JOURNAL OF SURGICAL RESEARCH 22, 97–108 (1977)

In Vivo Microscopic Observations of Intra-Arterial Injections of Barbiturates[1]

MARY P. WIEDEMAN, Ph.D.,[2] RONALD F. TUMA, Ph.D., AND HARVEY N. MAYROVITZ, Ph.D.

Department of Physiology, Temple Medical School, Philadelphia, Pennsylvania 19140

Submitted for publication April 5, 1976

In the past few years reports regarding accidental intra-arterial injections of drugs by addicts have appeared in the literature [1–4]. In many instances, the injected drug was in a form prepared for oral use, which might have added to the damaging effect and now raises a new problem in relation to drug abuse.

The clinical features seen in patients at varying times following the inadvertent intra-arterial injections of common drugs such as heroin, cocaine, methadone, and barbiturates include an intense burning pain, edema, cyanosis, and, often, a contraction of the muscles of the hand and arm. Ischemia and the subsequent development of necrosis may ultimately require amputation of the fingers, hand, or forearm. Attempts to reinstitute blood flow and to prevent tissue necrosis are seldom successful.

A number of theories has been proposed to explain the resultant ischemia and gangrene. They include release of norepinephrine [5], formation of crystals following contact of a drug with blood [6], red blood cell hemolysis and platelet aggregation [7], and vascular spasm [8].

Kinmonth and Shepard in 1959 [9] studied the effects of intra-arterial injections of thiopentone in rabbits and demonstrated that arterial spasm did not last long enough to cause necrosis, that the degree of damage was related to the concentration of thiopentone, and that ischemia was the result of occlusion of the damaged artery due to thrombosis. These authors also stated that intra-arterial injections of procaine had no therapeutic effect but that heparinization and sympathetic denervation reduced the amount of gangrene.

In a lengthy review article by Stone and Donnelly [10] in 1961, it was reasserted that damage to the arterial vessel lining was a primary cause of impaired blood flow that resulted in gangrene. They point out that stasis and intimal damage together favor thrombosis and that aggregation of blood platelets at the site of injury activates thromboplastin and initiates the clotting mechanism. In 1966 Waters [6] presented evidence that thiopentone mixed with blood forms a precipitate and that clumps of the precipitate in the form of crystals block small blood vessels. Stagnation of the blood leads to thrombosis, and gangrene is the end result. Furthermore, a 5% solution of thiopentone will form more than twice as much precipitate as a 2.5% solution. A second study of this feature was carried out by Brown *et al.* in 1968 [7] with confirmation of Waters' results and the additional information that platelet aggregates formed in the presence of the crystals and that red blood cells were hemolyzed.

In spite of the fact that there seemed to be mutual agreement that damage to the arterial wall which had been in contact with the barbiturate was the primary cause of the ischemia and subsequent gangrene,

[1] Supported in part by USPHS Grants No. HL 17767-01 and SCOR, Thrombosis HL-14217-05.

[2] To whom reprint requests should be addressed.

ISSN 0022-4804

no reports appeared which indicated that a means of overcoming vascular damage had been sought. Therapy continued to consist of using vasodilators, sympathetic blockade, and anticoagulants, particularly heparin.

A new interest came with reports in the literature about the increasing number of accidental intra-arterial injections of drugs by addicts. Many of the reported cases were the result of intra-arterial injections of oral preparations of barbiturates, most notably secobarbital, which contained materials such as talc or corn starch as additional damaging agents [1–4]. Albo *et al.* [1] in 1970 reported two cases of intra-arterial secobarbital injections and treated the patients on the basis of the work of Kinmonth and Shepard [9], using papaverine and sympathetic blockade in one case, with a poor result, and infusion of heparin in the second case, with success. In 1972, Lindell *et al.* [2] reported three cases, one of which was the result of oral secobarbital. Heparin, dextran, lidocaine, and nerve block were tried during the course of treatment without successful prevention of gangrene. In 1974 Ryan *et al.* [4] reported that 38 patients were seen in a 4-month period at the Johns Hopkins Hospital who had hand or arm problems resulting from drug injections. Response to treatment with adrenergic blocking agents and anticoagulants was not dramatic. The inability to overcome the adverse effects of intra-arterial injections of drugs makes some of these proposed explanations for the damage suspect. In this investigation, microscopic observation of blood vessels in the living, unanesthetized animal at the time of, and subsequent to, intra-arterial injection of selected drugs was used to investigate the cause of the damage produced. Because barbiturates, in both oral and parenteral preparations, were the most frequent offenders [1, 2, 9, 10] as well as the subject of the majority of investigations, sodium Pentothal and secobarbital were selected as the drugs for this study.

METHOD AND MATERIALS

Preparation of animals. Unanesthatized bats (*Myotis lucifugus*) were prepared for observation according to the technique described by Wiedeman [11]. The bats were placed face down in a Plexiglas holder with one wing extended and held in place on a glass slide. The epidermis in the area chosen for observation was teased away with jewelers' forceps to improve visual clarity and give good resolution at high optical magnifications. The exposed vessels were covered with buffered saline and a coverslip.

Measurement of vascular changes. The blood vessels were observed through a Leitz Ortholux trinocular microscope that permitted simultaneous recording of the field by cinephotomicrography or videotape. Optical magnifications of 400, 800, and 1200× (achieved by combining 20× wide-field eyepieces with 20, 40, and 60× objectives) were used depending on the vascular area studied. Vessel diameters were measured by an eyepiece micrometer or by the image-shearing method with an I P M Inc. image-shearing instrument which gives a digital readout of the diameter. The frequency of venous vasomotion and lymphatic contractions was determined by counting contractions for several minutes before and after drug injection.

