The Participation of the Platelet in the Vascular Response to Endotoxemia in the Rabbit Eye

Edward L. Howes Jr, MD, Donald G. McKay, MD and
William Margaretten, MD

Platelet depletion by a specific goat antirabbit platelet antiserum profoundly affected the response of the ocular blood vessels to intravenous endotoxin. The altered permeability of the vessels of the iridial portion of the ciliary processes in the thrombocytopenic animals was reduced by 50% at 1½ hours and by 30 to 58% at 4 hours after endotoxin administration. Intravascular fibrin at the 4-hour period could be eliminated by a properly timed platelet depletion. By preventing platelet aggregations and fibrin formation, the permeability alteration could be reduced by 66% and the usual stromal hemorrhages practically eliminated. Underlying these effects of the platelet was evidence of a vascular trauma unrelated to platelet activity and probably dependent to some extent on adrenergic stimulation. Platelet aggregation and/or fibrin formation had to be superimposed on the underlying vascular trauma to produce endothelial denudation (Am J Pathol 70:25–44, 1973).

THIS STUDY was undertaken to determine the role of the platelet in ocular response to endotoxemia in the rabbit. A single intravenous injection of bacterial endotoxin produces an acute prolonged alteration in the permeability of the vessels of the iridial portion of the ciliary process,[1,2] that portion of the process apparently responsible for the elaboration of aqueous humor.[3] An initial edema of the processes is followed by congestion, hemorrhages, occlusive platelet-fibrin thrombi and strands of fibrin within vessels and around endothelial cells.[4] Intravenous endotoxin is known to cause a profound and immediate decrease in the number of circulating platelets,[5] with the consequent appearance of platelet aggregates in the lung[6] and also in the small vessels of the ear[7] and mesentery[8] of the rabbit. Both *in vitro*[9] and *in vivo*,[5] the interaction of endotoxin and platelet results in the release of serotonin and probably other vassoactive substances, as well as platelet factor 3.[10] Recent studies have indicated that the release of vasoactive substances from the platelet is a key event in causing an initial alteration in vascular permeability in both

From the Department of Pathology, University of California School of Medicine, San Francisco General Hospital, San Francisco, Calif.

Supported by Grants EY-00056 and HL-12033 from the National Institutes of Health.
Accepted for publication Sept 19, 1972.

Address reprint requests to Dr. Edward L. Howes Jr, Department of Pathology, University of California School of Medicine, San Francisco General Hospital, San Francisco, Calif 94110.

the active Arthus reaction [11] and probably in serum sickness.[12] At the same time, the platelet has been found to be essential for the formation of glomerular fibrin in the generalized Shwartzman reaction in the rabbit.[13]

In this study, rabbit platelets were depleted by a specific immune serum and the ocular response examined during the period of initial edema of the ciliary processes and the period of fibrin formation, 1½ and 4 hours after endotoxin administration, respectively. The altered vascular permeability was readily quantitated by labeled albumin,[2,4,14] and histopathologic changes were evaluated. It was found that platelet depletion reduced the permeability alteration by 50% at 1½ hours and from 30 to 58% at 4 hours. By a proper timing of immune platelet depletion, intravascular fibrin formation was virtually absent at 4 hours and histopathologic changes were reduced, but some subendothelial fibrin formation still occurred. Heparin administration in platelet-depleted animals reduced the permeability alteration by 66% and practically prevented histopathologic changes at 4 hours. In the absence of platelets and fibrin formation, evidence of endothelial change was limited to a subendothelial edema and a few scattered small hemorrhages.

Materials and Methods

Albino rabbits of either sex, weighing 1800 to 2000 g, were used throughout these experiments. Endotoxin, *Escherichia coli* 055:B5 (Difco Laboratories, Detroit, Michigan), was injected into the marginal ear vein in 200 μg quantities dissolved in 2.0 ml of saline. Animals were studied at 1½ and 4 hours after endotoxin injection. Human serum albumin labeled with iodine (^{125}I-HSA) was given intravenously in 10- to 15-μl quantities 15 minutes before killing the animals. Cardiac blood was obtained at time of sacrifice, and the eyes enucleated. In some cases, both eyes were used for isotope quantitation and then processed for light microscopy; but, usually, one eye was used for isotope determination and the other processed for light and electron microscopic study.

Isotope Determinations

The accumulation of ocular ^{125}I-HSA relative to that in heart plasma was expressed as a theoretical volume, the total albumin space (TAS),[2,14] and was determined in the following fashion:

$$\text{TAS } (\mu\text{l/g of eye tissue}) = \frac{\text{counts/min of } ^{125}\text{I-HSA/g of eye tissue}}{\text{counts/min of } ^{125}\text{I-HSA/ml of cardiac plasma}}$$

A second theoretical space, the extravascular albumin space (EVAS) was then calculated: [2,14]

$$\text{EVAS } (\mu\text{l/g of eye tissue}) = \text{TAS} - \text{plasma volume } (2.90\ \mu\text{l/g})$$

In previous studies, blood volume and plasma volume had been determined using a dual radio-isotope technic, and there was no difference in control and endotoxin-injected animals.[2,4] The average volume for plasma volume in all these

studies was 2.90 μl/g of eye tissue; this figure was used as a constant plasma volume in the present study.

Light and Electron Microscopy

For routine light microscopy, tissues were fixed in 4% buffered formalin. For quantitation of histopathologic change and for electron microscopy, fixation was initially carried out in 1.75% glutaraldehyde in 0.05 M cacodylate buffer. After enucleation, the eye was immediately bisected, lens removed, and the anterior segment subdivided and immersed in fixative at 4 C. Tissues were fixed overnight, and sections then made of the iridial portion of the ciliary processes throughout its circumference. The sections were postfixed in 2% osmium tetroxide, dehydrated and embedded in Araldite. Sections 1 to 2 μ in thickness were stained with methylene blue and Azure II and studied by light microscopy. Appropriate specimens were then further examined with an RCA EMU-3G microscope after thin-sectioning and staining.

Experimental Design

Immune Platelet Depletion

Thrombocytopenia was produced with goat antirabbit platelet antiserum which had been prepared in a single goat from washed rabbit platelets, as reported previously.[11,13] White blood cell and platelet counts were determined in many cases prior to endotoxin injection and in all cases at the time of sacrifice. Marked thrombocytopenia was produced in 30 minutes and was maintained for at least 8 hours. Platelet counts were usually less than 5000/cu mm and often 1 to 2000/cu mm in animals receiving platelet antiserum, and depressed to the same level in both endotoxin-treated test and control animals.

During the course of the experiments, it was found that platelet antiserum given ½ to 1 hour before endotoxin administration enhanced intravascular fibrin formation at 4 hours after endotoxin administration. To evaluate the effect of this formation, three different groups of animals were established.

Group I. Animals were injected intravenously with 1.0 ml of platelet antiserum over a 10-minute period, 30 to 60 minutes prior to intravenous endotoxin injection. Controls were given endotoxin alone.

Group II. Animals were given platelet antiserum in a similar fashion but additionally received intravenous heparin (3000 units/kg) at the time of endotoxin administration and 2 hours later. Animals given only heparin, or heparin and endotoxin, in a similar dosage schedule were available for comparison.[15]

Group III. Animals were injected with platelet antiserum 4 hours prior to endotoxin administration. One intravenous injection of heparin (3000 units/kg) was also given at the time of platelet antiserum injection. Controls were given heparin and 4 hours later endotoxin was injected.

