

PASSAGE OF FOREIGN PARTICLES THROUGH THE SINUSOIDAL WALL OF THE RABBIT BONE MARROW
—AN ELECTRON MICROSCOPIC STUDY

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Thorotrast, India ink, OsO_4 -fixed staphylococci and candida were injected into rabbit ear vein. Electron microscopic observation of the marrow revealed the thorotrast and carbon particles in the cytoplasm of the sinusoidal endothelial cells and reticulum cells as well as in the extracellular space of the marrow parenchyma, while staphylococci and candidas were demonstrated only in the cytoplasm of reticulum cells. This difference seems to be related to the size difference of the particles.

Reticulum cells frequently projected a cytoplasmic process into the sinusoidal lumen through the endothelial gap, and injected particles were frequently seen in these intraluminal projections. This finding strongly suggests that the particulate matter in the blood flow can be directly taken up by means of intraluminal projection of the reticulum cell and then transported into the extraluminal portion of the cell. This mechanism may be only one possible way by which the particles larger than staphylococci enter the marrow parenchyma across the sinusoidal wall. The passage of erythrocytes from blood flow into marrow parenchyma is assumed to be made by the same mechanism. ACTA PATH. JAP. 21: 349~367, 1971.

Introduction

The passage of the cell and particulate matter through the sinusoidal wall reveals one of the essential problems in bone marrow function. It is widely accepted that mature blood cells migrate from the marrow parenchyma into the lumen of sinusoid passing through the sinusoidal wall^{9,16,28,29,31,32,35}. In this event the passage of the cell is thought to be made through the endothelial gap. However, concerning the passage of particulate matter in inverse direction, i.e.

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from sinusoidal lumen into marrow parenchyma, there are some controversies between investigators. It has been recognized by many authors that the carbon particles introduced into blood stream are readily taken up by the sinusoidal endothelium and later on by reticulum cell in the marrow parenchyma. HUDSON and YORFFEY¹¹ have observed the passage of carbon particles through the sinusoidal wall by means of electron microscopy. According to these authors the leakage of carbon particles through the gap of sinusoidal endothelium is not recognized and that transport across the cytoplasm of the sinusoidal endothelium may only be a possible way by which carbon particles enter the marrow parenchyma. In contrast to this work, ZAMBONI and PEASE³⁵ have shown that thorotrast particles introduced into the abdominal aorta disperse throughout the marrow parenchyma within 10 minutes, and they interpreted this phenomenon as a sufficient evidence of functional patency of the sinusoidal wall. WATANABE^{28,29,30} has demonstrated that the reticulum cell near the sinusoidal wall frequently send a cytoplasmic projection through the endothelial gap into the sinusoidal lumen. He suggested that the particulate matter, including carbon particles and erythrocytes, may be directly taken up by the intraluminal projection of reticulum cells and transported into the extraluminal portion of the reticulum cell.

To summarize the results of these works, there seems to be at least three possible ways by which particulate matter passes across the sinusoidal wall from blood stream into the marrow parenchyma, i.e. 1) leakage through the endothelial gap, 2) transport across the cytoplasm of the endothelial cell, and 3) direct capture by the intraluminal projection of the reticulum cell. The present author has assumed that the size of the particle may be an important factor for the different modes in passage of particulate matter and attempted to carry out an experiment using foreign particles of various sizes, including thorotrast, carbon, cocci, and fungi, to confirm this assumption.

The sinusoidal wall of the bone marrow is a labile structure easily destroyed by improper treatment during specimen preparation. In order to minimize the artificial break down of the sinusoidal wall, the bone marrow was fixed by perfusion method using glutaraldehyde throughout the experiment.

Materials and Methods

Albino rabbits weighing 600 to 900 g were used. The foreign particles were injected into the ear vein with or without ether anesthesia. One or two hours after the injection, the abdomen was opened and 2.5% glutaraldehyde buffered at pH 7.2-7.4 with cacodylate buffer was perfused through the abdominal aorta. At least four rabbits were used for each group of foreign particles.

The injected foreign particles were:

1. Thorotrast: Stabilized colloidal thorium dioxide (Fellows Testagar Co. 24-26% by volume) was used at the rate of 5 ml per kg of body weight.

2. Carbon particle: India ink (Pilot Ind Co.) was diluted by an equal volume of normal saline and the solution was filtered by No. 101 filter paper (Toyo Filter Paper Co. Ltd.). It was injected at the rate of 5 ml per kg of body weight.

3. *Staphylococcus aureus* No. 209 P strain and *Candida albicans* (a strain isolated from a patient), both kept in the Department of Microbiology, Keio University were used. The former was cultured in brain heart infusion media and the latter in Sabouraud glucose media. To the 48-hour-cultures, 1% trypsin solution was added. After three repeated washings by normal saline (each by 20 minutes 3,000 r.p.m. centrifuge), an equal volume of 2% OsO_4 -phosphate buffer was added and fixed for 15 minutes. Another three washings were repeated. The final concentration of the bacteria and the fungi were collected in a 5 ml saline solution for 200 ml of the original 48-hour culture per one rabbit.

Method of perfusion: The rabbit was anesthetized by intraperitoneal injection of Nembutal (Abott Lab.) 3 ml per kg of body weight. After laparotomy, both the abdominal aorta and vena cava posterior were dissected. No. 12 to No. 16 Teflon tubes were inserted to the aorta and the vena cava respectively after making a small incision on each blood vessel. The tube inserted in the aorta was connected to an ordinary infusion apparatus containing cooled fixative. The tube inserted in the vena cava was led to a mess cylinder. The perfusion pressure was controlled as to keep the fluid height at 120–140 cm. The perfused fixative used was 2.5% glutaraldehyde-cacodylate buffer solution cooled at 4°C. In the majority of cases, glucose and calcium bichloride were added to 4 g% and 0.05 g%, respectively. Each rabbit was perfused for 30 minutes. The inflow volume of the fixative in this method was usually between 400 to 450 ml. The outlet fluid volume was about 50 ml less than the inflow volume.

