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Reticulo-endothelial cells in the bone marrow of the guinea-pig

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In the light of earlier studies of the blood and blood-forming organs of the 400 g. guinea-pig (Yoffey, 1960), and of the increased interest shown in the possible reactions of the reticulo-endothelial (RE) cells in the bone marrow (Miescher, 1957; von Ehrenstein & Lockner, 1959), it was thought advisable to pay further attention to these elements. Since the early work of Hoffman & Langerhans (1869), Hoyer (1869) and Ponfick (1869), who made studies of the bone marrow after intravenous injection of particulate matter, the presence of injected particles in the cells of the marrow has been described by many investigators (e.g. Cousin, 1898; Kiyono, 1914; Evans, 1915; Nagao, 1920; Wislocki, 1921, 1924; Hashimoto, 1936; Huggins & Noonan, 1936; Patek & Bernick, 1960). However, published illustrations of the RE cells of the marrow are surprisingly few in number and in general show little detail. Furthermore, in many instances the suspensions used have been open to criticism. It seemed important, therefore, that further studies of the RE cells of the bone marrow should be carried out, using one of the newer and more satisfactory suspensions now available. In the present investigation, the non-toxic colloidal suspension of carbon first described by Halpern, Benacerraf & Biozzi (1953) and Biozzi, Benacerraf & Halpern (1953) has been employed. The distribution of carbon particles was studied in the bone marrow of normal guinea-pigs at intervals from 5 min. to 28 days after intravenous injection.

MATERIALS AND METHODS

The work was performed on male albino guinea-pigs of the Dunklin–Hartley strain, weighing approximately 400 g. Injections of the carbon suspension C 11/1431 *a* (Gunther Wagner, Hanover) were made into the external jugular or cephalic vein through a small skin incision. This was carried out under general anaesthesia, induced by ether. Details relating to the preparation of the suspension have been given by Halpern *et al.* (1953). A dosage of either 25 or 50 mg. per 100 g. body weight (1 or 2 ml.) was employed. A total of forty animals was used, and at least three animals were killed at each of the following intervals after injection: 5, 15, 30, 60, 90 and 180 min., 2, 14 and 28 days. Plugs of red marrow were removed from the medullary cavity of the femur, fixed in Zenker-Formol, and sectioned at 3 μ after embedding in ester wax. Staining was carried out by Dominici's Eosin Orange G–Toluidin Blue. In a few experiments specimens of fatty marrow from the lower tibia were also studied.

Smears were also made from teased preparations. For this purpose femoral marrow from the opposite side was used and gently teased in serum. Smears were made in the usual way and stained with MacNeal's tetrachrome.

RESULTS

5 min. after injection

The carbon is intravascular and present in each type of vessel. It maps out the vascular bed in a very striking manner and shows, for example, the way in which some sinusoids are related to fat vacuoles (Pl. 1, fig. 1). In some of the sinusoids, as also in the vein, the particles show a tendency to assume a marginal or peripheral position.

Particles are not present in the parenchyma at this stage.

15 min.

There is still a great deal of carbon in the lumen of the vessels, mapping out the vascular bed of the bone marrow. The tendency for the particles to assume a marginal position in the veins and sinusoids is even more marked, and many particles appear to be either adhering to the sinusoidal wall or occupying an intracellular position. Some small groups of particles now lie in the parenchyma, but from the study of sectioned material it is difficult to be absolutely certain that these lie in macrophages.

In teased marrow preparations, however, macrophages laden with carbon particles can be clearly identified (Pl. 1, fig. 3).

30 min.

The general picture is very similar to that at 15 min., although less carbon is present in the lumen of the vessels. There is still very little carbon in the parenchyma. In the sections, a few laden macrophages may be seen in the peripheral part of the marrow, although in sections it is still difficult to identify them with certainty at this stage. Macrophages containing particles are readily identified in teased preparations. Many macrophages without particles may, however, be noted.

60 min.

Carbon particles are still present in the lumen of the vessels, but the amount of carbon present is noticeably less. Most of the particles lie either on or in the endothelium of the sinusoids or veins. Small collections of carbon occur in the parenchyma and it is probable that these are within the cytoplasm of macrophages as suggested by the presence of a surrounding ring of erythroblasts (Pl. 2, fig. 5).