Within each group, animals were examined at 1½ and 4 hours after endotoxin injection. Each test and each control group was comprised of at least 6 animals.

Leukocyte Depletion

To evaluate and compare the effects of a similar type of immune depletion of formed blood elements, an antibody to rabbit granulocytes was employed. The antibody was also prepared in goats following multiple injections of washed heterophiles in complete Freund's adjuvant.[16] Immune granulocytopenia lasted several hours. White blood cell counts 15 minutes after administration of anti-

serum ranged from 500 to 1000/cu mm, at 1 hour from 1000 to 1500/cu mm, and at 2 hours from 1500 to 2000/cu mm. Results of a differential count showed that most of the cells were lymphocytes and monocytes. Heterophiles comprised less than 50 of the total number of cells at 15 minutes and less than 150 at 2 hours. Platelet counts were virtually unchanged. Leukocyte antibody was given 15 minutes prior to endotoxin injection; evaluations were only made at 1½ hours after endotoxin. At time of sacrifice, average white blood cell counts were 510/cu mm in animals that received leukocyte antiserum and endotoxin and 2300/cu mm in animals injected with endotoxin alone. No attempt was made to study the 4-hour period because of the short duration of action of the granulocyte antiserum.

Results

As in previous studies, circulating endotoxins profoundly altered ocular vascular permeability. This effect was markedly evident at 1½ hours, and the alteration was maintained for at least 4 hours and returned toward normal by 24 hours.[2,4] The anatomic location of this altered permeability was only in the iridial portion of the ciliary process, thus making it possible to make a complete histopathologic evaluation of the change.[4,17] Microscopic change in this region at 1½ hours was primarily an edema of stroma and ciliary epithelium. In examining many processes in the present study, it was apparent that approximately one third of all animals also showed some evidence of intravascular platelet aggregation focally in a few processes. At 4 hours, numerous platelet aggregates, intravascular and mural fibrin, congestion of vessels and stromal hemorrhages were seen in addition to edema.

Early Period

1½ Hours After Endotoxin Injection

There was some variation in the time of onset of the ocular response after endotoxin injection. In both control and test groups a few animals had not as yet responded, and this was reflected in the large standard deviations (Table 1). The average ocular albumin space for each of the experimental thrombocytopenic groups was 50% of control values (Table 1 and Text-figure 1). Heparin alone had little effect at this time.

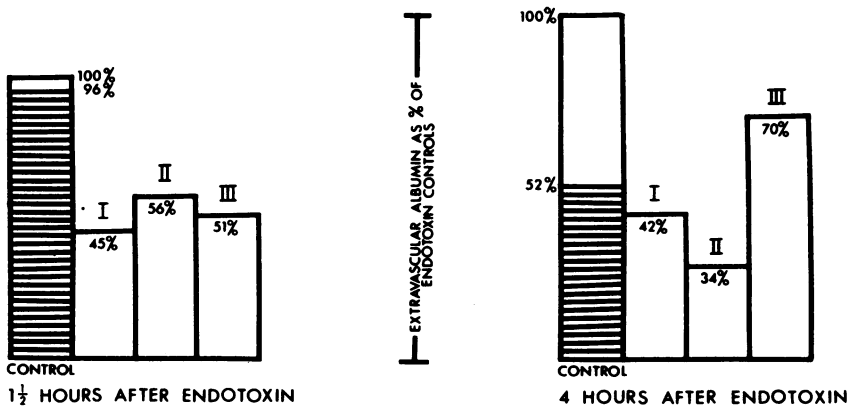
By contrast, the average ocular albumin space of leukopenic animals after endotoxin (19.60 ± 9.18 μl/g) was virtually the same as the respective control group (19.53 ± 11.18 μl/kg), indicating that the immune reaction, used to deplete formed elements, by itself did not alter the ocular response to systemic endotoxin and that, parenthetically, the leukocyte was not an important factor at this time.

Microscopic examination of the ciliary processes of thrombocyto-

Table 1—Ocular Response to Systemic Endotoxin, Effect of Immune Platelet Depletion on Total Albumin Space (TAS)

Experimental group*	Time of sacrifice post-endotoxin (hrs)	TAS experimental ± SD (μl/g eye tissue)	TAS control ± SD (μl/g eye tissue)	P
I. Platelet antiserum 30 min before endotoxin	1½	9.55 ± 3.98	17.36 ± 7.79	$P < .005$
	4	14.69 ± 6.41	31.24 ± 12.39	$P < .001$
II. Same as in I but with heparin	1½	11.61 ± 6.29	17.93 ± 8.91	$.025 < P < .05$
during endotoxemia	4	8.46 ± 5.71	23.34 ± 6.66	$P < .001$
III. Platelet antiserum and heparin 4 hrs before endotoxin	1½	13.26 ± 6.60	23.16 ± 11.12	$.005 < P < .01$
	4	18.85 ± 11.29	25.78 ± 11.48	$.025 < P < .05$
Heparin during endotoxemia	1½	26.20 ± 10.14	27.19 ± 11.97	—
	4	15.45 ± 7.25	27.65 ± 10.68	$P < .001$
Platelet antiserum only	—		4.42 ± 1.70	
Normals	—		5.03 ± 1.75	

* Each value represents a group of at least 6 animals



TEXT-FIG 1—Effect of immune platelet depletion on extravascular ^{125}I-albumin, expressed as per cent of control of group receiving endotoxin alone. ^{125}I-HSA injected 15 minutes before sacrifice. Endotoxin (*open columns*); endotoxin plus heparin (*hatched columns*), bar on left in each grouping represents endotoxin and endotoxin-and-heparin controls.

penic animals showed no evidence of platelet aggregation, but 5 out of 15 control animals given endotoxin alone had platelet aggregates focally in at least some processes. A comparison of the average ocular albumin space of those control animals with platelet aggregates to those without demonstrated that the presence of platelets locally was

associated with a higher average TAS (23.74 μl/g) than was found in their absence (14.32 μl/g) (Table 2). A typical thrombocytopenic group had still lower values (Group I: 9.55 μl/g) (Table 2).

Platelet aggregates were seen in small vessels just beneath the ciliary epithelium (Figure 1). By electron microscopy, damage to endothelial cells was usually minimal in regions of such aggregates (Figure 2). Occasionally, a single platelet was found beneath endothelium accompanied by ghost-like remnants of platelets in the stroma (Figure 3). In a few vessels, a platelet aggregate was seen in association with denuded endothelium, the endothelial cell pulled back from its basement membrane (Figure 4). Although no fibrin was seen by light microscopy, small quantities were found in some platelet aggregates (Figure 4). Additionally, degranulated platelets and platelet remnants were seen within vascular lumens, beneath endothelial cells and again most commonly in surrounding stromal tissues (Figure 4). The large number of platelet remnants in perivascular tissue suggested that a local release of platelet factors might have occurred.

Later Period

4 Hours After Endotoxin Injection

Two-thirds of control animals receiving endotoxin alone had platelets focally in some processes; each animal examined also showed evidence of intravascular fibrin of varying quantity as well. Thrombocytopenic animals had no evidence of platelet aggregation and demonstrated a 30 to 60% reduction in the accumulation of ocular albumin, the magnitude of the reduction depending on the timing of platelet depletion (Groups I and III) and whether or not the animals were treated with the anticoagulant heparin as well (Group II; Table 1 and Text-figure 1). In all platelet-depleted animals, there was a marked reduction in the number of ciliary processes showing stromal hemorrhage (Table 2).