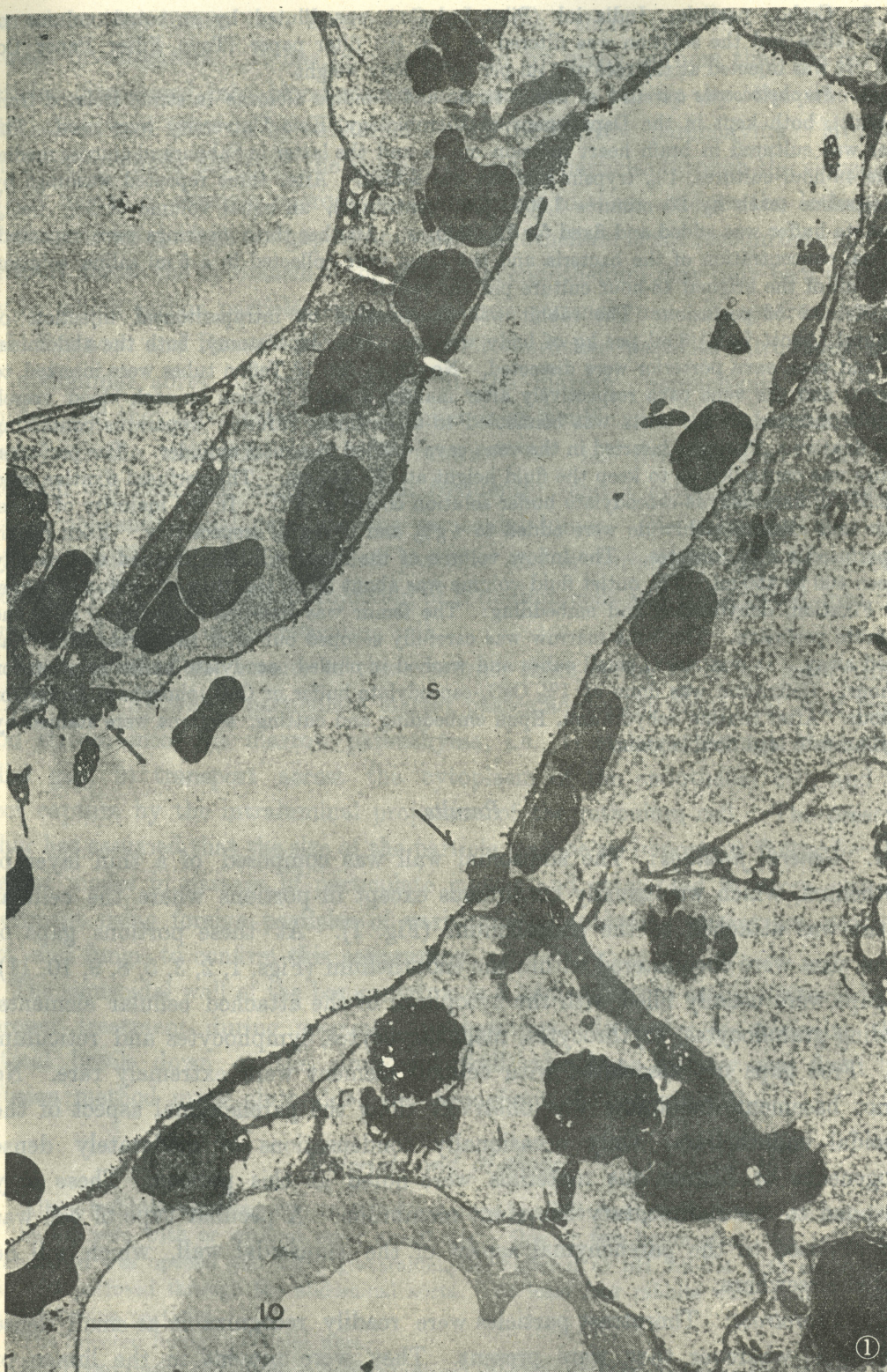
Fixation, dehydration and embedding: The femur was dissected immediately after the 30 minute perfusion. The bone marrow was carefully scooped out after the cortex bone was nibbled away. It was cut in small cubes and washed in chilled cacodylate buffer solution for one hour. Refixation was done by 1% OsO_4 -cacodylate buffer or OsO_4 -phosphate buffer for one hour. After routine dehydration, Epon embedding and cutting, sections were stained by uranyl-acetate lead double stain.

Results

1. *General findings*: The sinusoidal wall was composed of a thin layer of endothelium, which was usually continuous except in portions where the cellular element was attached to the endothelium (Fig. 1). At these portions gaps of various sizes were encountered within the endothelium (Figs. 1, 2, 3, 5, 8, 9, 10, 11). The gaps were usually filled with the cytoplasm of the attached cellular elements, including granulocytes, erythrocytes, megakaryocytes, lymphocytes and reticulum cells. Free gaps not associated with cellular element were extremely rare. No distinct basement membrane was observed along the parenchymal aspect of the sinusoidal endothelium. Only occasionally condensations of moderately dense homogeneous or filamentous materials were noted at this portion. These may correspond to the rudimentary basement membrane. A peculiar type of cell was noted apposed to the parenchymal aspect of the sinusoidal wall, which will be reported elsewhere.

2. *Thorotrast*: Thorotrast particles were readily recognizable as small dense dots (200 Å) wherever they were present. They were present in the lumen of sinusoid, in the sinusoidal endothelium and the reticulum cell, and freely

SINUSOIDAL PASSAGE OF PARTICLES



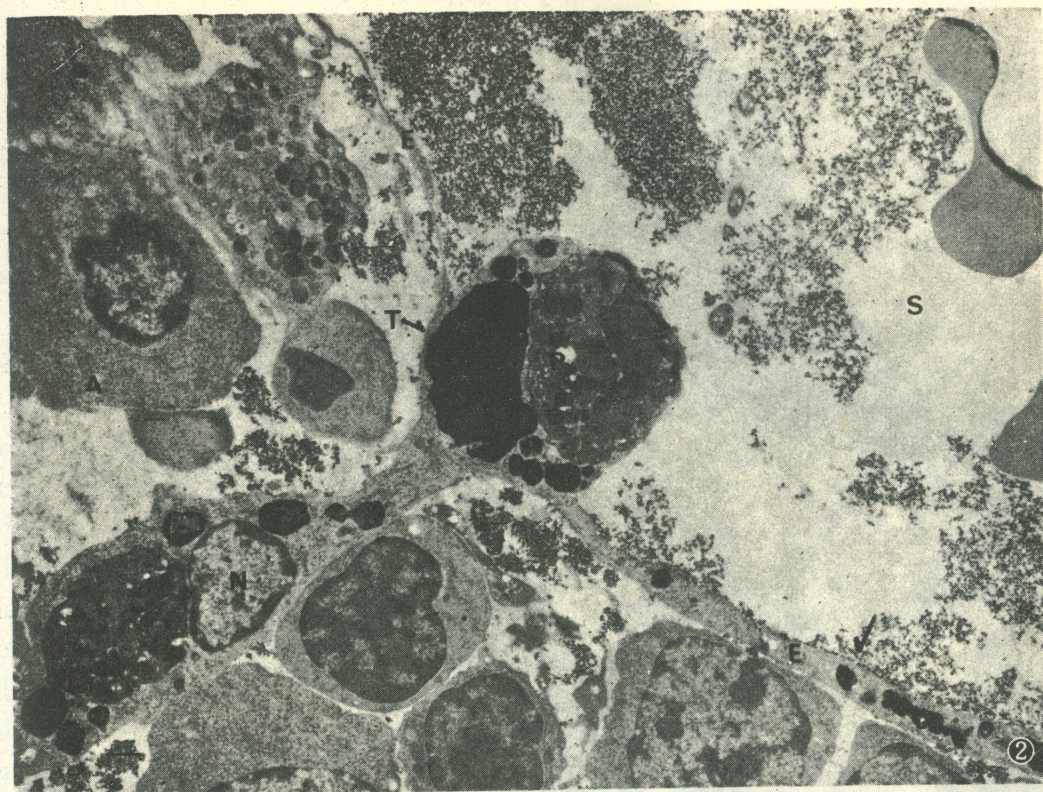


Fig. 2. A portion of the bone marrow from the rabbit killed 2 hours after thorotrast injection. The sinusoid (S) is seen in right upper half of the figure. Numerous thorotrast particles are seen within the sinusoidal lumen. In the sinusoidal endothelium (E) aggregates of thorotrast are seen (arrows). In the left lower corner a reticulum cell is seen, which sends into the sinusoidal lumen a large cytoplasmic projection (P) in which aggregates of thorotrast (T) and degenerated cell are phagocytized. Thorotrast particles are also seen in the extracellular space of parenchyma.