Infusion of drugs. Oral secobarbital was prepared for injection by dissolving the contents of a sodium secobarbital capsule (Seconal; Lilly, 100 mg) in 2 ml of tap water, to conform to the method used by drug addicts. The solution was drawn into a 1-ml tuberculin syringe which was then connected to the T-tube that joined the glass cannula for injection retrograde into the major artery of the bat's wing. The solution was injected against the inflowing arterial blood so that it cleared the major artery of blood and entered the arterial branches (Fig. 1). The vessels under observation were exposed to the secobarbital solution for a period of 2.5 to 3 sec. On cessation of perfusion the inflow of

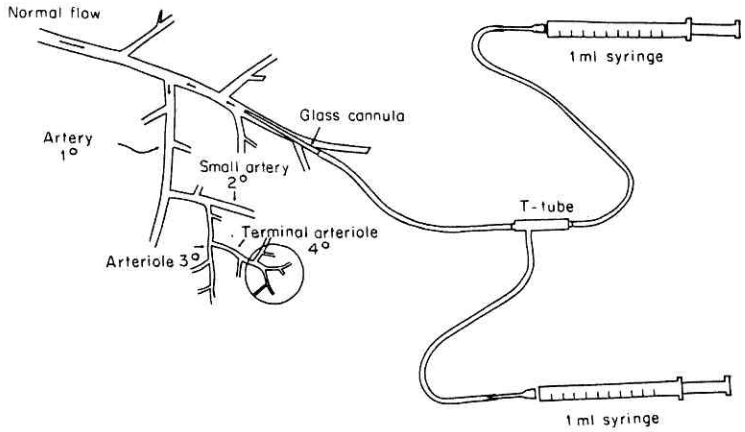

FIG. 1. A diagrammatic representation of the procedure for intra-arterial injection of barbiturates. The main artery of the bat wing was cannulated, and drugs were infused retrograde up to the branch site of a first-order (1°) artery, at which point the vasculature was perfused in the normal direction of blood flow.

arterial blood resumed its normal pathway.

The intravenous preparation of sodium secobarbital used in the study was manufactured by Wyeth (Philadelphia), 50 mg/ml. Sodium Pentothal (Abbott, sterile powder) was prepared in concentrations of 2.5, 5, and 10%.

Osmolarity and pH. The osmolarity of the oral secobarbital solution was determined with a freezing-point depression osmometer. The pH of the solution was measured in a Radiometer PHM 71 pH meter.

Aggregometer studies. The analysis of possible platelet aggregation following the mixing of the sodium secobarbital solution with washed platelets was made in a Payton aggregation module and was recorded on a Bausch and Lomb recorder. The aggregometer studies were done in the Thrombosis Center of Temple University School of Medicine.

Electron micrography. To prepare vessels for election micrography after exposure to the secobarbital solution, the arterial bed was perfused with 2.5% glutaraldehyde in cacodylate buffer. A section of the perfused wing was pinned to a Silastic rubber pad and was submerged in glutaraldehyde for overnight fixation. The specimen was postfixed in 1% osmium tetraoxide for 1 hr, dehydrated, and embedded in Epon 812. Thick and thin sections were cut along the length of a vessel known by light microscopic inspection to contain aggregates. Thick sections were examined by light microscopy and thin sections by electron microscopy. The electron micrography was done in the Thrombosis Center of Temple University School of Medicine.

RESULTS

1. Arterial Vessel Response to Intra-Arterial Injections of Oral Secobarbital

In 27 animals, the solution of oral secobarbital was injected into the major wing artery. Following exposure of the arterial vessels to the drug there was an immediate reaction. The first response was often a squeak from the bat, indicating a painful stimulus. Second, numerous areas in which arteriolar vessels branched from the parent arterial vessel became occluded with small clumps of particles (perhaps crystals and platelets), and, when inflow of arterial blood returned following cessation of perfusion of the drug, large numbers of platelets accumulated at these sites and effectively blocked flow into the areas beyond (Figs. 2–5). In a very short time, patchy spots along the wall of the parent artery showed distortion of red blood cell shapes when the

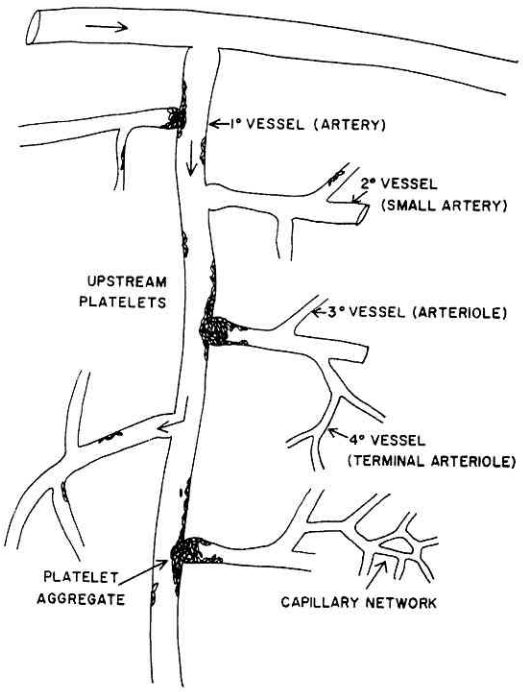

FIG. 2. The arterial distribution is represented by diagram; the sites of platelet aggregate formation and the areas where platelets are seen adhering to the arterial wall are shown.