90 min.

In most cases the circulation is now free of carbon. The actual time needed to clear the blood stream seems to vary somewhat with the size of the dose and with different animals. At this stage, the role of the endothelium of the sinusoids and veins is seen to best advantage. Fine particles are present, often extending in linear fashion along the whole length of the sinusoidal or vein wall (Pl. 1, fig. 4). The particles, though intracellular, are not confined to any part of the endothelial cell, being found in relation to the nucleus as well as elsewhere in the cytoplasm (Pl. 2, fig. 6). The nuclei of the endothelial cells are in most cases flattened, but occasionally a more rounded nucleus is seen. There is no indication of mitosis in the endothelial cells. There are no carbon particles in the endothelium of the arteries.

This is true both of the usual thick-walled arteries and of the peculiar thin-walled arteries which consist of only two cell layers (Yoffey, 1962). Clumps of fine particles, many lying clearly within the cytoplasm of macrophages, can now be seen in the parenchyma (Pl. 2, fig. 7).

3 hours

The most striking feature in the sections is the presence of small accumulations of carbon, fairly evenly distributed through the parenchyma (Pl. 3, fig. 8). It is impossible to state whether these all lie within the cytoplasm of macrophages, though presumably they do. The walls of the sinusoids still contain some particles but carbon particles no longer 'outline' them.

2 days

The general appearance is similar to that at 3 hr., although little carbon now remains in the sinusoidal walls. Laden macrophages are clearly seen in sections at this stage, some in an intrasinusoidal position (Pl. 1, fig. 2).

14 and 28 days

The marrow shows large well-localized aggregates of carbon scattered quite evenly throughout the parenchyma. The size of these aggregates is a striking feature (Pl. 3, fig. 9). Many are closely related to fat vacuoles. In isolated places a few carbon granules can be found in the sinusoidal endothelium, but the endothelium is largely free of particles at this stage.

Other findings

In smears made from teased marrow, all the lymphocytes, and the majority of the granulocytes, are free from particles. However, intracellular particles can occasionally be observed in a neutrophil.

At all stages, the haemopoietic marrow is black to the naked eye, whereas the fatty marrow is still pale even after the intravenous injection of 200 mg. of carbon. This can be clearly seen in the tibia, where the marrow of the upper two-thirds is black, while the fatty marrow of the lower one-third is pale.

DISCUSSION

How effective is the barrier between blood and parenchyma?

Until relatively recently, workers on the marrow circulation concerned themselves repeatedly with the problem of a 'closed' or 'open' circulation. Observations on the living marrow (Kinosita, Ohno & Bierman, 1956; Brånemark, 1959) have shown clearly that, as far as the red and white cells are concerned, the circulation is effectively closed in relation to the passage of cells from blood to parenchyma, though permitting their free movement in the reverse direction.

The position with particulate matter, however, is not quite so simple. In the early stages of the present experiment, the particles are confined to the vascular bed. They do not immediately obtain access to the parenchyma. This is in agreement with the findings of Drinker, Drinker & Lund (1922), who carried out perfusions of the bone marrow with India ink. However, electron microscopic observations (Pease, 1956; Zamboni & Pease, 1961) have indicated that the sinusoidal endothelium has

no basement membrane and that there are numerous breaks in its continuity. Furthermore, when colloidal thorium dioxide is introduced into the bloodstream, the particles become dispersed throughout the marrow parenchyma within 10 min. (Zamboni & Pease, 1961). The discrepancy between our own observations and those of Zamboni & Pease (1961) may be due to difference in particle size. Most of the thorotrast particles were said to be less than 10 mμ in diameter, whereas the size of particles in the present preparation is about 25mμ (Biozzi *et al.* 1953; Freeman, Gordon & Humphrey, 1958), although the possibility that some may aggregate to form larger particles cannot be excluded (Heller, 1958). One does not know the extent to which the effective size of the particles may also be influenced by the presence of protein molecules on their surface.