dispersed in the intercellular space of marrow parenchyma (Fig. 2). In the sinusoidal lumen, thorotrast particles tended to attach on the cell membrane of endothelial cells. In the endothelial cells the majority of thorotrast particles were already forming large aggregates probably of lysosomal nature (Fig. 2). Particles engulfed within pinocytotic vesicles were only occasionally observed. The distribution of particle aggregates in the endoplasm as well as in the pinocytotic vesicles in the ectoplasm was rather diffuse, although in the former they were usually formed in the thick portions of the cells. In the reticulum cells, as in the case of endothelial cells, thorotrast particles formed aggregates of varying sizes

Fig. 1. A low power view of a sinusoid. One hour after injection of India ink. The sinusoidal endothelium shows no discontinuity except in portions where reticulum cells are in contact with the sinusoid (arrows). In these portions the reticulum cells project small cytoplasmic process into the sinusoidal lumen (S) through the endothelial gap. Carbon particles are seen adhered to the surface of the endothelium.

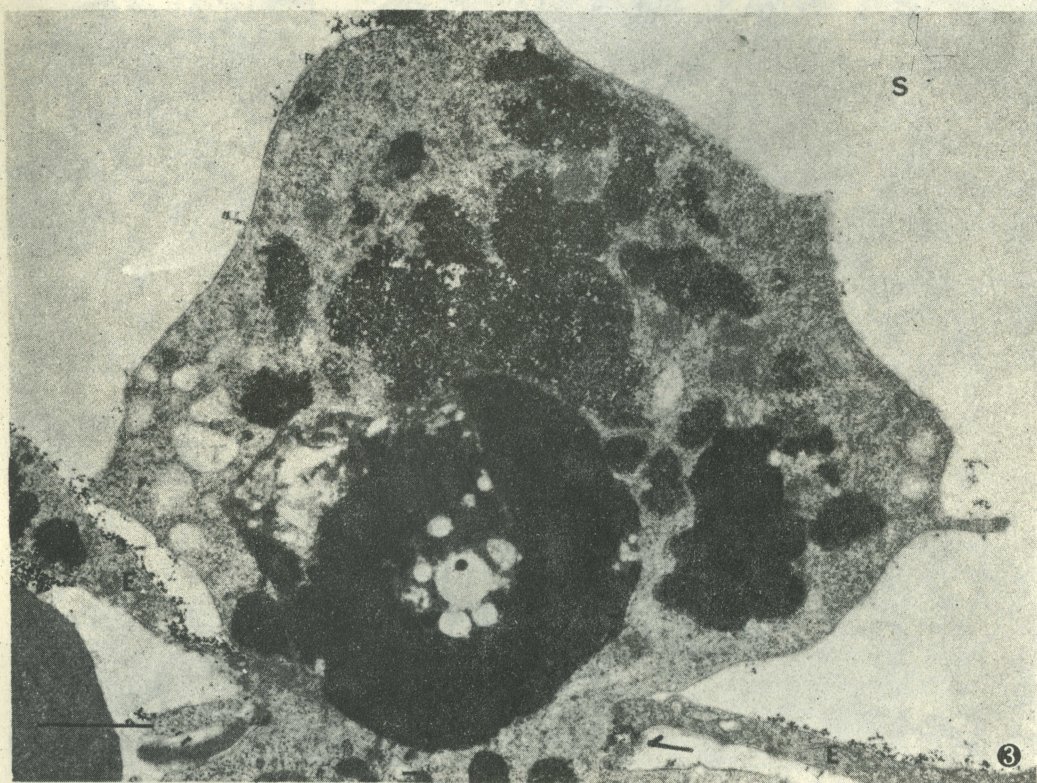


Fig. 3. An intraluminal projection of a reticulum cell. Two hours after the thorotrast injection. A number of aggregates of thorotrast particles are seen in this portion. In very dense aggregates individual particles are obscured. Thorotrast particles are seen on the luminal surface of the sinusoidal endothelium (E), within the endothelium and freely dispersed in the extracellular space of the marrow parenchyma. Small numbers of the thorotrast particles are observed in the narrow space between the edge of the endothelial gap and the plasma membrane of the reticulum cell filling the gap (arrow).

(Figs. 2 and 3). In many instances, the reticulum cell protruded a part of its cytoplasm through the gap of endothelial lining into the sinusoidal lumen. The thorotrast particles were frequently seen in the intraluminal projection of the reticulum cell (Figs. 2 and 3). The thorotrast was never found in the granulocytes, lymphocytes, plasma cells, megakaryocytes, fat cells as well as mature or immature erythrocytes.