Platelet antiserum given 30 to 60 minutes prior to endotoxin injection (Group I) reduced the albumin space by 58% (Table 1 and

Table 2—Ocular Response to Systemic Endotoxin 1½ Hours After Endotoxin

	No. of animals	TAS (μl/g eye tissue)
Overall average TAS	15	17.46 ± 7.21
Platelets evident microscopically	5	23.74 ± 6.72
No platelets evident	10	14.32 ± 5.08
Group I: thrombocytopenia	6	9.55 ± 3.98

Text-figure 1) but also enhanced the amount of fibrin deposition by both quantitative (Table 3) and qualitative estimation. Intermingled with the fibrin were fragments of platelets, degranulated platelets and some intact platelets (Figures 5 and 6). In some areas of fibrin formation the endothelium was absent (Figures 5 and 6); similar findings were seen in animals receiving endotoxin alone.

Thrombocytopenic animals treated with heparin during endotoxemia (Group II) had a 66% reduction in the accumulation of ocular albumin, no evidence of intravascular fibrin and very few stromal hemorrhages within ciliary process (Table 3). By comparison, normal animals treated with heparin during endotoxemia had a 52% reduction in ocular albumin and no evidence of intravascular fibrin,[15] but 2 of 8 animals had numerous platelet aggregates associated with striking hemorrhages.

Platelet antiserum accompanied by heparin 4 hours prior to endotoxin injection (Group III) resulted in a 30% reduction in the intraocular accumulation of albumin; microscopically, only minimal intravascular fibrin formation was found (Table 3). Three of 9 animals had a peculiar material in a subendothelial location (Figure 7). By electron microscopy, this material was fibrillar or finely granular, without periodicity (Figures 8 and 9) and often associated with cell remnants and cell granules (Figures 8 and 9), most likely representing an incompletely formed fibrin. Excluding the 3 animals with this change, the average albumin accumulation of Group III animals was comparable to that of Group II. All animals pretreated with heparin alone (Control Group III) had evidence of platelet aggregation and intravascular fibrin formation 4 hours after endotoxin injection (Table 3).

Table 3—Microscopic Evaluation of Ciliary Processes 4 Hours After Systemic Endotoxin*

	Stromal hemorrhages	Congestion	Thrombi (fibrin-platelets)	Fibrin IV†
Group I	24	23	18 (fibrin)	50
Group II	8	1	0	0
Group III	24	15	0	6
Control III	62	33	29 (mixed)	43

* Expressed as average percent of ciliary processes per eye showing change; average of 6 animals per experimental group; >30 ciliary processes counted per eye

† Fibrin IV refers to strands of fibrin within vascular lumens or along endothelial cells

Endothelial Cell Damage

Endothelial cells were examined microscopically for evidence of damage in the absence of platelets and fibrin formation in both Group II and some Group III animals. Vessels from over 300 processes were examined, primarily by light microscopy of stained 1- to 2-μ Araldite sections at the resolving power seen in Figures 10 and 11. Where questionable changes were observed, electron microscopy was done. The most pronounced finding was an elevation of the endothelial cell away from underlying basement membrane, collagen and perithelial cell (Figures 10–13). In some cases, this change was marked (Figure 11). It was more striking in animals with a greater protein leak and greater at 4 hours than at 1 hour. This "blister" formation contained a finely particulate protein material, occasional granules and sometimes an individual red blood cell (Figures 10 and 12). In each case, the bleb appeared immediately beneath or adjacent to the junction of endothelial cells (Figure 13). In no case was there evidence of endothelial denudation such as had been found in some areas of platelet aggregation (Figure 4) and fibrin formation (Figures 5 and 6). Examination of animals given platelet antiserum alone showed no vascular changes.

Discussion

Several lines of evidence in this study indicate that the presence of platelet aggregates locally enhances alteration in vascular permeability and is associated with a more pronounced endothelial damage. These include: a) 1½ hours after endotoxin administration, animals with platelet aggregates in vessels of the iridial processes had a higher ocular albumin accumulation than animals without evidence of platelet aggregates. b) Immune platelet depletion prevented the development of platelet aggregates and reduced ocular albumin leakage by 50% at 1½ hours and 30 to 50% by 4 hours. c) In platelet-depleted animals, hemorrhages were evident in less than 25% of iridial processes compared to more than 60% in controls. d) Platelet aggregation was associated with endothelial denudation in some vessels while no evidence of this change was observed in platelet-depleted animals in the absence of fibrin formation.

Platelet aggregates have been the apparent initiators of endothelial damage and tissue injury in other studies.[18–20] A deleterious effect of transient ADP-induced platelet aggregates has been seen in vessels of the myocardium, kidneys, and lungs of pigs and rabbits.[18] Gaps between endothelial cells appeared immediately and actual endothelial

denudation occurred beneath platelet emboli over a 24-hour period.[18] Similarly, it has been demonstrated that platelet embolization to the kidney from a source in the thoracic aorta of rabbits has led to both acute and chronic endothelial damage in renal arterioles.[19] Recently, platelet aggregation has been implicated as the initial event in acute renal allograft rejection in mongrel dogs, that this change preceded evidence of vascular damage, and that endothelial damage was delayed by inhibiting platelet aggregation.[20]

In the present study, platelets did not appear to be the only factor in the initiation of vascular change. In the virtual absence of platelets and all evidence of clotting, there remained an alteration in vascular permeability and evidence of vascular trauma. This underlying change could itself stimulate some local platelet aggregation by release of ADP from endothelium, and possibly by basement membrane and collagen exposure.[21] But platelets and platelet aggregation did porvide an enhancement of the underlying permeability change and a greater degree of vascular damage. Participation thus differs from that in the active Arthus reaction where the platelet is responsible for the initiation of alteration in vascular permeability that leads to immune vasculitis.[11] The underlying vascular permeability is important in considering the degree of damage caused by platelet aggregation, for endothelial injury does not always occur adjacent to platelet aggregates. For instance, damage to endothelial cells in foci of platelet aggregation could not be demonstrated in vessels of lung following the intravenous injection of endotoxin in pregnant rats.[22]

How do platelets enhance the underlying vascular injury? To some extent, the changes may be due to a mechanical obstruction and enhanced proximal leakage of plasma, but the demonstration of perivascular platelet remnants locally suggests there has been a local release of platelet constituents. It is well known that the interaction of platelets and endotoxin both *in vivo* [5] and *in vitro* [9] results in platelet aggregation and the release of some vasoactive substances, particularly serotonin. In all likelihood, other vasoactive substances (perhaps the platelet permeability factor described by Mustard,[23] and cationic protein such as has been described in human platelets by Nachman) [24] are released as well. Precisely how endotoxin interacts with platelets is not fully understood, but complement seems to play an essential role. This has been strongly suggested both *in vitro* [25] and in the pathogenesis of the generalized Shwartzman reaction *in vivo*.[26] From the studies of Des Prez [27] and Müller-Eberhard,[28] endotoxin-

induced platelet injury appears to occur utilizing the alternate complement pathway.

The definition of a selective platelet effect in this reaction was made difficult by the formation of intravascular fibrin 4 hours after endotoxin injection. Interpretation was further complicated by the fact that, in all probability, platelet interaction with antiserum stimulates intravascular coagulation when platelets are destroyed. In the present study, platelet antiserum administered 30 minutes to 1 hour prior to endotoxin seemed to enhance the deposition of intravascular fibrin (Group I). Similarly, Levin and Cluff [29] actually produced the generalized Shwartzman reaction with platelet antiserum in suitably prepared animals. Notwithstanding these complications, several features of the present study indicate that platelets are essential for most intravascular fibrin formation in this ocular response, and that intravascular fibrin by itself makes a small contribution to the altered vascular permeability.