inflowing blood was mixing with the secobarbital solution. When blood flow through the arterial vessel was completely restored and the vessel was scanned, it was possible to predict the appearance of a platelet aggregate at an arteriolar opening downstream by observing the adherence of platelets to the wall before the arteriolar branch site was in the microscopic field. It has previously been reported from this laboratory by Wiedeman [12] that a cluster of platelets adhering to a vessel wall serves as an attraction for platelets in the flowing blood. In addition, the cluster seems to interact with the wall to cause platelets to adhere a distance of approximately 55 μm upstream. The same phenomenon was seen in this preparation. Blood flow in most sections of the parent artery appeared to be completely normal after injection of secobarbital, while, in areas of the vessel wall just preceding an arteriolar branch origin which was plugged with platelet clumps, platelets were visible rolling along the wall.

Arterial vessel diameters were measured before and after injections of oral secobarbital into seven animals. The first-order arterial vessels showed a 41% increase in diameter after injection. Second-, third-, and fourth-order vessels either increased in diameter slightly or not at all, and, by analysis, the diameter changes were not statistically significant.

2. *Venous and Lymphatic Vessel Response to Intra-Arterial Injections of Oral Secobarbital*

Measurements of venous vessel and lymphatic vessel diameters were made, and the rates of venous vasomotion and lymphatic contractions were determined. There were no changes in the diameter of venous vessels that were statistically significant, but the rate of venous contractions was markedly reduced from a mean rate of 14.1/min to a mean rate of 3.8/min. There was an increase in the diameter of lymphatic vessels from a mean of 16.2 μm to a mean of 21.9 μm, representing an increase of 35%. Lymphatic vessel contractility was also decreased from a mean rate of 5.9/min to a mean rate of 2.5/min (Fig. 6).

3. *Characteristics of the Oral Secobarbital Solution*

Consideration was then given to the nature of the injected solution for some explanation of its damaging properties. Because some of the vascular and cellular component changes were reminiscent of results from injections of hyperosmolar contrast materials, the osmolality of the solution was determined. [13]. The osmolality was found to be within the normal range (330 mosm).

The possibility that using tap water as the diluent may have produced some adverse effects was tested by injecting tap water alone into the arterial vessels. Except for a momentary constriction of an irregular

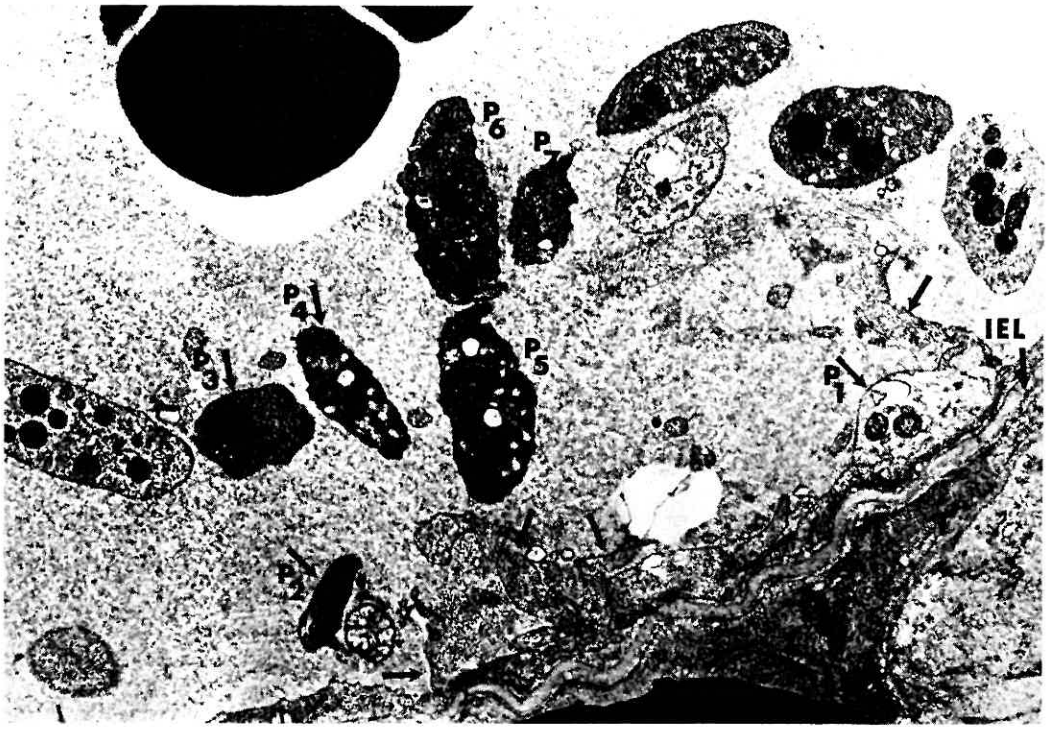

FIG. 3. Transmission electron micrograph of a section of thrombosed artery in the bat wing. The endothelium can be seen just luminal to the internal elastic lumina (IEL).

pattern, no changes in blood flow, blood cells, or vessel wall could be distinguished. No platelet aggregates or adhering platelets were seen.

A determination of the pH of the oral secobarbital solution showed it to have a pH between 9.0 and 10.0. A solution of glycine–NaOH with a pH of 9.5 was injected into the arterial vessels, resulting in an impairment of flow beyond second-order arteries as a result of vasoconstriction. However, in less than 60 sec the arterial vessels relaxed and flow was returned to its normal state. At no time were deformed red cells, platelet aggregates, or platelets adhering to vessel walls seen.