Speed of passage into parenchyma

It is clear that there is no instantaneous passage of the ink particles through the sinusoidal endothelium. It is, however, equally apparent that within a relatively short time some of the particles do begin to pass through into the parenchyma, though most of them are still intraluminal. In sections a few ink-containing macrophages are seen in the peripheral portion of the marrow 30 min. after injection. In smears occasional ink-containing macrophages are seen in as short a time as 15 min. (Pl. 1, fig. 3).

The role of the sinusoidal endothelium

The fact that many injected particles come to lie within the cytoplasm of the endothelial cells has been reported by numerous investigators (e.g. Cousin, 1898; Kiyono, 1914; Nagao, 1920; Wislocki, 1921; Hashimoto, 1936; Patek & Bernick, 1960). The presence of these particles can be seen to the best advantage at the stage where the blood has just been cleared (Pl. 1, fig. 4). Thereafter their number decreases. In view of the progressive increase in the amount of carbon in the macrophages of the parenchyma, one is tempted to speculate that there is a steady movement of particulate matter from the lumen to the endothelium and thence to the interior of the marrow. While our findings would accord with such a concept, they provide no evidence of the method by which such a transfer could be effected. One cannot state whether the considerable pinocytotic activity of sinusoidal cells, reported by Zamboni & Pease (1961) and Weiss (1961), is important here. The possibility that laden endothelial cells become mobilized and migrate into the surrounding parenchyma should also be considered. In tissue culture studies, Woodard & Pomerat (1953) have shown sprouting of tubes of sinusoidal endothelium, the cells apparently maintaining continuity all the time. However, no evidence of such a process has been seen in the present experiments.

While it is true that the endothelium is not normally called upon to deal with ink particles, the fate of such particles may throw light on that of the particulate matter with which the endothelium is normally concerned, namely, fragments of broken-down red cells. The work of von Ehrenstein & Lockner (1959) suggests that the ingestion of erythrocytic fragments occurs on quite a large scale in the bone marrow. There may, however, be a species difference in this. The studies of Hughes Jones (1961) confirm that in the rabbit the bone marrow is of considerable importance in removing effete red cells or their fragments from the circulation, whereas in the

rat the marrow seems to play a relatively minor role (Hughes Jones & Cheney, 1961).

It is noted that Wislocki (1921) working mainly with rabbits observed that 'even after a period of months...the carbon deposited in endothelial cells has not diminished in amount...'. He made a few observations on guinea-pigs, but, apart from the fact that the reticulo-endothelial cells of the marrow seemed less active, he did not mention any essential species difference. On the other hand, Nagao (1920), working with both guinea-pigs and rabbits, noted that the endothelial cells of the marrow sinusoids gradually lose their ink, although he gave no details of the speed with which this occurred. Our own observations show that, although the number of particles in the endothelium diminishes after the blood has been cleared, occasional particles still remain in the endothelium after one month.

The endothelium of the veins

Pl. 1, fig. 4 and Pl. 2, fig. 5 show the endothelium of a large marrow vein. As seen with the light microscope, its structure and properties are identical with those of the sinusoidal endothelium. The fact that the marrow veins are very thin-walled was noted as far back as 1868 by Bizzozero. The apparent identity in structure and properties of venous and sinusoidal endothelium has been noted by Nagao (1920) and Hashimoto (1936).

The role of the macrophages

In recent years Bessis (see review by Bessis and Breton-Gorius, 1962) has attributed an essential role to the marrow macrophages as centres for the provision of ferritin to developing erythroid cells. The appearance of an ink-laden macrophage surrounded by young red cells (Pl. 2, fig. 5) is not an infrequent phenomenon and can be interpreted as supporting Bessis's view. The movement of particles from the lumen of the sinusoids to the parenchymal macrophages takes place in a remarkably short time and, if Bessis's view is correct, in the case of red cell fragments this movement might be a continuous process. However, the evidence already noted, that in the rat the bone marrow is of much less importance in the removal of red cell fragments from the blood stream (Hughes Jones & Cheney, 1961), suggests that some caution should be exercised before regarding this concept as generally applicable.

Pl. 2, fig. 7 and Pl. 3, figs. 8 and 9 indicate that inert particles gradually accumulate in the macrophages and become aggregated into larger masses, as was noted by Nagao (1921) in other situations. This is presumably not the fate of physiological particles such as red cell fragments which the cell can metabolize and then be ready to deal with further particles. The accumulation of carbon does, however, suggest an analogy with the presence of lipoid and other masses in certain pathological conditions.