Heparin treatment during immune platelet depletion 4 hours before endotoxin administration (Group III) effectively prevented most intravascular fibrin formation. This was the result of thrombocytopenia, not anticoagulation, since heparin by itself 4 hours before endotoxin injection did not prevent platelet aggregates or fibrin formation. A similar method of platelet depletion prior to the second injection of endotoxin had been found effective in preventing the generalized Shwartzman reaction.[13]

That intravascular fibrin formation might contribute to the altered vascular permeability was suggested by findings in Groups II and III and the effect of heparin alone. In Group II, heparin treatment during endotoxemia prevented any fibrin formation and reduced the ocular albumin by an additional 8%. In Group III, one-third of the animals had evidence of a presumed fibrin, incompletely polymerized, in a subendothelial location. These animals had the highest ocular accumulations of albumin. Excluding these animals, the average reduction in albumin accumulation in Group III was comparable to Group II. Finally, in animals with normal platelets, heparin treatment had prevented the formation of fibrin and significantly reduced the ocular albumin 4 hours after endotoxin injection.[15]

Subendothelial edema of varying extent was commonly found in the absence of both platelet aggregation and fibrin deposition in the present study. This finding was indicative of a greater endothelial damage than that reported following the usual vasoactive substances

(histamine, serotonin, bradykinin) where gaps only appeared between endothelial cells.[30] To some extent, this change might have been magnified by the use of platelet antiserum. Certainly, platelet depletion is known to affect vascular integrity and the response to minor trauma, the endothelial supportive function of the platelet.[31] Although platelet antiserum alone had no effect on vessels, presumably the combination of endotoxin and platelet antiserum could have a greater effect than endotoxin alone. In all likelihood, adrenergic stimulation plays a significant role in this underlying vascular change. Cervical ganglionectomy depletes catecholamines locally and significantly decreases the ocular response to endotoxin,[17] and α-adrenergic stimulation is an important factor in other responses to endotoxin as well.[32,34]

Previous studies have indicated that an endothelial lesion occurs in endotoxemia in the rabbit with both low and high doses of endotoxin.[35,36] In the present study, in the absence of platelet aggregates and fibrin deposition, no evidence of endothelial denudation was found in vessels of the iridial process within 4 hours after intravenous endotoxin injection, suggesting that either local platelet aggregation and/or fibrin formation must be superimposed on the underlying vascular trauma to produce damage of sufficient severity to result in endothelial denudation.

References

1. Ayo C: A toxic ocular reaction. I. New property of Shwartzman toxins. J Immunol 46:113–126, 1943
2. Howes EL Jr, Aronson SB, McKay DG: Quantitation of the effect of systemic bacterial endotoxin on ocular vascular permeability. Arch Ophthalmol 84:360–367, 1970
3. Kozart DM: Light and electron microscopic study of regional morphologic differences in the processes of the ciliary body in the rabbit. Invest Ophtalmol 7:15–33, 1968
4. Howes EL Jr, McKay DG, Aronson SB: An ultrastructual study of the ciliary process in the rabbit following systemic administration of bacterial endotoxin. Lab Invest 24:217–228, 1971
5. Davis RB, Meeker WR Jr, Bailey WL: Serotonin release by bacterial endotoxin. Proc Soc Exp Biol Med 108:774–780, 1961
6. Statson CA: Studies on the mechanism of the Shwartzman phenomenon: certain factors involved in the production of the local hemorrhagic necrosis. J Exp Med 93:489–504, 1951
7. Silver MD, Stehbens WT: The behaviour of platelets *in vivo*. Q J Exp Physiol 50:241–247, 1965
8. Urbaschek B: The effects of endotoxins in the microcirculation, Microbial Toxins, Vol 5. Edited by S Kadis, G Weinbaum, SJ Ajl. New York, Academic Press, Inc, 1971, pp 261–275

9. Des Prez RM, Horowitz HI, Hook EW: Effect of bacterial endotoxin on rabbit platelets. I. Platelet aggregation and release of platelet factors *in vitro*. J Exp Med 114:857–873, 1961
10. Horowitz HI, Des Prez RM, Hook EW: Effects of bacterial endotoxin on rabbit platelets. II. Enhancement of platelet factor 3 activity *in vitro* and *in vivo*. J Exp Med 116:619–633, 1962
11. Margaretten W, McKay DG: The requirement for platelets in the active Arthus reaction. Am J Pathol 64:257–270, 1971
12. Cochrane CG: Mechanisms involved in the deposition of immune complexes in tissues. J Exp Med 34:75s–89s, 1971
13. Margaretten W, McKay DG: The role of the platelet in the generalized Shwartzman reaction. J Exp Med 129:585–590, 1969
14. Fish MB, Aronson SB, Pollycove M, Coon MA: Ocular blood volume. Arch Ophthalmol 82:377–380, 1969
15. Howes EL Jr, Aronson SB, McKay DG: The effects of bacterial endotoxin on ocular vascular permeability. Fed Proc 29:690, 1970
16. Margaretten W, McKay DG: The effect of leukocyte antiserum on the generalized Shwartzman reaction. Am J Pathol 57:299–305, 1969
17. Howes EL Jr, McKay DG: Effect of cervical sympathectomy on the ocular response to systemic endotoxin. Proc Soc Exp Biol Med 139:839–844, 1972
18. Jørgensen L, Hovig T, Rowswell HC, Mustard JF: Adenosine diphosphate-induced platelet aggregation and vascular injury in swine and rabbits. Am J Pathol 61:161–177, 1970
19. Moore S, Lough J: Lipid accumulation in renal arterioles due to platelet aggregate embolism. Am J Pathol 58:283–294, 1970
20. Sharma HM, Moore S, Merrick HW, Smith MR: Platelets in early hyperacute allograft rejection in kidneys and their modification by sulfinpyrazone therapy. Am J Pathol 66:445–460, 1972
21. Johnson SA: Platelets in hemostasis and thrombosis, Chap 12, The Circulating Platelet. Edited by SA Johnson. New York, Academic Press, Inc, 1971, pp 355–394
22. McKay DG, Margaretten W, Csavossy I: An electron microscopic study of the effects of bacterial endotoxin on the blood vascular system. Lab Invest 15:1815–1829, 1966
23. Packham MA, Nishizawa EE, Mustard JF: Response of platelets to tissue injury. Biochem Pharmacol (Spec Suppl): 171–184, 1968
24. Nachman RL, Weksler B, Ferris B: Increased vascular permeability produced by human platelet granule cationic extracts. J Clin Invest 49: 274–281, 1970
25. Spielvogel AR: An ultrastructural study of the mechanisms of platelet-endotoxin interaction. J Exp Med 126:235–250, 1967
26. Fong JSC, Good RA: Prevention of the localized and generalized Shwartzman reactions by an anticomplementary agent, cobra venom factor. J Exp Med 134:642–655, 1971
27. Des Prez RM: The effect of bacterial endotoxin or rabbit platelets. V. Heat labile plasma factor requirements of endotoxin-induced platelet injury. J Immunol 99:966–973, 1967
28. Götze O, Müller-Eberhard HJ: The C3-activator system: an alternate pathway of complement activation. J Exp Med 34:90s–108s, 1971