A final test was done to evaluate the effect of the secobarbital solution on washed platelets *in vitro*. After mixing the two solutions together, no evidence of platelet aggregation could be demonstrated in the platelet aggregometer. Because the formation of very small aggregates may not be detected by the aggregometer, secobarbital was tested along with ADP to determine if the secobarbital would potentiate ADP-induced aggregation. In three trials, however, it was found that secobarbital inhibited ADP-induced aggregation.

4. Electron Microscopy

Electron micrographs of arterial vessels exposed to the solution of oral secobarbital showed numerous changes. The endothelial sheet was present with no areas of missing cells in the material that was examined, thus establishing the fact that loss of endothelium was not common. Some of the endothelial cells appeared to be normal, while others were considerably altered (Figs. 6–9).

At the electron microscope level, the platelets were not densely packed or, in some cases, not in contact with one another (Fig. 7). They maintained the normal disk shape although some showed pseudopods

FIG. 4. Arterial vessel and a branching arteriole showing the accumulation of platelets at the origin of the branch. These platelets prevented flow of blood from the parent vessel into the capillary network beyond.

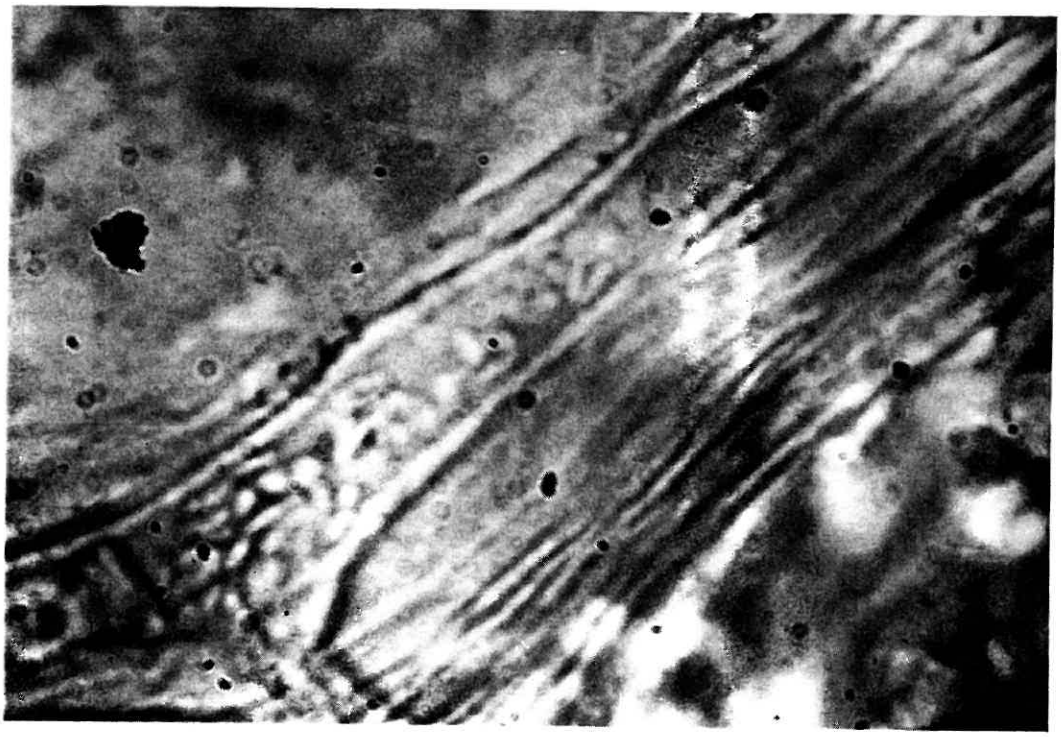

FIG. 5. Platelets adhering to the wall of an arteriole.

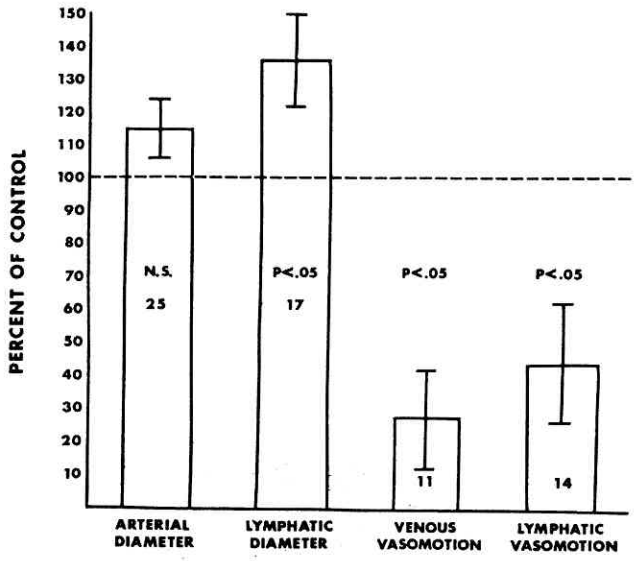

FIG. 6. Arterial diameter (measurements of first- to fourth-order vessels), lymphatic diameter, venous vasomotion, and lymphatic vasomotion values are expressed as percentages of the control values prior to injection of barbiturates. Bars indicate the standard error of the mean. The numerals represent the number of vessels studied for each parameter. *P* values were determined by a paired *t* test.

and unusually dark cytoplasm (Fig. 8). Platelets exposed to the calcium ionophore A23187 show an increased density of cytoplasm, indicating an increase in cytoplasmic calcium [16].