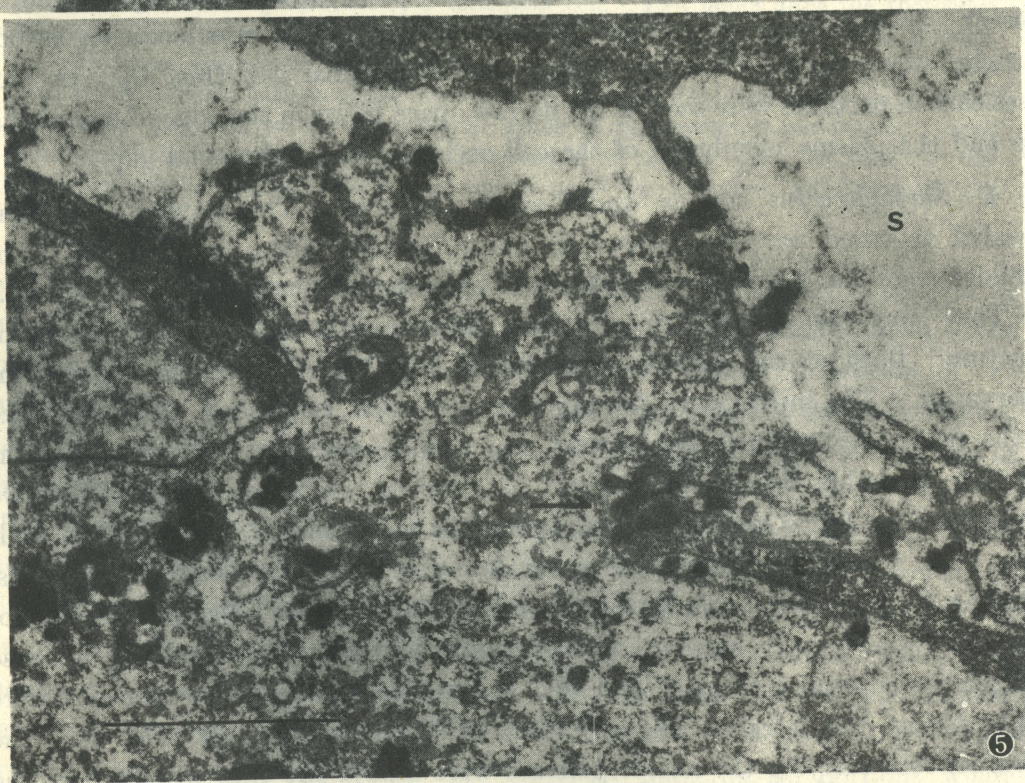
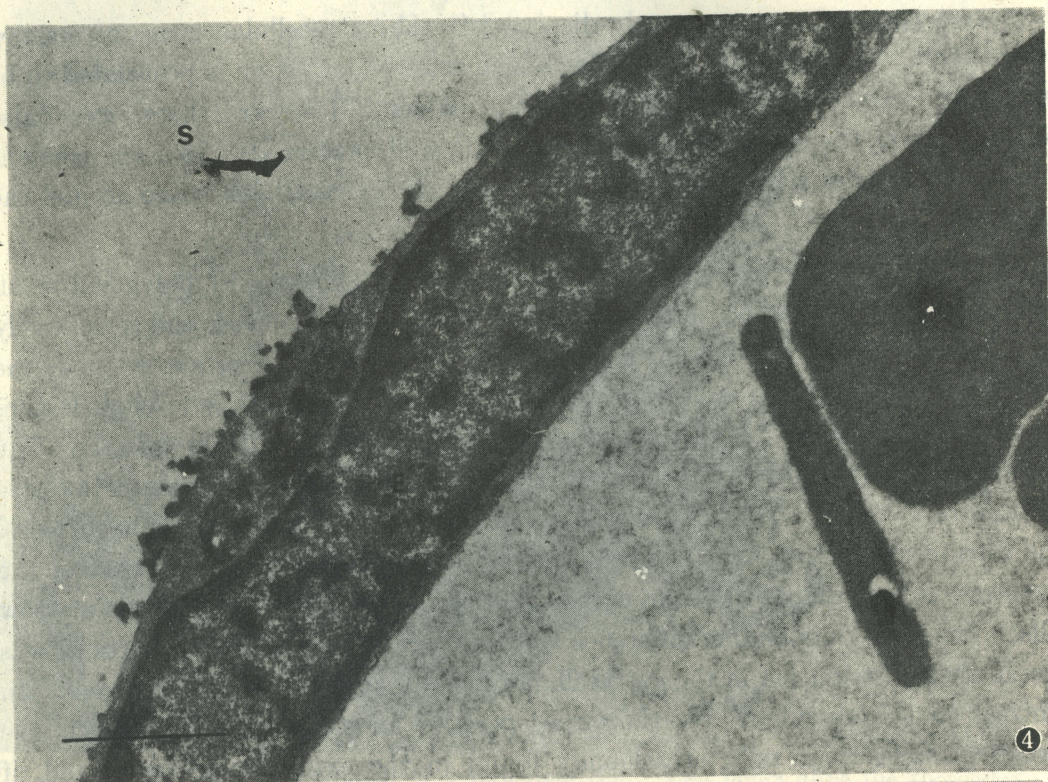
Dispersed thorotrast particles were also found in the ground substance of extracellular space. They were usually present in the area near the sinusoids but could also be demonstrated in part 20 to 30 μ away from the sinusoidal wall. It seems noteworthy that the free openings of the sinusoidal endothelium were frequently encountered in the bone marrow from the thorotrast-treated animals, though such openings were only rarely found in animals from control and other experimental groups. The openings were especially numerous in portions where

thorotrast particles were massively dispersed in the extracellular space of marrow parenchyma. The majority of the free openings of endothelium is postulated to be artificially formed by thorotrast administration. However, there were also portions where the sinusoidal endothelium was well preserved. In such portions thorotrast particles were also found dispersed in the extracellular space around the sinusoid.

3. *Carbon*: The carbon particles were found as spherical or polygonal small electron dense particles measuring about 0.03 to $0.05\ \mu$ in diameter and were easily identified under the electron microscope. By the perfusion fixation method, the carbon particles were almost completely washed away from the sinusoidal lumen, except those attached to the endothelial cell membrane (Figs. 1 and 4). The endothelial cells phagocytized carbon particles in the same manner as in the case of thorotrast (Fig. 4). Carbon particles were usually enveloped within phagosomes. The distribution of the carbon in reticulum cells was also the same as that of the thorotrast in reticulum cells. No carbon particles were found in other bone marrow cells. The most outstanding difference between carbon and thorotrast particles was seen in their distribution in the extracellular space of the marrow parenchyma. In contrast to the abundant free thorotrast particles in the extracellular space, the carbon particles were only occasionally found in the extracellular space and, if present, always near the sinusoidal area (Fig. 5). On rare occasion carbon particles were seen in transient through the endothelial gap (Fig. 5). In such cases carbon particles were seen in the narrow space between the edge of endothelial gap and the plasma membrane of the cell occluding the endothelial gap.

4. *Staphylococcus aureus* and *Candida albicans*: The staphylococci were revealed as ovoid particles measuring $0.6\sim0.7\times0.76\sim0.88\ \mu$ in diameter. The candidas were also found as roughly ovoid particles measuring $2.2\sim3.7\times3.7\sim4.7\ \mu$. They were easily identifiable by their characteristic trilamellated membrane structure. Both of the particles were found in a few numbers throughout the observation. The injection of the more concentrated suspension than that used in the present experiment had caused the sudden death immediately after the injection, probably due to pulmonary or cerebral embolism. All of the two organisms were found only restricted within the cytoplasm of the reticulum cells (Figs. 6, 7, 8 and 9). They were never found in the other bone marrow cells, including the endothelial cell, and in the extracellular space. The engulfed microorganisms were usually present in phagosomes and were enveloped by a dense homogenous substance (Figs. 7 and 9). A number of the microorganisms were caught within the intraluminal projection of the reticulum cell (Fig. 6). The reticulum cells carrying the microorganisms were always the ones located near the sinusoid.