29. Levin J, Cluff LE: Platelets and the Shwartzman phenomenon. J Exp Med 121:235–246, 1965
30. Majno G: Mechanisms of abnormal vascular permeability in acute inflammation, Chap 4, Injury, Inflammation and Immunity. Edited by L Thomas, JW Uhr, L Grant. Baltimore, Williams and Wilkins Co, 1964, pp 58–93
31. Johnson SA: Endothelial supporting function of platelets, Chap 10.[21]
32. Latour J-G, McKay DG: Requirement of the adrenal glands for provocation of the generalized Shwartzman reaction. Lab Invest 22:281–285, 1970
33. McKay DG, Müller-Berghaus G, Cruse V: Activation of Hageman factor by ellagic acid and the generalized Shwartzman reaction. Am J Pathol 54:393–420, 1969
34. Müller-Berghaus G, McKay DG: Prevention of the generalized Shwartzman reaction in pregnant rats by alpha-adrenergic blocking agents. Lab Invest 17:276–280, 1967
35. Gaynor E: Increased mitotic activity in rabbit endothelium after endotoxin. Lab Invest 24:318–320, 1971
36. McGrath JM, Stewart GJ: The effects of endotoxin on vascular endothelium. J Exp Med 129:833–848, 1969

Acknowledgments

The authors are greatly indebted for the technical assistance provided by Gregory B. McHolm and Virginia K. Cruse.

Legends for Figures

Fig 1—Iridial portion of ciliary process 1½ hours after endotoxin injection. Edematous epithelium is to right. A small vessel is filled by platelet aggregates and a few red blood cells (× 1200).

Fig 2—A similar blood vessel to that in Figure 1. Platelets contain varying numbers of granules. Endothelium is intact except for wide gap (*arrow*) (× 8000).

Fig 3—Portion of another blood vessel. Endothelium is intact, but a single platelet is present beneath the endothelium. Several degranulated platelets are in the stroma (*arrows*). Basal part of the epithelium is below (× 13,000).

Fig 4—Vessel of ciliary process 1½ hours after endotoxin injection. Endothelium is intact above and below, but beneath the platelet aggregate it is absent or has been peeled off (*large arrow*). Platelets are in various stages of degranulation. Completely degranulated platelets are in stroma below. Fine strands of fibrin are seen as well. Nucleus of an epithelial cell is seen in lower right (× 13,500).

Fig 5—Vessel of ciliary process, 4 hours after endotoxin injection in platelet-depleted animal (Group I). Densely packed fibrin is seen within the vessel lumen, between two endothelial cells (*arrows*) and in a subendothelial location. A partially degranulated platelet is to the right and a membrane fragment to the left in the lumen (× 17,000).

Fig 6—Four hours after endotoxin injection in thrombocytopenic animal (Group I). Condensed strands of fibrin are present against basement membrane between a wide gap in endothelial cells (*arrows*) (× 16,000).

Fig 7—Four hours after endotoxin injection in thrombocytopenic animal (Group III). Edematous ciliary epithelium is to right. A small blood vessel is seen centrally and several extravasated red blood cells above. An amorphous material is seen in the wall of the blood vessel (*arrow*) (× 1100).

Fig 8—Portion of a blood vessel similar to that in Figure 7. The lumen is above, and the endothelium is intact. Between endothelial and perithelial cells is some granular material with suggestion of finely fibrillar structure. Scattered cell granules and fragments are seen as well. A nonmyelinated axon is in the lower left (× 11,000).

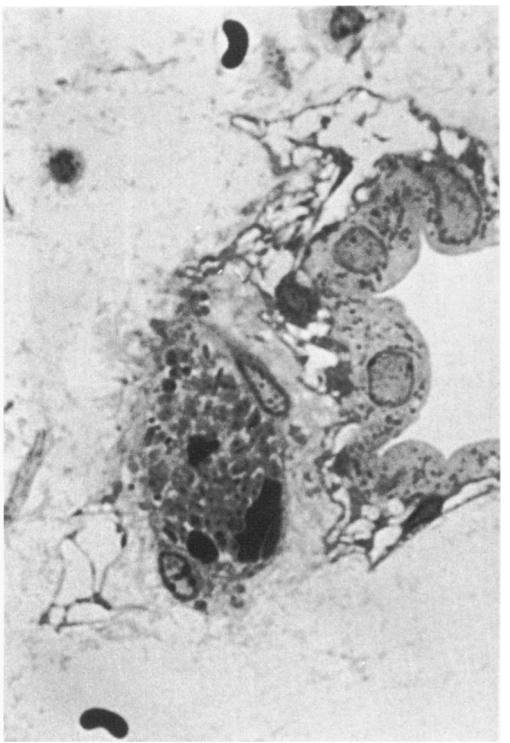
Fig 9—Higher power of subendothelial material similar to that found in Figures 7 and 8. At this magnification the loose fibrillar nature of the material can be seen. No periodicity or condensation of this material was found, but it is presumed to be a form of fibrin. Lumen is to right; cell fragments, granules and a part of a red blood cell can be seen in this subendothelial location (× 24,500).

Fig 10—Portion of a ciliary process, 4 hours after endotoxin injection in Group II animal. Edematous ciliary epithelium is to right. Two blood vessels and several extravasated red blood cells can be seen. In both vessels, endothelium is lifted away from the underlying connective tissue, basement membrane and perithelial cell (*arrows*). In the bleb found in the vessel above, several granules can be seen, and a red blood cell can be found in the subendothelial pocket below (× 1000).

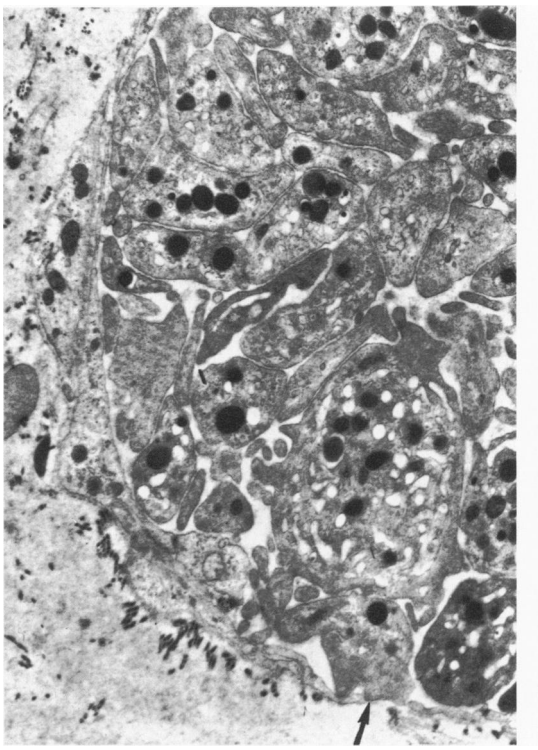
Fig 11—A similar vascular change to that seen in Figure 10. The endothelial cell is lifted away from underlying structures (*arrow*) (× 1200).

Fig 12—Vessel of ciliary process from Group II animal, 4 hours after endotoxin injection. A subendothelial pocket contains finely stippled proteinaceous material as well as several granules and a partially extravasated red blood cell (× 14,800).