Leukocytes also adhered to the luminal surface of an arterial vessel exposed to an oral secobarbital solution and frequently became entrapped between the endothelium and the basement membrane (Figs. 9, 10).

5. Intra-Arterial Injections of Parenteral Secobarbital

A solution of secobarbital prepared for intravenous injection (50 mg/ml) was injected intra-arterially into the bat wing to determine to what degree additives in the oral preparation might contribute to the damaging effects. It was found that platelet aggregates did not form readily at the arteriolar branch sites, and, therefore, complete occlusion of these small vessels did not occur as in the case of oral preparations of secobarbital. Platelet clumps did form, however, in the circulating blood and eventually shut off blood flow in small distal vessels when clumps which were too large to pass lodged in the lumen.

6. Intra-Arterial Injections of Thiopental

It is well-known that thiopental, commonly used as an anesthetic, has been injected intra-arterially into surgical patients by mistake with consequences similar to those described for secobarbital. Clinical treatment, which can be initiated immediately in most cases, has also not been successful, even though anticoagulants, diluents, and vasodilator drugs were injected through the same cannula after the first sign of trauma.

Sodium pentothal in concentrations of 2.5, 5, and 10% were injected intra-arterially in the bat wing, and microscopic observation showed the degree of damage to be directly proportional to the concentration. When a 2.5% solution was injected into an artery, no adverse effects were seen. A 5% solution produced a moderate degree of change in the bed perfused with the solution, a change that consisted of small platelet clumps in the flowing blood and some platelets adhering and rolling along the vessel walls. The immediate response involved the vessel which showed irregular vasoconstriction and then relaxation.

An injection of a 10% solution was very

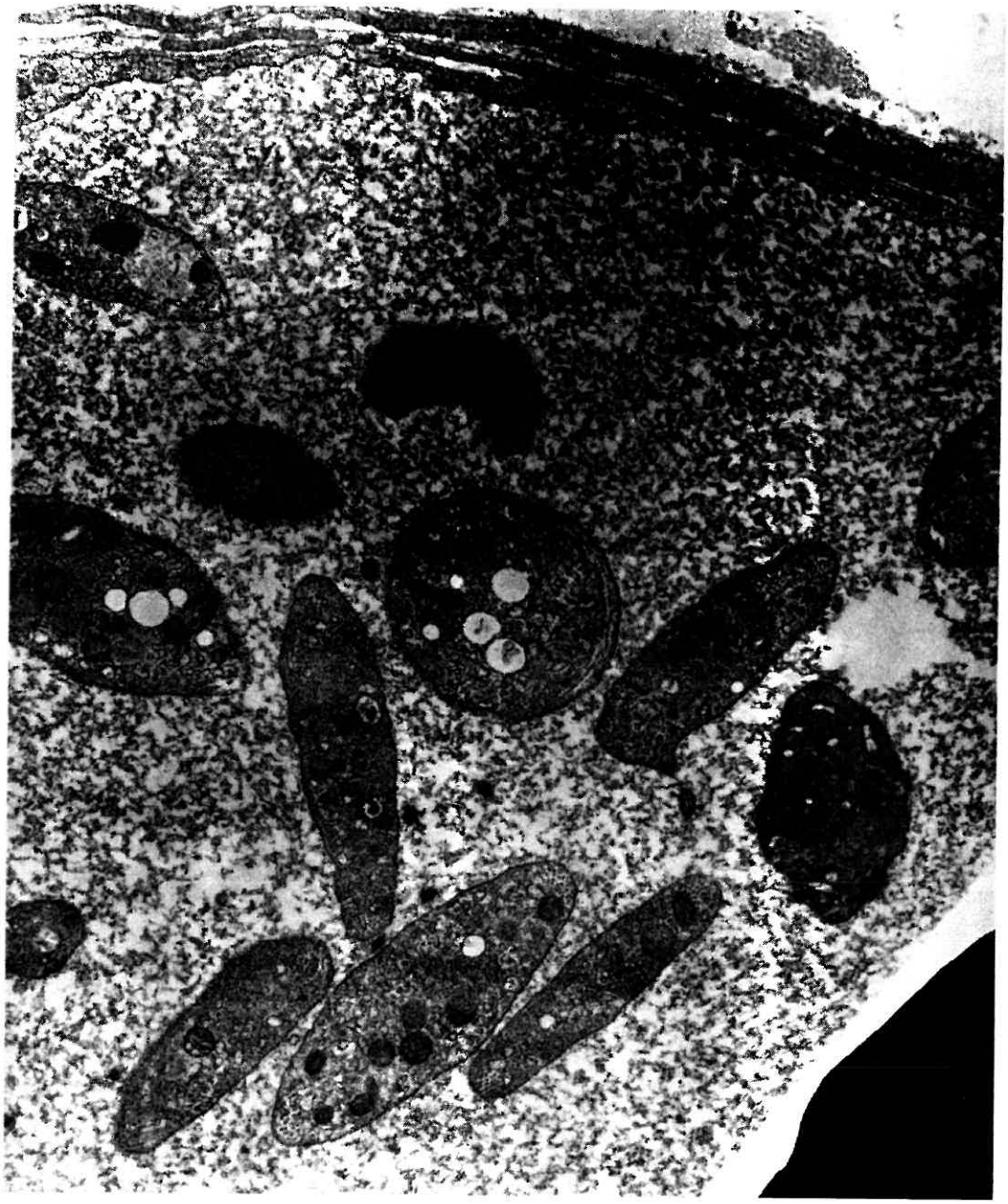

FIG. 7. Electron micrograph of an artery showing an accumulation of platelets. The platelets are not in contact with one another in this plane of section. The platelets have not undergone a shape change. As can be seen most are disk shaped. The endothelium is intact and appears quite normal in this area. ×12,100.

damaging and resulted in large accumulations of platelets at arteriolar branch sites. Massive clumps of platelets plugged origins of arteriolar branches and, in a short time, caused occlusion of so many arterial vessels, both large and small, that no blood flowed into the previously perfused areas.