Do ink-laden macrophages migrate?

Pl. 1, fig. 2 depicts a particle-laden macrophage in a marrow sinusoid at 48 hr. after injection. Such an appearance gives no indication of the direction in which the macrophage is moving, whether from blood to marrow, or marrow to blood. It does, however, indicate that distribution of carbon in the organism is not static. Such macrophages could of course leave the blood stream in a number of situations besides the marrow.

Particles in cells other than macrophages

No particles have been observed in marrow lymphocytes whether in sections or smears. Very occasionally a few ink granules can be found in a neutrophil polymorph. In blood smears made 48 hr. after injection, large mononuclear cells containing particles are also seen. This is in agreement with the findings of Bimes (1962), Downey (1917) and Nagao (1920) but not with those of Koszewski, Emerick and Dicus (1957), who observed ink granules in circulating small lymphocytes.

SUMMARY

The reticulo-endothelial elements of the bone marrow have been studied in a series of guinea-pigs following the intravenous injection of a fine carbon suspension. Observations have been made at intervals over a period of 28 days.

At 5 min. after injection the carbon is confined to the vascular bed, but the appearance of laden macrophages in the parenchyma shortly afterwards indicates that the sinusoidal endothelium permits the rapid passage of particles. In teased preparations laden macrophages are clearly seen in as short a time as 15 min. after injection. By 90 min. almost all the injected particles have been removed from the circulation. Fine particles are present in the endothelial cells of the sinusoids and also the veins, but no phagocytic activity is shown by the arteries, including those which possess the thin-walled structure. After 90 min. the particle content of the sinusoidal endothelium decreases and a striking redistribution of carbon takes place. By the 14th and 28th day the carbon has been deposited in the form of large aggregates, sometimes closely related to fat vacuoles. The possibility that carbon is also moved from one organ to another is suggested by the appearance of laden macrophages in the lumen of the blood vessels in the 2-day observations.

Particles are occasionally noted in a neutrophil polymorph, but no examples of marrow lymphocytes containing particles have been observed.

The possible physiological significance of these observations has been discussed.

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EXPLANATION OF PLATES

All the illustrations except Fig. 3 are from histological sections of femoral marrow stained with Dominici's Eosin Orange G–Toluidin Blue.

PLATE 1

Fig. 1. General view, 5 min. after injection. The carbon appears to be confined to the vascular bed. Part of a large central vein and numerous sinusoids may be noted. The particles in the central vein show a tendency to concentrate towards the periphery of the vessel. × 240.

Fig. 2. Intrasinusoidal macrophage, 48 hr. after injection. The lumen of the sinusoid contains a macrophage laden with carbon particles. A few particles may be noted in the sinusoidal endothelium. × 500.

Fig. 3. A laden macrophage in a preparation of teased marrow, 15 min. after injection. The remains of a red cell, staining rather faintly, and other nuclear debris may be noted in its cytoplasm. The other cells present do not contain particles. × 1000.

Fig. 4. Endothelium of a vein and a sinusoid, 90 min. after injection. The lumen of the vein, containing a few red cells but no particles, is to the right of the figure. The attenuated endothelium shows the presence of a number of fine particles. The sinusoid to the left of the figure shows similar features. An occasional endothelial nucleus may be noted. × 1000.

PLATE 2

Fig. 5. A parenchymal macrophage, 60 min. after injection. An accumulation of carbon is seen, surrounded by a number of erythroblasts (arrowed). The ink is presumably within the cytoplasm of a macrophage. × 1000.