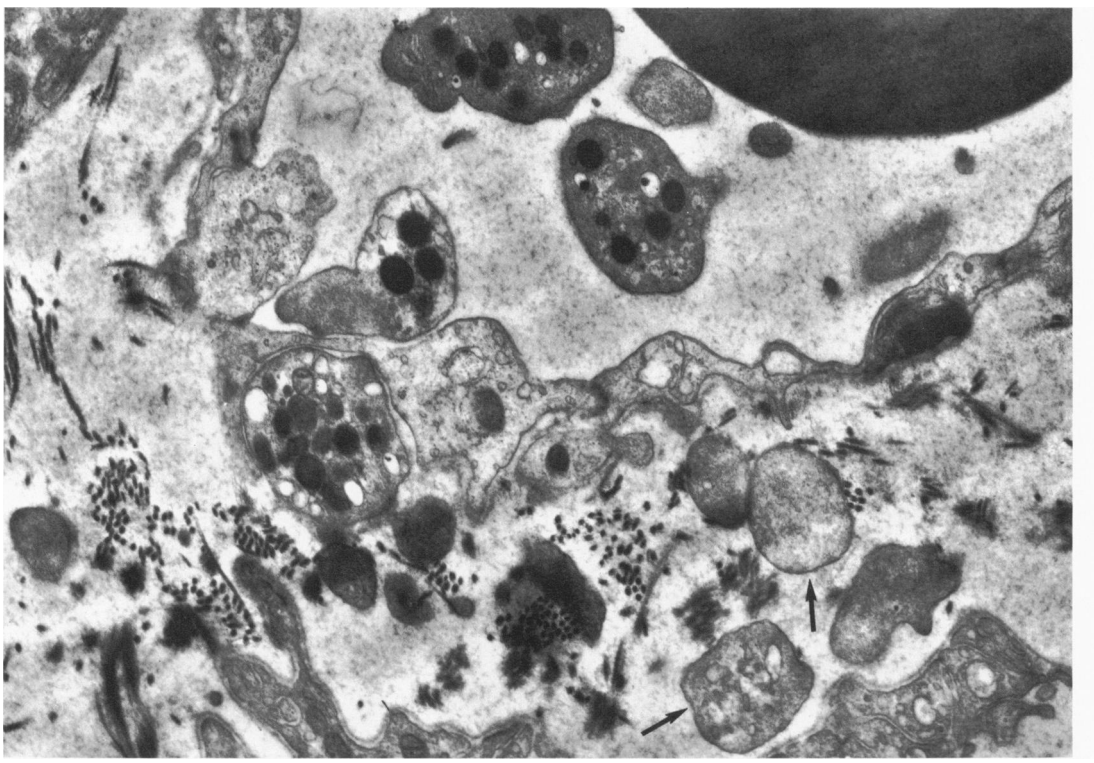
Fig 13—Subendothelial pockets usually occurred beneath junctions of endothelial cells in Group II animals. The bleb found contains a proteinaceous material similar to plasma (× 23,500).