DISCUSSION

The damaging effects of intra-arterial injections of barbiturates in both parenteral and oral preparations has been under investigation for many years. Most aspects of the resultant injury have been explored, although the findings have not led to any

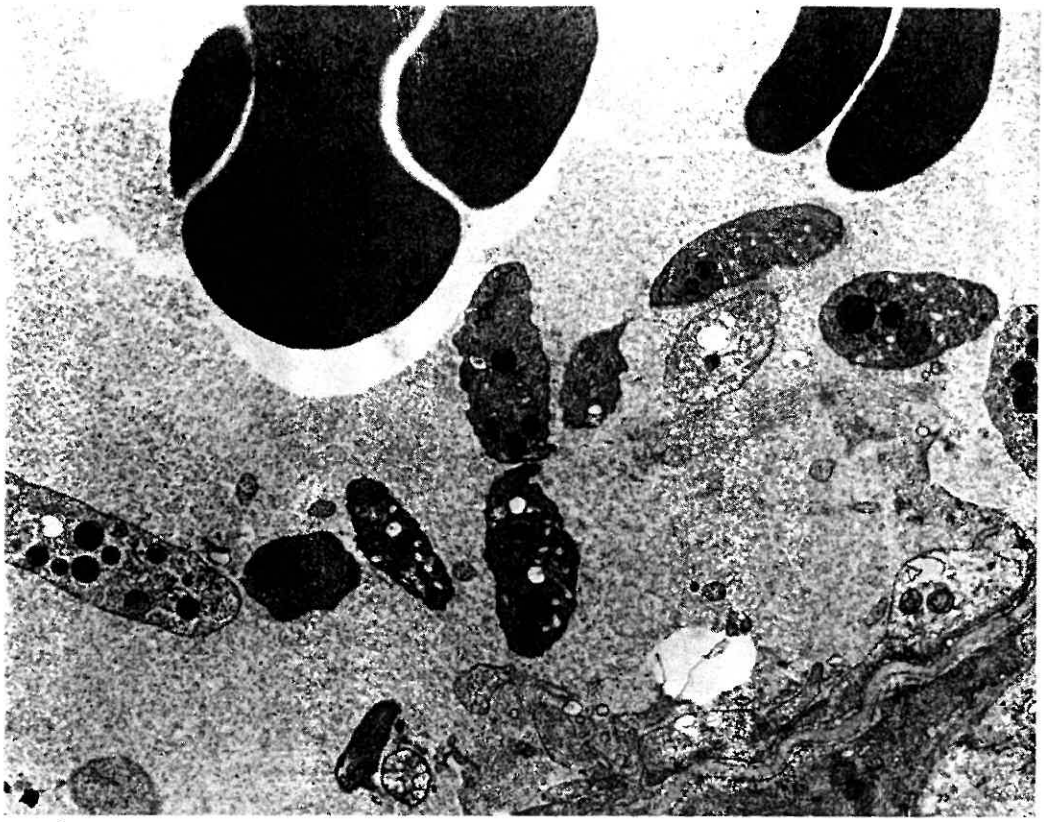

FIG. 8. Electron micrograph of an artery showing an accumulation of platelets in an area with intact but altered endothelium. ×9560.

generally accepted reason for the ischemia and gangrene that develop. Furthermore, no conclusions have been reached regarding the most effective treatment.

A hypothesis that has been confirmed in this study by direct microscopic observation of arterial vessels during injection is that the cause of the irreversible damage is the production of platelet aggregates and thrombosis.

Interestingly, platelets adhered to and accumulated on the vessel wall in the absence of gross disruption of the endothelial sheet. While the sectioning technique was not adequate to exclude missing single cells or even a very small patch of cells, it was adequate to establish that the endothelium was mostly intact. The close proximity of platelets and endothelium does not prove adhesion, but adhesion is strongly indicated since platelets were selectively retained in the area during perfusion.

The adherence of platelets to a vessel wall in which little change in the endothelial lining has occurred would suggest that platelets were not activated, in this instance by exposure to collagen or basement membrane. One possible explanation for platelet aggregation with subsequent accumulation and adherence at arteriolar branch sites is that the drugs alter the permeability of the red blood cell membrane to an extent that small quantities of liberated ADP can accumulate and initiate aggregate production. The circulating aggregate then plugs a small arteriolar branch orifice where the release reaction continues to perpetuate the aggregate. Although highly speculative, the possibility exists that such an event may occur. Further studies are needed to confirm or refute this proposed explanation.