SINUSOIDAL PASSAGE OF PARTICLES



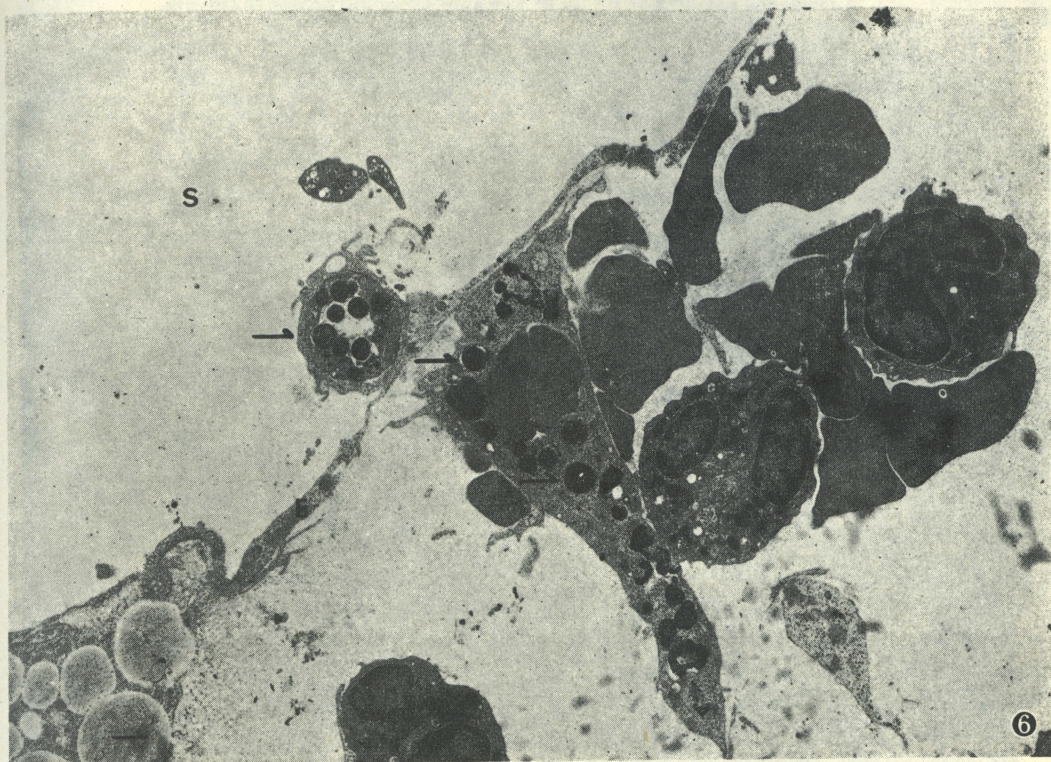
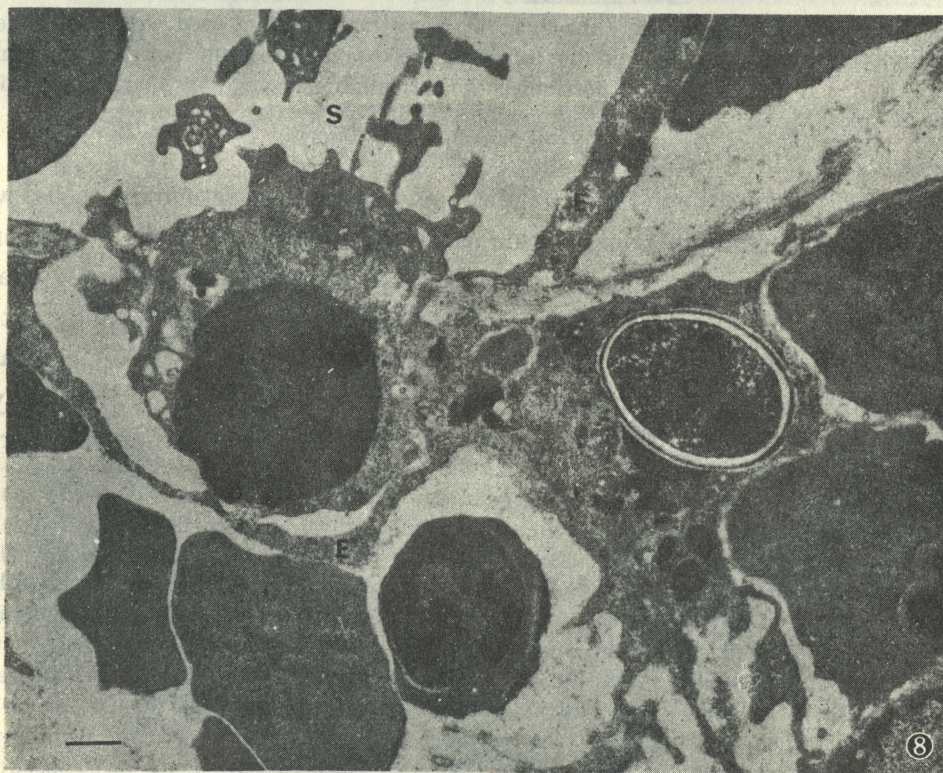
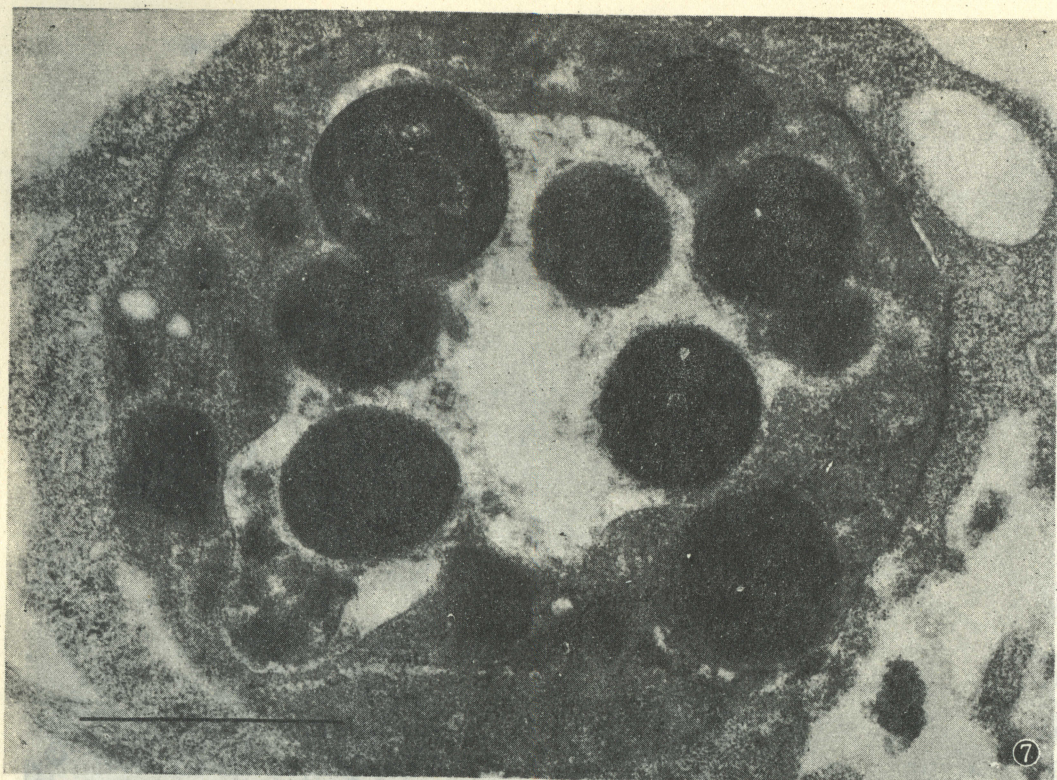


Fig. 6. A low power view of the bone marrow, from the rabbit injected with OsO_4 -fixed staphylococci, 2 hours. A number of staphylococci are seen in both intraluminal projection and extrasinusoidal portion of the reticulum cell (arrows). No staphylococcus is seen within the endothelial cell.

5. *Erythrophagocytosis*: Erythrocytes enveloped by reticulum cells were frequently found during the observation (Figs. 10 and 11). This phenomenon cannot be categorized as the phagocytosis of foreign particles, but is closely related to the problem under discussion in that the erythrophagocytosis is a phagocytosis of particles of larger size. From this point of view the author has made a detailed observation on the erythrophagocytosis in the present materials. Erythrocytes were engulfed by reticulum cells but not other cellular elements in the bone marrow

Fig. 4. A portion of the sinusoidal endothelium. India ink injection, 1 hour. Carbon particles are seen attached to the luminal surface of the endothelial cell. Particles are also present in the membrane-bound vacuoles in the cytoplasm of the endothelial cell. No basement membrane is observed on the parenchymal aspect of the endothelium. No carbon particles are seen in the extracellular space of the marrow parenchyma.