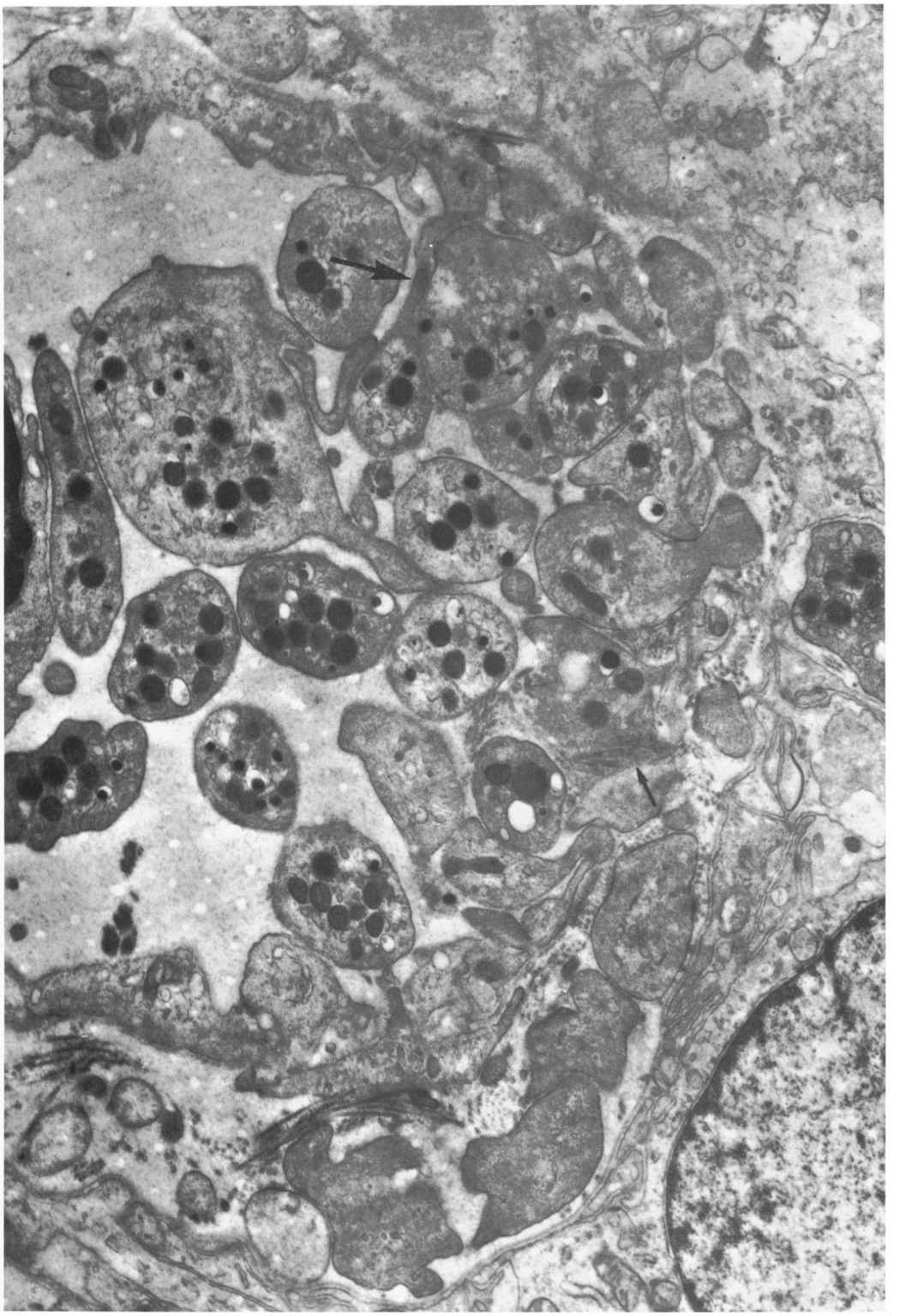
1



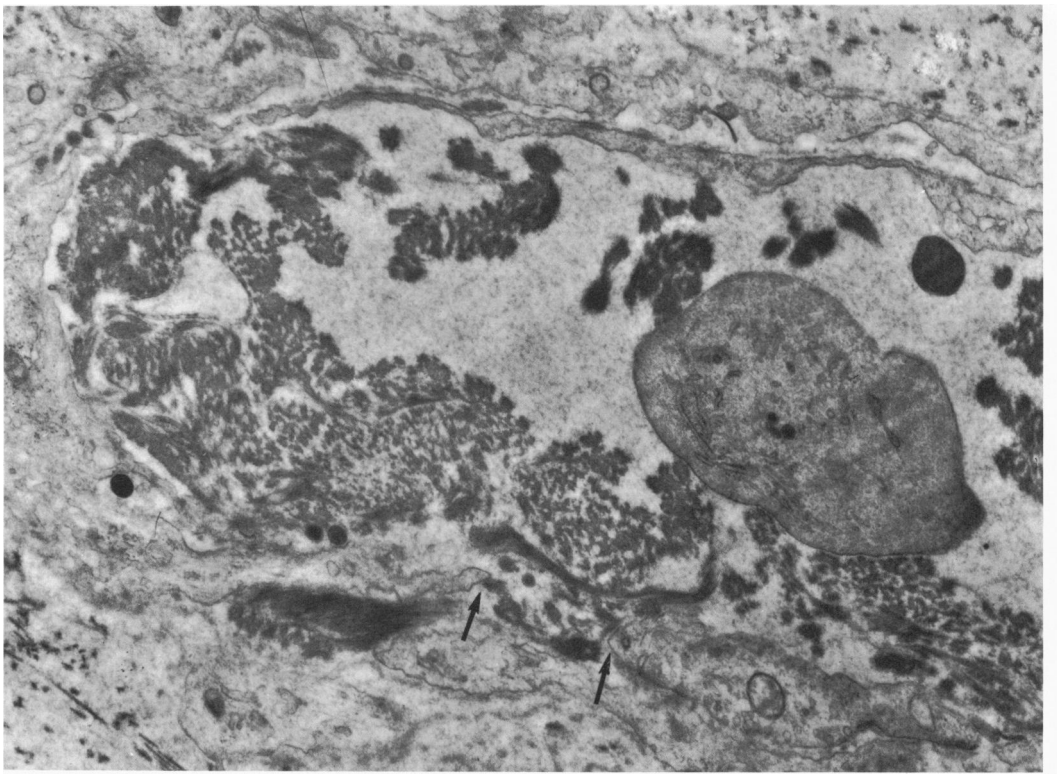
2



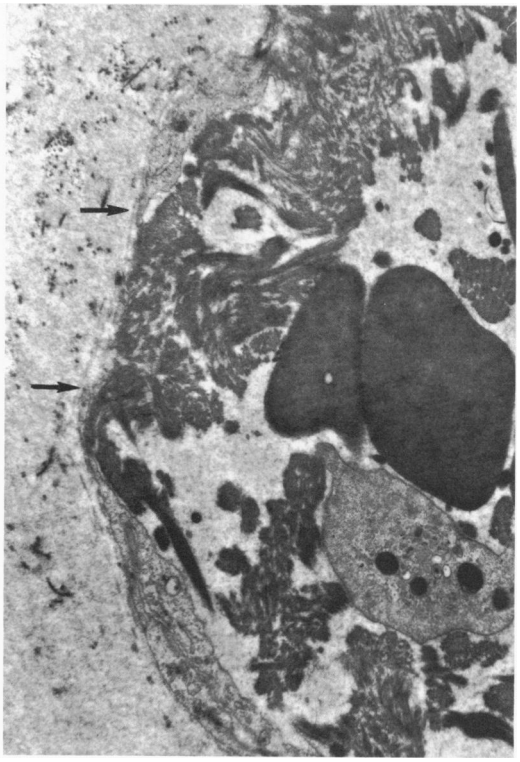
3



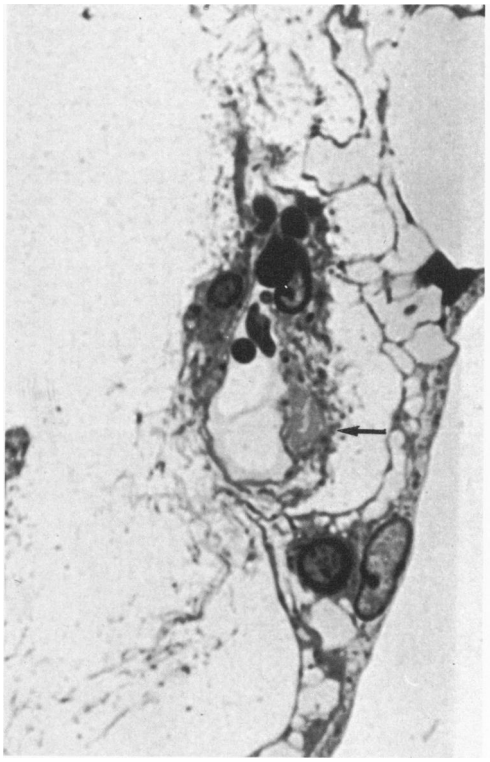
4



5

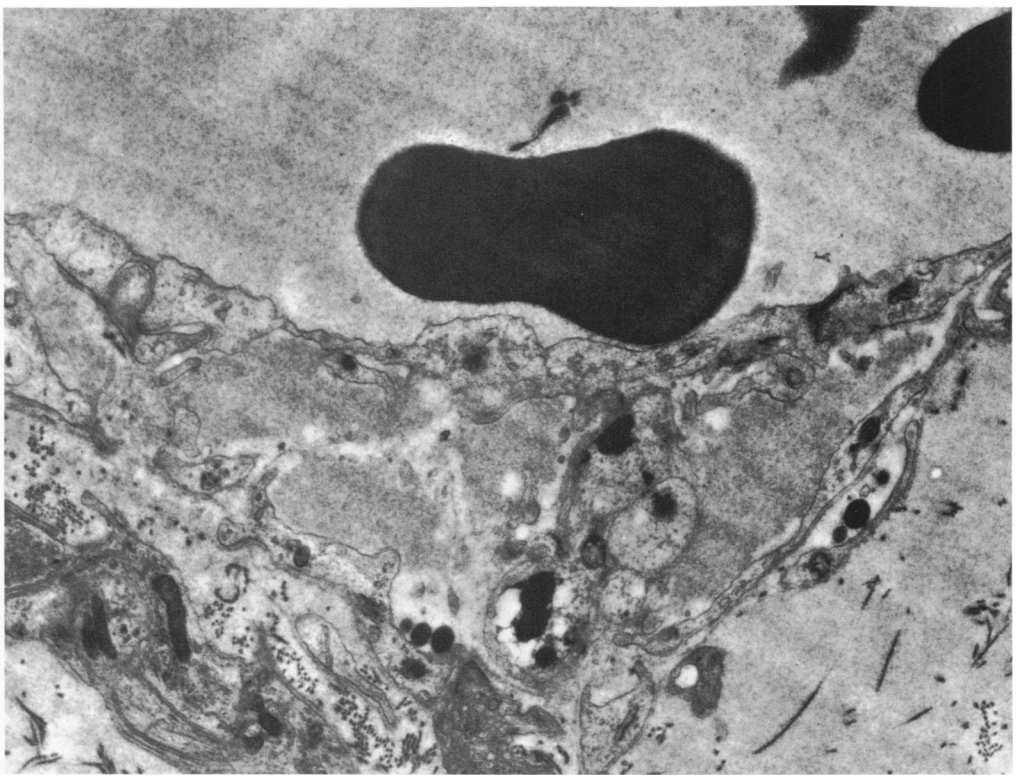