After an intra-arterial injection of an oral secobarbital solution, the appearance

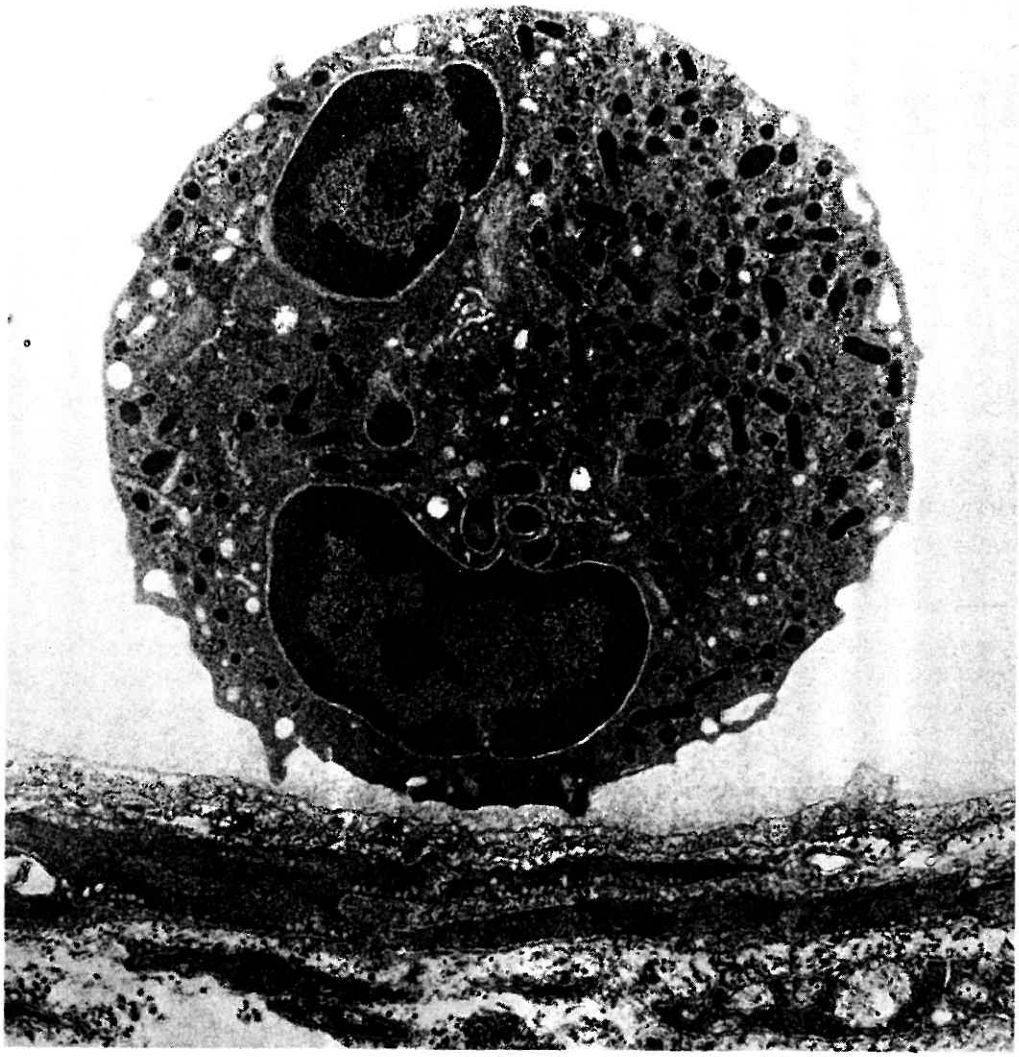

FIG. 9. Transmission electron micrograph of an arterial wall with an adhering leukocyte. The endothelium in this area appears to be quite normal (thin, with many caveole) and is closely attached to the underlying vessel. The adhering polymorphonuclear granulocyte appears normal with no evidence of release of granular contents. ×12,900.

of clumps of platelets at the sites of branching of arteriolar vessels from their parent arteries was a very effective means of stopping blood flow into the capillary network beyond. Although the physical signs seen in patients could be interpreted as a response to vasospasm, large arterial vessels in the bat continued with a normal flow of blood, and no evidence of vasoconstriction was seen at any level of arterial distribution.

It was concluded from these observations that the major cause of ischemia is the occlusion of the arteriolar branch openings by platelets which prevents distribution of blood into the capillary network beyond. Arterial vasoconstriction, as postulated by others, is not a factor, and, therefore,