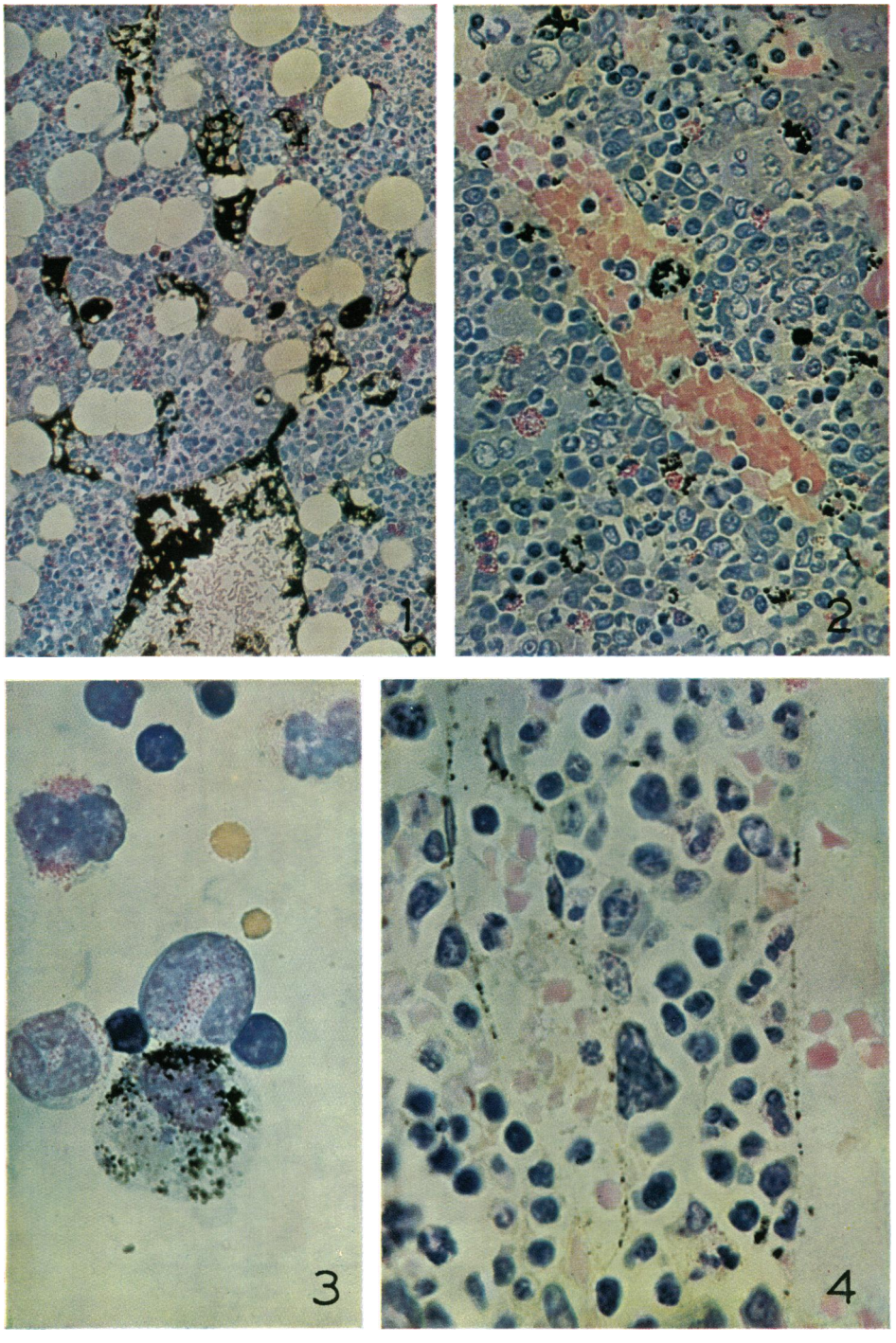
Fig. 6. Endothelium of a vein, 90 min. after injection. The lower part of the figure is occupied by a mass of blood cells lying within the lumen of a large vein. A number of particles are present in an endothelial cell (E), some in the region of the nucleus. × 1050

Fig. 7. Parenchymal macrophage, 90 min. after injection. The arrow indicates the nucleus of a macrophage (M) which is laden with carbon particles. × 850.

PLATE 3

Fig. 8. General view, 3 hr. after injection, showing small accumulations of carbon scattered throughout the parenchyma. × 325.