6

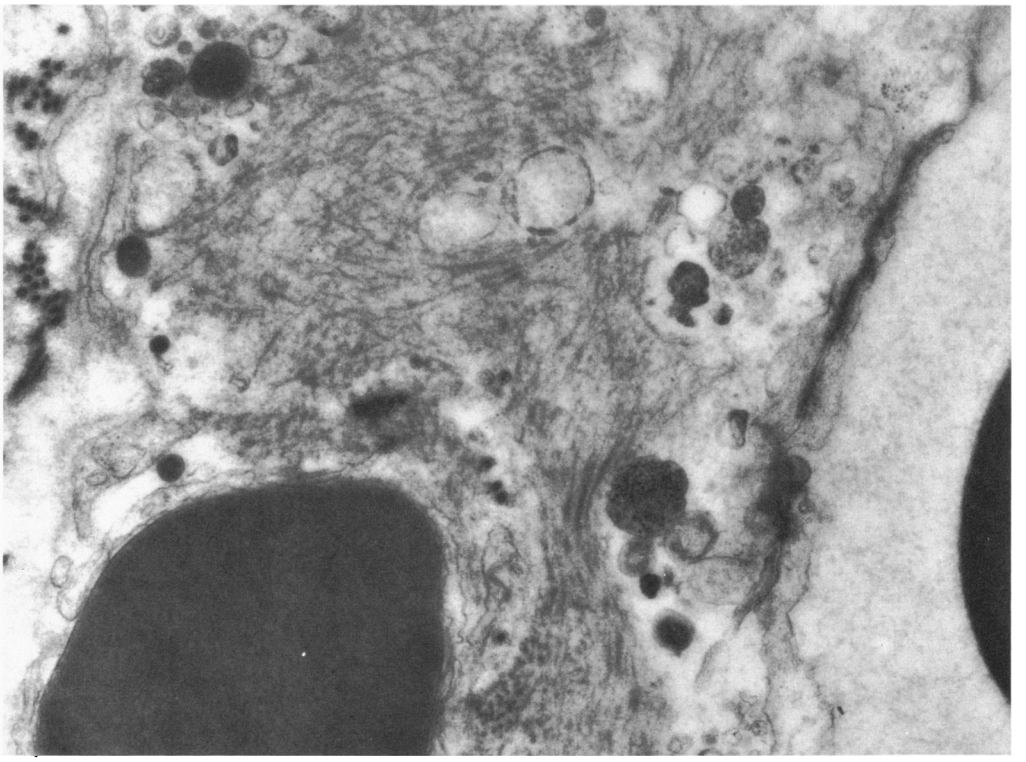


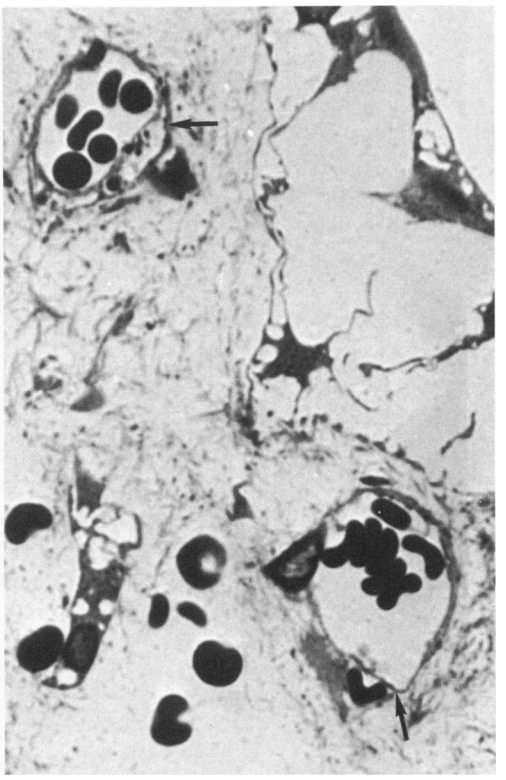
7

8

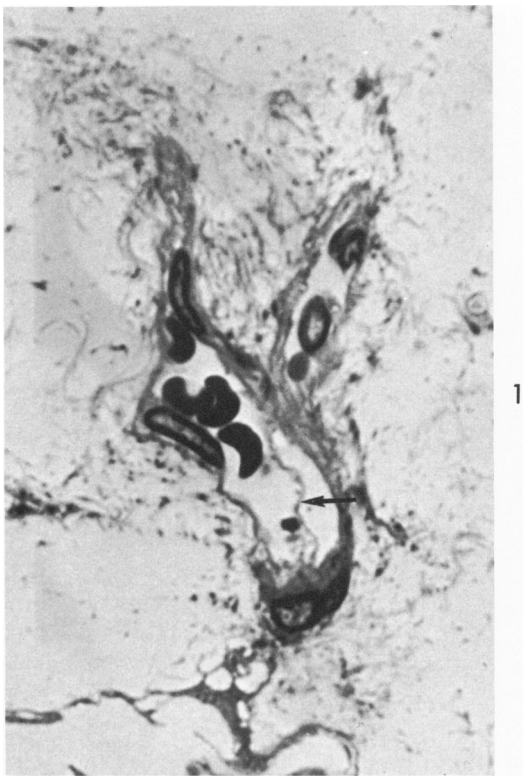


9

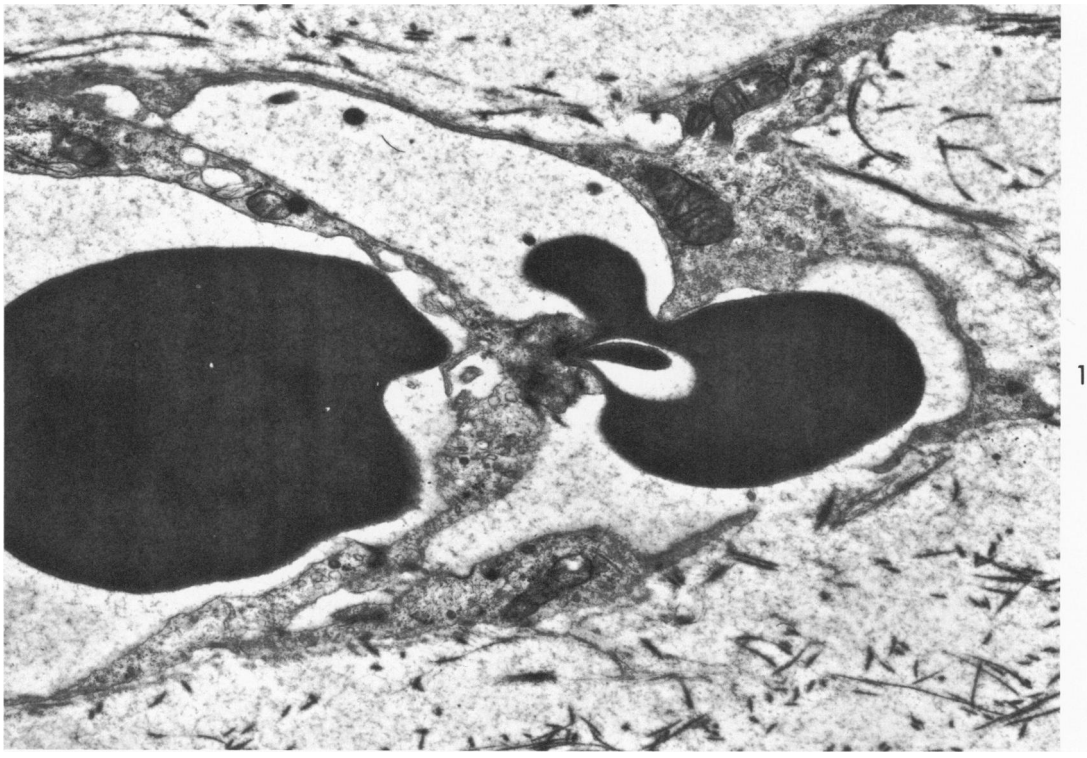




10



11



12

13