Fig. 5. An intraluminal projection of a reticulum cell. India ink injection, 1 hour. Sinusoidal lumen is seen in the upper half of the figure. Carbon particles are seen attached to the surface of the intraluminal projection and within the cytoplasm of the reticulum cell. In the latter, the particles are enveloped by a thin membrane. Several carbon particles are seen just in transient through the narrow space between the edge of the endothelial gap and the plasma membrane of the reticulum cell (arrow).



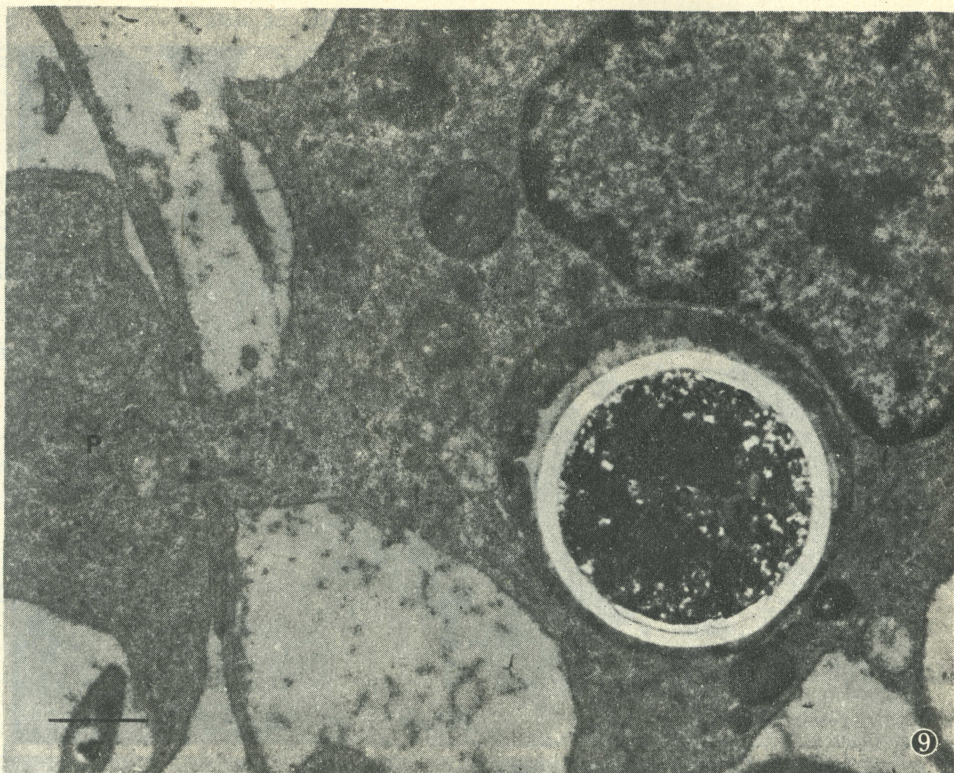


Fig. 9. A portion of a reticulum cell of the bone marrow from a rabbit killed 1 hour after the injection of OsO_4 -fixed candidas. A candida is observed in the extrasinusoidal portion of the reticulum cell. S: sinusoidal lumen, P: intraluminal projection of the reticulum cell.

including sinusoidal endothelium. This indicates that erythrocytes are only phagocytized by reticulum cells in the bone marrow. Engulfed erythrocytes were present both in the intraluminal projection and in the extrasinusoidal portion of the reticulum cell. The erythrocytes present in the extrasinusoidal portion of the reticulum cell usually showed more or less advanced state of digestion. In a favorable section (Fig. 11) was observed reticulum cell which engulfed a relatively unaffected erythrocyte in its intraluminal projection and some degenerated erythrocytes, some of them were already enveloped within a lysosomal granule, in its extrasinusoidal portion. Except in few instances the nucleus was always situated within the extrasinusoidal portion of the cell.

6. *Summary of findings:* The distribution of the injected foreign particles

Fig. 7. A higher magnification figure of the intraluminal projection of the reticulum cell shown in Fig. 6. Bacteria are already enveloped within the vacuolar structure.

Fig. 8. A reticulum cell in the bone marrow from the animal killed 1 hour after the injection of OsO_4 -fixed candidas. A candida is seen in the extrasinusoidal portion of the reticulum cell (C). This cell projects into sinusoidal lumen a cytoplasmic process in which an erythrocyte is phagocytized.

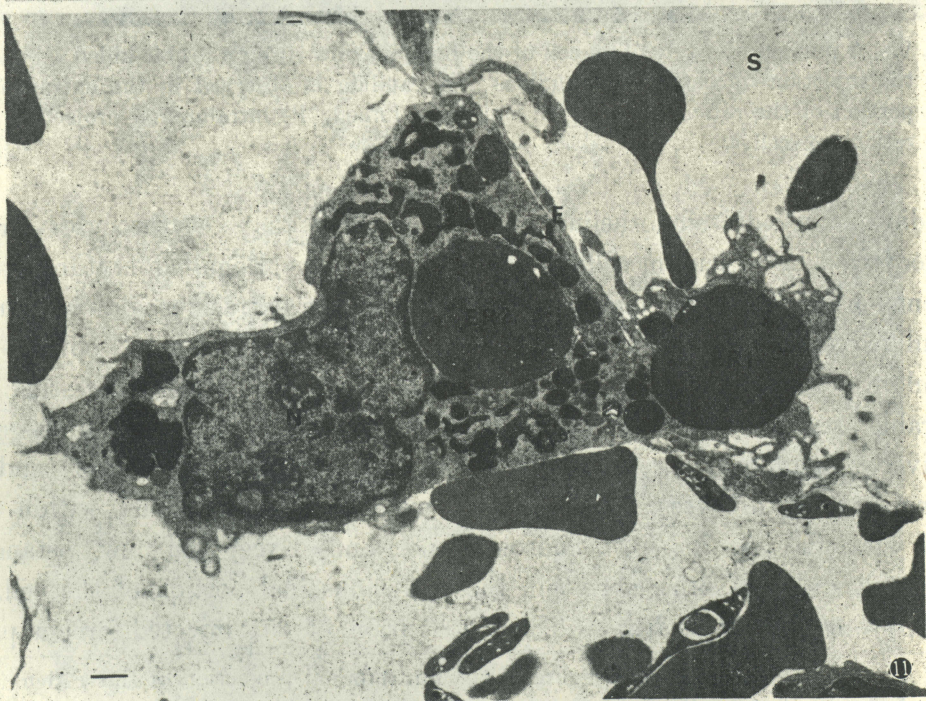
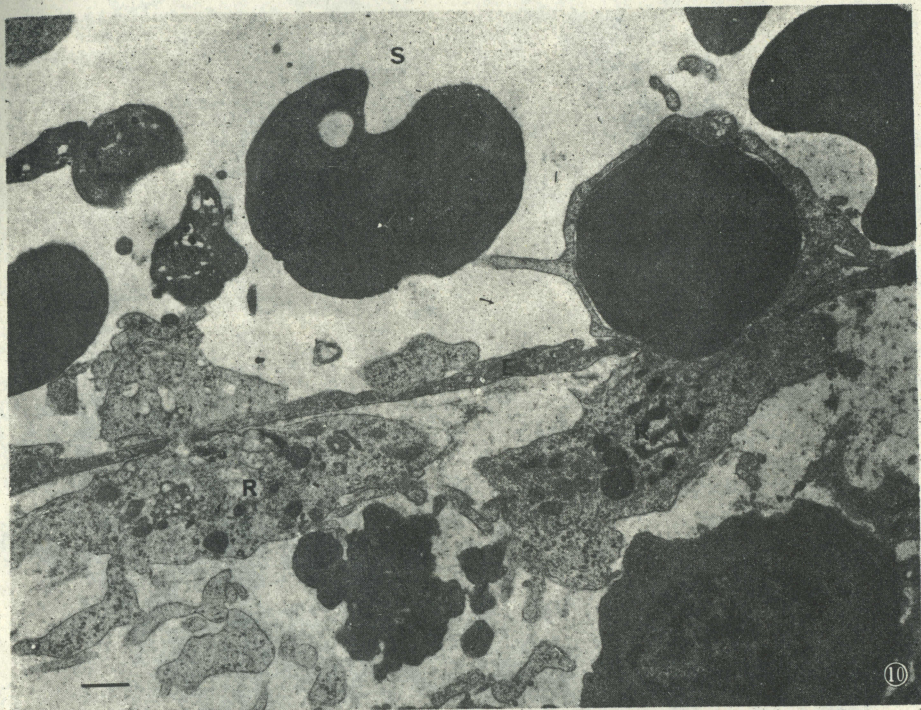