Fig. 9. Aggregations of carbon, 14 days after injection. Some of these aggregations are closely related to fat vacuoles. × 500.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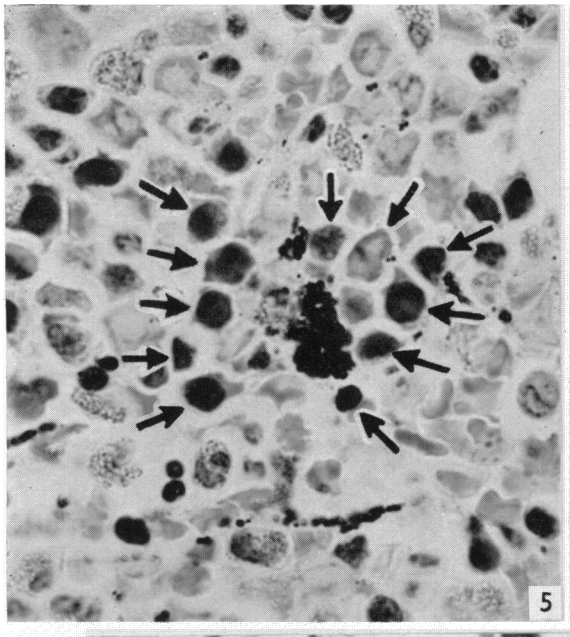
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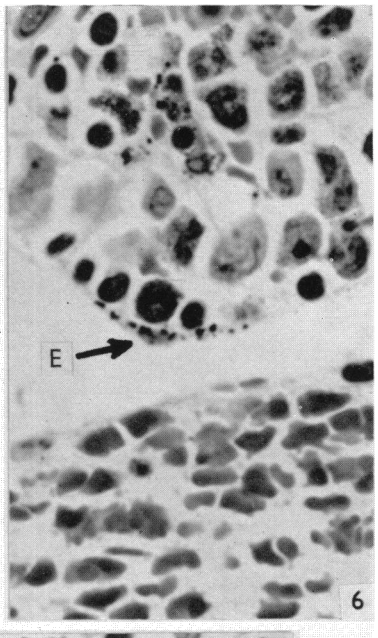