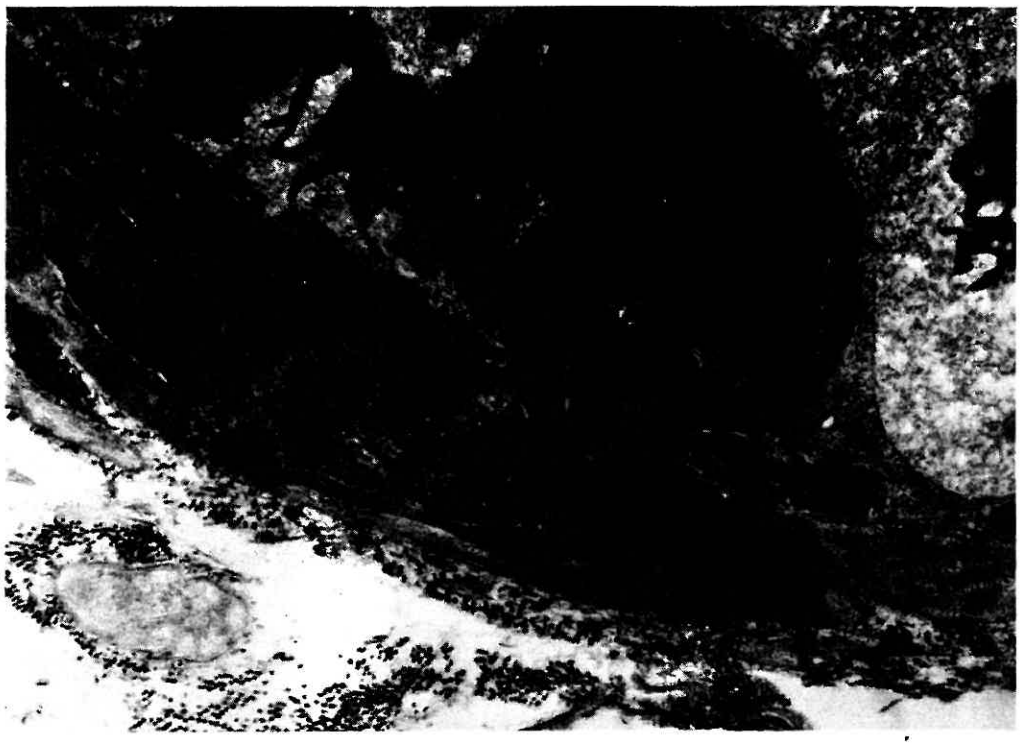

FIG. 10. Transmission electron micrograph of an artery with invading polymorphonuclear leukocyte. The endothelium lies over the surface of the leukocyte which has sent a pseudopod out into the vessel wall. One polymorphonuclear leukocyte was flattened and already within the vessel wall. Two altered platelets are seen attached to the surface of the artery. ×9500.

attempts to reinstitute blood flow with adrenergic blocking agents or vasodilators cannot be expected to be effective.

Although there was no obvious swelling indicating that tissue fluid was accumulating in the thin membrane of the bat wing, the fact that normally contractile lymphatic vessels were quiescent and distended indicated that tissue fluid removal was impeded. With the additional loss of venous vasomotion, which is important in assuring appropriate venous outflow from an area, venous pressure may increase. This could contribute to a restriction of capillary outflow from beds still perfused with blood through open arteriolar vessels or arcades. The demonstrated loss of contractile activity from both venous and lymphatic vessels could be expected to lead to an accumulation of tissue fluid. The edema seen clinically subsequent to intra-arterial injections of barbiturates may be the result of this decrease in the function of mechanisms which normally prevent the accumulation of tissue fluid. It was possible also that an increase in vascular permeability may have occurred, but a few attempts to demonstrate increased permeability in the terminal vascular beds by injecting carbon black were not successful.

Although the characteristic pattern of damage produced by oral preparations of secobarbital differs slightly from that produced by intravenous preparations, the secobarbital itself has been shown to be responsible for the pathological changes which occur following intra-arterial injection.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the aid of Miss Robin Bozian in the preparation of illustrations as

well as for her technical assistance. We would also like to thank Dr. Gwendolyn Stewart for the preparation and evaluation of electron micrographs and Dr. Stephen Niewiarowski for his assistance in the aggregometer studies.

REFERENCES

1. Albo, D., Cheung, L., Ruth, L., Snyder, C., and Reemtsa, K. Effect of intra-arterial injections of barbiturates. *Amer. J. Surg.* **120:** 676, 1970.
2. Lindell, T. D., Porter, J. M., and Langston, C. Intra-arterial injections of oral medications. *N. Engl. J. Med.* **287:** 1132, 1972.
3. Hawkins, L. G., Lischer, C. G., and Sweeney, M. The main line accidental intra-arterial drug injection. *Clin. Orthop.* **94:** 268, 1973.
4. Ryan, J. J., Hoopes, J. E., and Jabaley, M. E. Drug injection injuries of the hands and forearms in addicts. *Plast. Reconstr. Surg.* **53:** 445, 1974.
5. Burn, J. H. Why thiopentone injected into an artery may cause gangrene. *Brit. Med. J.* **2:** 414, 1960.
6. Waters, D. J. Intra-arterial thiopentone. *Anesthesiology* **21:** 346, 1966.
7. Brown, S. S., Lyons, S. M., and Dundee, J. W. Intra-arterial barbiturates. *Brit. J. Anesthesiol.* **40:** 13, 1968.
8. Burn, J. H., and Hobbs, R. Mechanism of arterial spasm following intraarterial injection of thiopentone. *Lancet* **1:** 1112, 1959.
9. Kinmonth, J. B., and Shepard, R. C. Accidental injection of thiopentone into arteries. *Brit. Med. J.* **2:** 914, 1959.
10. Stone, H. H., and Donnelly, C. C. The accidental intra-arterial injection of thiopental. *Anesthesiology* **22:** 995, 1961.
11. Wiedeman, M. P. Preparation of the bat wing for *in vivo* microscopy. *Microvasc. Res.* **5:** 417, 1973.
12. Wiedeman, M. P. Vascular reactions to laser *in vivo*. *Microvasc. Res.* **8:** 132, 1974.
13. Almen, T., and Wiedeman, M. P. Application of contrast media to the external surface of the vasculature. *Invest. Radiol.* **3:** 151, 1968.
14. Kott, I., Ginsburg, H. N., and Jacobson, J. H. Intra-arterial barbiturate injection in a drug addict. *JAMA* **218:** 884, 1971.
15. Mürer, E. H., Stewart, G. J., Rausch, M. A., and Day, J. Calcium iontophore A23187 (Eli Lilly) effect on platelet function, structure and metabolism. *Thromb. Diath. Haemorrh.* **30:** 72, 1975.