsequent figures are photomicrographs, Zeiss, apochromatic, eyepieces 2 and 4, objectives 16 and 4 mm.

FIG. 2. — Bone marrow from Case 2, dying on the fourth day, high power. Note: *a*, pyknotic giant cell; *b*, cells containing blood pigment.

FIG. 3. — Bone marrow from Case 6, dying on the eighth day, two hours after venesection. Relatively large amount of hyperplastic tissue.

FIG. 4. — Same case, high power. Note: *a*, five giant cells in the field. At the upper margin; *b*, five eosinophile myelocytes can barely be detected by their coarse granules.

FIG. 5. — Bone marrow from Pte. R. F., dying on the tenth day. Leucocytic count had dropped from 36,000 to 14,000 four days before death. Normoblasts and mitotic cells (small dark dots) numerous.

FIG. 6. — Same, high power. Note: *a*, myelocyte; *b*, eosinophile myelocyte; *c*, normoblast; *d*, mitotic cell.

PLATE XXII., FIG. 7. — Bone marrow from Case 7, dying on the eleventh day. Note absence of normoblasts and polys. No edema or necrosis shown in this field, which was one of the few that showed cell increase.

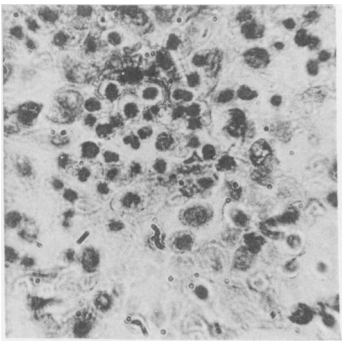
FIG. 8. — Same case, high power. Note the pyknotic phagocyte and absence of polys.

FIG. 9. — Bone marrow from Case 8, dying on twelfth day. Clumps of normoblasts and scattered myelocytes can be distinguished.

FIG. 10. — Bone marrow from Case 9, dying on fifteenth day. Almost complete absence of hemopoietic cells.

FIG. 11. — Bone marrow from Case 10, dying on twentieth day. Note larger spaces filled with cells. Myelocytes, polys, normoblasts and erythrocytes can be distinguished.

FIG. 12. — Drawing of bone marrow of Case 10, to show a cellular hyperplasia, due to a mixture of various types of cells; *a*, primordial cells (large lymphocyte); *b*, myeloblasts and myelocytes; *c*, polymorphonuclear leucocytes; *d*, eosinophile myelocyte; *e*, eosinophile leucocyte; *f*, normoblasts; *g*, small lymphocytes; *h*, multinuclear giant cell; *i*, phagocyte containing two erythrocytes and nuclear remains; *j*. small pigment-bearing cells; *k*, fat and connective tissue cells.



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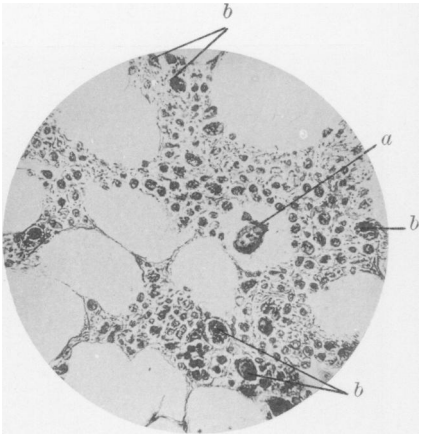

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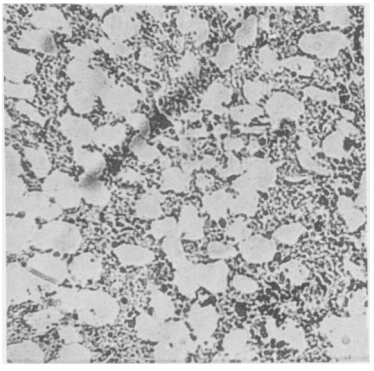

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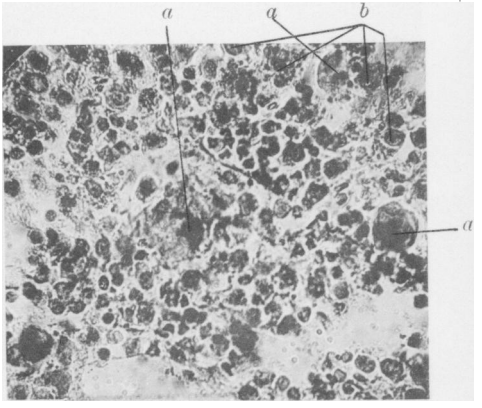