Table 1. *Distribution of Particles in Bone Marrow*

Location	Particle Size	Thorotrast		Carbon		Staphylococcus Aureus		Candida Albicans		Erythrocyte
		200Å		0.05-0.03μ		0.6-0.8μ		3-5μ		6-8μ
	Time(hr)	1	2	1	2	1	2	1	2	
Sinus Lumen		+	+	+	-	0	0	0	0	
Endothelial Cell:										
1) Cell surface		+	+	+	+	0	0	0	0	0
2) Intra-cellular		++	++	+	++	0	0	0	0	0
Reticulum Cell:										
1) Intra-luminal portion		++	++	+	±	3	2	1	6	34
2) Near Sinus		+	++	++	++	10	8	14	8	13
3) Distant from Sinus		++	+	+	++	0	1	0	2	0
Marrow Space:										
1) Near Sinus		+	+	+	±	0	0	0	0	
2) Distant from Sinus		+	+	±	-	0	0	0	0	

and erythrophagocytosis in the bone marrow are summarized in Table 1. While thorotrast and carbon particles were seen in both endothelial cell and reticulum cell, staphylococci and candidas were found only restricted within reticulum cell. In the extracellular space of marrow parenchyma both thorotrast and carbon particles were present, though the latter particles were far less in number than the former. Staphylococci and candidas were never observed in the extracellular space in marrow parenchyma. Erythrophagocytosis was seen in the reticulum cell but not in the sinusoidal endothelium.

Discussion

The technique of fixing animal tissue by injecting fixatives into the blood vessel for electron microscopic study was first introduced by PALAY *et al.*²² in 1962.

Fig. 10. The bone marrow from a rabbit killed 1 hour after the injection of India ink. Two reticulum cells are present in contact with sinusoidal endothelium. Both cells send cytoplasmic process into the sinusoidal lumen through the endothelial gap. In one of these intraluminal projections an erythrocyte is engulfed. S: sinusoidal lumen, E: sinusoidal endothelium.

Fig. 11. A reticulum cell seen in the bone marrow from the rabbit killed one hour after the injection of India ink. An erythrocyte (ER 1) is caught in the intraluminal projection of the reticulum cell. In the extrasinusoidal portion of the cell there is an erythrocyte in a more advanced state of digestion (ER 2) than that in the intraluminal portion. A number of phagosomes are present in the extraluminal cytoplasm. S: sinusoidal lumen, E: sinusoidal endothelium, N: nucleus of the reticulum cell.

They intended to fix neural tissue by perfusing OsO_4 in acetate-veronal buffer from the aorta and prepared a balanced solution for the purpose of washing the pre-existing blood. They also estimated that the suitable pressure for the perfusion was about 5 ft. Later, KARLSSON and SCHULTZ^{17,18}, and PEASE²⁵ have studied this problem. The former investigators concluded that the aldehyde fixation was better than the osmium and that the use of prewash, vasodilator, cooling, etc., were not necessary. Although the perfusion method has been used for fixing a variety of tissues, no attempt has previously been made for the fixation of the bone marrow. In the present study, the sinusoidal wall of the bone marrow fixed by the perfusion method showed far less discontinuities, except in the bone marrow from animals injected with thorotrast, than that seen in the preliminary experiment using the conventional immersion method. Several authors^{4,5,16} have maintained that the endothelial gap is an artifact produced by mechanical damage during excision and/or improper fixation, and this artifact is largely prevented by the use of glutaraldehyde fixation. In the present materials practically no endothelial gaps were observed except in portions where the cellular elements including blood cells and reticulum cells were in close contact with the sinusoidal wall. This finding seems to support the view expressed by HUDSON and YOFFEY¹² that the gaps may only be transient in nature and may eventually disappear after the cell has passed through.

Thorotrast and carbon have been most commonly used for studies on the passage of materials through the endothelial lining of the marrow sinusoid. ZAMBONI and PEASE³⁵ have intravenously injected the thorotrast in guinea pig and observed the bone marrow after conventional fixation. They found that the thorotrast particles dispersed throughout the marrow parenchyma within 10 minutes and interpreted this phenomenon as sufficient evidence of functional patency of the sinusoidal wall. They concluded that these vessel walls are incomplete, and that an "open" circulation exists. In my experiment discontinuities were frequently seen in the sinusoidal endothelium in those materials injected with thorotrast, especially in portions where the dispersion of thorotrast particles was intensive. Since such openings were not observed in other experimental groups, they were postulated to be of non-physiological nature induced by the thorotrast administration. HUHN¹⁶ has stressed the mast cell- and blood vessel-activating effect of dextrin contained in thorotrast. However, the possibility of actual leakage of thorotrast particles through endothelial gaps was not completely excluded, because the extracellular particles were also observed around the sinusoidal wall showing no discontinuity.

ZAMBONI and PEASE³⁵ have described the presence of thorotrast particles in the cytoplasm of immature granulocyte and megakaryocytes, but the present

author failed to demonstrate the thorotrast within the marrow parenchymal cells other than reticulum cells and endothelial cells.