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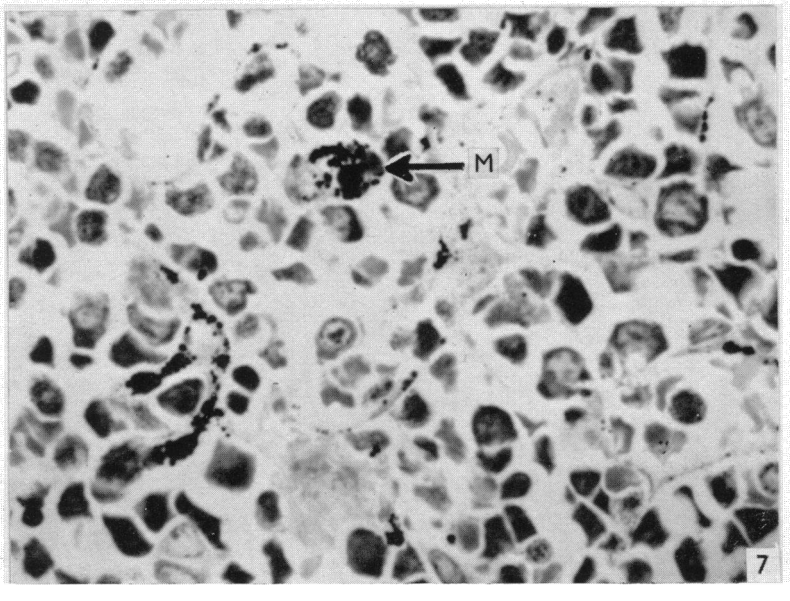
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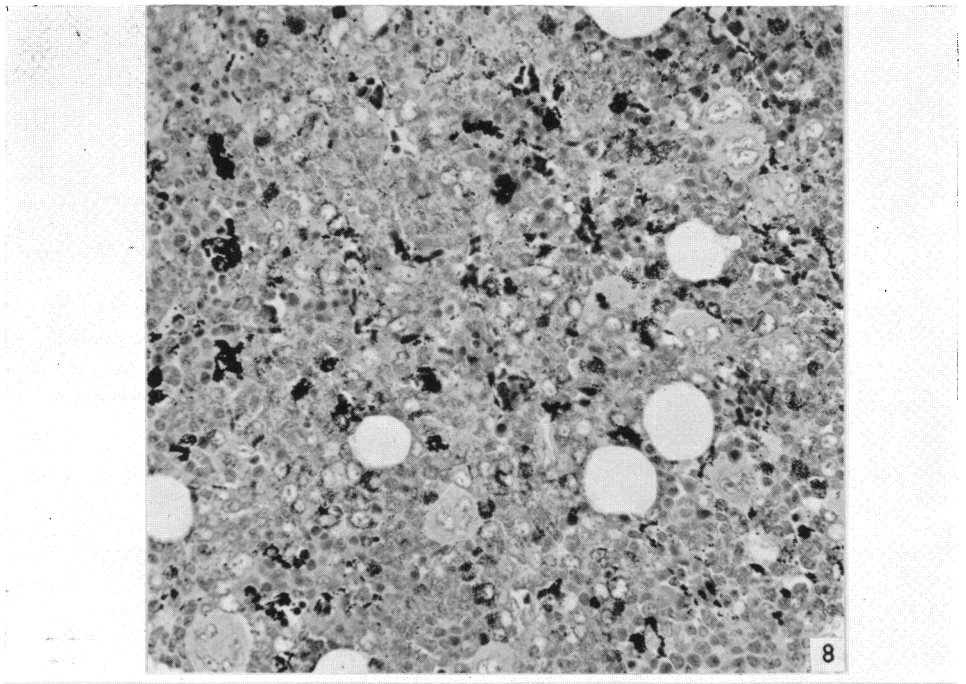
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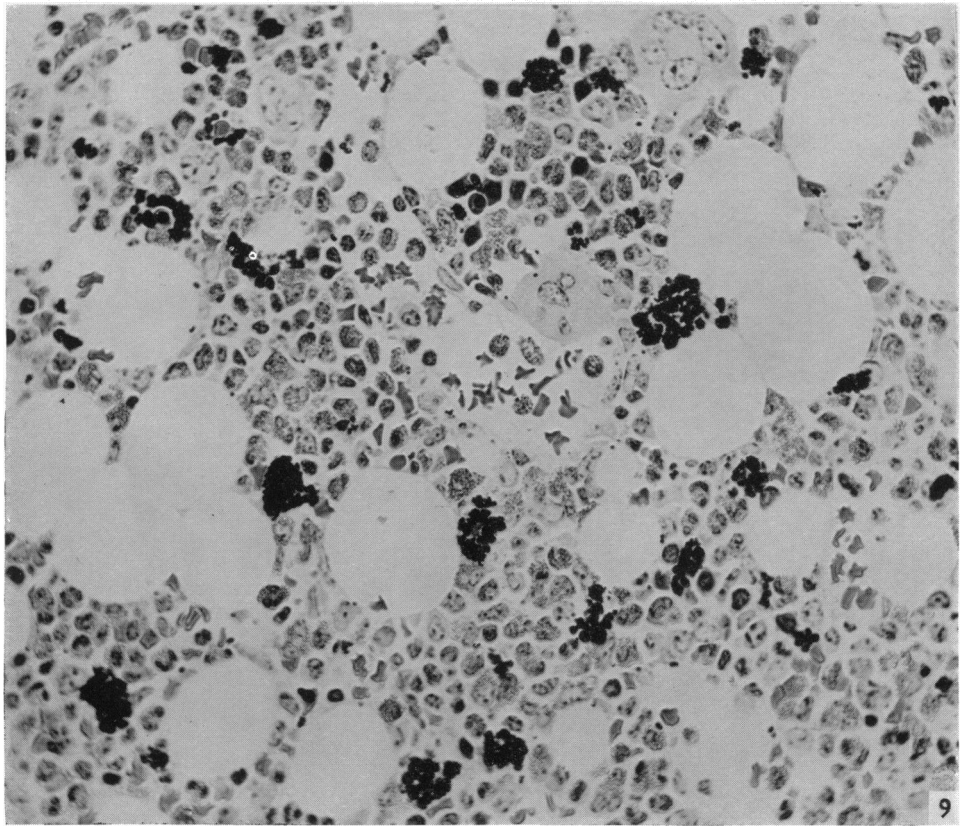
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