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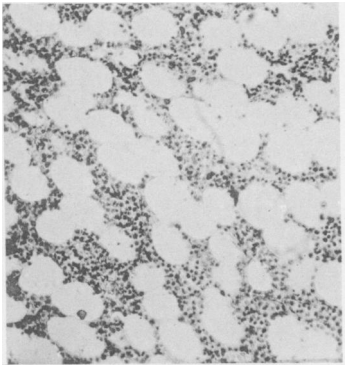

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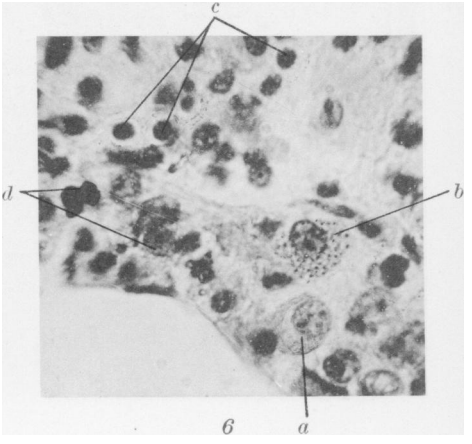
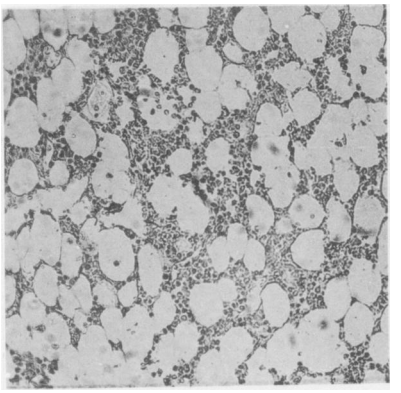


Yellow Cross Gas.



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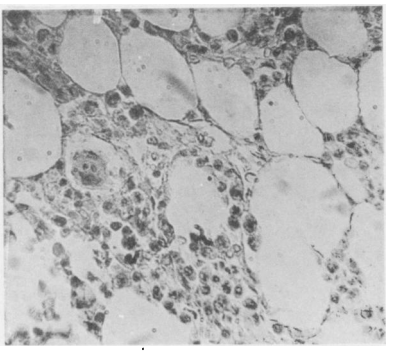

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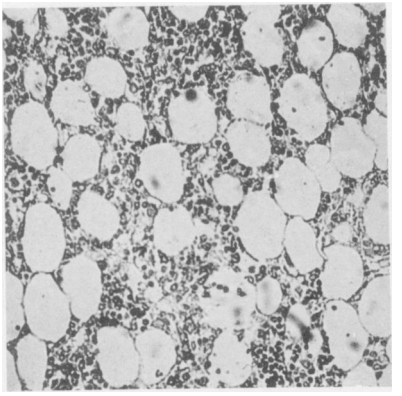

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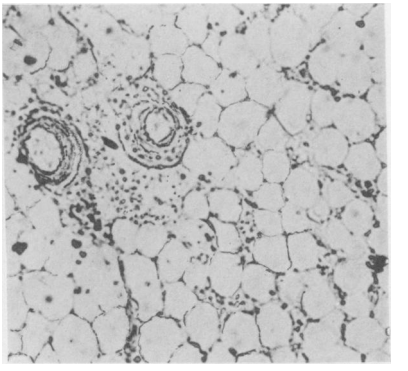

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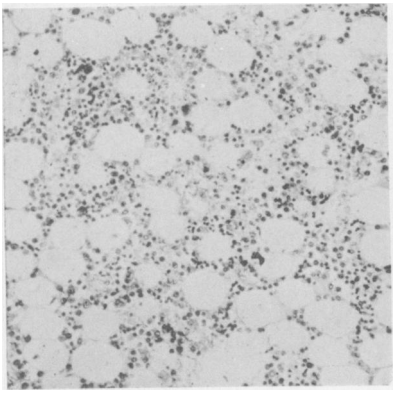

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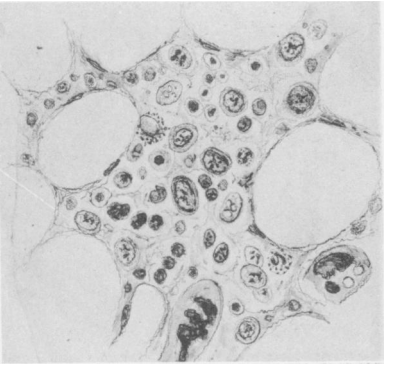

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Yellow Cross Gas.