The distribution and time sequence study of carbon particles in the bone marrow have been minutely studied since the light microscopic era^{9,10,11,20,23}. It seems that all investigators have reached an agreement on this subject. At the very early stage, - 10 minutes after the injection of the particles - the carbon particles are engulfed only by the sinusoidal endothelium. Phagocytosis of carbon particles can be found in parenchymal reticulum cells near the sinusoids about one hour later. Carbonladen cells which are far away from the sinusoid are not observed until two to three hours later. The phagocytized carbon particles then remain in the cytoplasm for a long time, but about one week later, the distribution of the carbon in the above three kinds of cells are reversed. The reticulum cells which are situated distant from sinusoids contained more carbon particles than those located near the sinusoids and the sinusoidal endothelium. HUDSON and YOFFEY¹³ have shown similar results by means of electron microscopy. They have shown that the carbon particles are confined within vascular lumen and sinusoidal endothelium 5 minutes after injection of India ink and appear in macrophages in the marrow parenchyma 15 minutes after injection. Since the aim of the present experiment was not to pursue the entire time sequence of the injected material, any further discussion on literature would not be appropriate. However, with regard to the certain time intervals, this experiment was in full agreement with those of previous authors.

Reports on the electron microscopic observation concerning the behavior of injected microorganisms in the bone marrow have not presently been available to the author. Electron microscopic observation on the phagocytosis of staphylococcus and other bacteria has appeared in literature^{6,7,26,33}. However, all dealt with the mechanism of phagocytosis of bacteria by phagocytes and the subsequent change in phagocytized bacteria or the attention was focused on the influence of the virulent strains on the cells. In this research, the microorganisms were all killed by OsO_4 and then washed thoroughly before being introduced into the animal and, therefore, they were assumed to act as non-active foreign particles of relatively large size. In the present experiments it was clearly shown that both microorganisms were phagocytized only by reticulum cells and not by endothelial cells. No free organisms were encountered in the extracellular space of marrow parenchyma.

Concerning the pathway through which the particulate matter enters from the sinusoidal lumen into the marrow parenchyma no definite conclusion has yet been obtained. Several possibilities have been discussed, i.e. 1) transfer of the particles from the endothelial cells to the reticulum cells, 2) transformation of sinusoidal

endothelium (littoral cell) into free reticulum cell and 3) leakage of particles through sinusoidal wall. The time sequence study of injected carbon particles in the bone marrow as described above seems to support the first possibility. However, the actual findings indicating the direct transfer of particles from cell to cell has not yet been demonstrated. It may be postulated that the particles are at first taken up by the endothelial cells and then released into the marrow space where they are ultimately phagocytized by reticulum cell. This explanation may be pertinent to the passage of thorotrast particles through sinusoidal wall but not fully adequate to carbon particles, because the latter particles were only occasionally observed in the extracellular space in marrow parenchyma. To explain the passage of microorganisms across the sinusoidal wall the transfer mechanism is not appropriate because the microorganisms were found neither in the endothelium nor in the extracellular space. WEISS^{31,32} has maintained that the sinusoidal endothelium and extravascular reticular cells form a reciprocating system wherein portions may become vascular or extravascular depending upon the requirement for hematopoiesis, blood flow, blood storage and delivery of cells to the blood. In the present material the sinusoidal wall revealed a continuous lining and the transition between endothelial cells and parenchymal reticulum cells was not observed. The discrepancy between WEISS' observation in rat bone marrow and the present one may be due to the species difference or the difference in functional state. However, so far as the untreated rabbit bone marrow is concerned, the author has been forced to conclude that the mutual transformation between sinusoidal endothelium and reticulum cell does not exist. As the third possible way of passage across sinusoidal wall the leakage of particulate matter through endothelial gaps has been assumed. HUDSON and YOFFEY¹³ have denied such a possibility for carbon particles. However, the carbon particles were, though in small numbers, clearly demonstrated in the extracellular matrix in the present material. It was also noted that the carbon particles were present in the narrow space between the edge of the endothelial gap and the plasma membrane of the reticulum cell nearly obstructing the gap. This finding strongly indicates that the carbon particles can pass through the endothelial gap. However, this mechanism does not seem to be the principal pathway of carbon particles, because such endothelial gaps were not observed so sufficiently in number to explain the massive movement of carbon particles from sinusoidal lumen into marrow parenchyma. This may work as a supplementary mechanism of other pathways. The leakage through endothelial gap may occur in the case of thorotrast, but the break-down of the endothelial lining due to thorotrast administration may be responsible for the massive leakage of the particles. For the passage of microorganism across the sinusoidal wall, the leakage through the gap may not be

operative, because the organisms were never found in the extracellular space of marrow parenchyma. Thus the three possible pathways mentioned above seem to be not applicable for the passage of the particles larger than the carbon. To explain the passage of these relatively large particles, other mechanism should be supposed. In this connection the direct capture of particles in the blood stream by means of the intraluminal projection of the reticulum cell, as suggested by WATANABE^{28,29,30}, seems to be worth to note. It was frequently observed in the present experiment that the reticulum cells in juxtasinusoidal position sent their cytoplasmic projection into the sinusoidal lumen through the endothelial gap. The foreign particles of all sizes used were frequently seen engulfed within the intraluminal projections of reticulum cells. It seems reasonable to assume that the materials taken up by means of the intraluminal projection are transported into the extrasinusoidal portion of the reticulum cell. HUDSON and YOFFEY¹³ have reported that they have observed a similar finding on infrequent occasions in guinea pig bone marrow, but they also mentioned the reverse possibility that such phagocytic cells were themselves in transient across the endothelium. Though it is difficult to reach any decisive conclusions concerning this problem, the author postulates the former possibility being more probable than the latter, because 1) in a majority of cases the nucleus of the reticulum cells situated in extrasinusoidal portion and 2) the extrasinusoidal portion of such reticulum cell usually contained phagocytized material in a more advanced state of digestion than those in intraluminal portion.

Based upon the assumption presented above, it seems that there is a distinct difference between smaller and larger particles in pathways by which the particulate matter passes across the sinusoidal wall. For the smaller particles, including thorotrast and carbon, all three pathways, i.e. *via* endothelium, *via* endothelial gap and *via* reticulum cell, are probably pertinent, whereas the larger particles including staphylococci and candidas, can enter into the marrow parenchyma only *via* reticulum cell. Particle size seems to be an essential factor for such difference in passage through the endothelial wall.

Erythrophagocytosis by reticulum cells was frequently encountered in the present materials, but erythrocytes were never found enclosed in endothelial cells. Similar results were reported by HAGINO⁸, using light microscope, in the bone marrow from a patient with sepsis. It was frequently observed that the erythrocytes were taken up by means of the intraluminal projection of reticulum cells. Since these findings are well in accord with the transport of the particles of larger size across sinusoidal wall, they are supposed to indicate the sequestration process of aged erythrocytes from the circulating blood. The presence of the erythrocytes in advanced destruction and of the digestive vacuoles filled with ferritin-like parti-

cles in the extrasinusoidal portion of such reticulum cells may support this view. It was occasionally observed that erythroblasts were closely apposed to the surface of the extrasinusoidal portion of the reticulum cells, simulating the erythroblastic islet proposed by BESSIS^{1,2,3,21} (Fig. 8). Contrary to the present observation, no descriptions have been made concerning the pictures suggesting the sequestration of erythrocytes by the reticulum cell in the bone marrow from animals other than rabbit. This may reflect the fact, as reported by HUGHES JONES and others^{14,15,19}, that the major site of red cell destruction is the bone marrow in the rabbit, while a small percentage may be destroyed in the bone marrow in the rat.

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