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Original Articles

Effects of Plasma and Serum on Arterial Endothelium

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Summary. Electron microscopic study of the femoral artery of rats following a single brief intra-arterial infusion of two different types of rat plasma and of rat serum led to the following findings and conclusions:

1. Plasma from heparinised blood caused no endothelial injury whatsoever whereas plasma from citrated blood damaged the arterial endothelium. Since the intra-arterial infusion of citrate alone produced an endothelial injury that was similar to that induced by plasma from citrated blood, it is very likely that the endothelium-injuring effect of the latter was due to its citrate content.

2. Serum obtained from clotted blood injured both the endothelium and some myocytes of the arteries into which it was infused. This injury was possibly due to the proteolytic or other actions of activated clotting factors, which are known to be present in serum but absent from plasma.

The implications of these findings for certain areas of experimental and human pathology are discussed.

Key words: Endothelial injury – Plasma – Serum – Citrate – Heparin.

Introduction

While studying the ultrastructural effects of various chemicals on arterial endothelium by direct intra-arterial infusion we also had to find solvents for these chemicals that had no effects of their own on endothelium. We thus found that isotonic solutions of phosphate buffer at neutral or slightly alkaline pH had no endothelial effects when infused into arteries in vivo (Constantinides and Robinson, 1969a) and were, therefore, very suitable solvents for any water soluble agents one might wish to study. We were, however, surprised to discover recently that plasma from citrated blood –which should be expected to be a much more "physiological" solvent than chemical buffers – significantly

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injured arterial endothelium. It was also interesting to discover that serum – which is still widely used as a "physiological solution", despite its proven ability to increase capillary permeability under certain conditions (Spector, 1958) – injures the arterial wall. The agent responsible for the endotheliotoxic effect of plasma from citrated blood appeared to be the very citrate we used to produce that plasma. The reason for the arterial injury produced by serum, however, remains unknown for the present.

Materials and Methods

Various solutions were infused for 2 min into the left femoral artery of male Wistar albino rats weighing 300–400 g. Each solution was infused into the artery of 6 rats. The infusion procedure, as repeatedly employed in this laboratory in the past and described in detail elsewhere (Constantinides and Robinson, 1969a), can be summarized as follows:

Each rat was anesthetized with ether, laparotomized, and a gauge 22 hypodermic needle was inserted into its left common iliac artery; using that needle as a cannula, 2 ml of each solution under study was infused into the artery within 2 min, followed immediately afterwards by 1 ml of 5% buffered glutaraldehyde within 60 s, in order to initiate fixation of the arterial endothelium right after the action of the investigated solution. Several 1 mm long cylindrical segments of each artery were then quickly secured by dissection under glutaraldehyde, fixed by simple immersion in 5% buffered glutaraldehyde overnight, post-fixed for 1 h in 2% buffered osmic acid, dehydrated and embedded in epon. Ultra-thin transverse sections made from these arterial segments were then stained with uranium acetate and examined in a JEOL-100B trnasmission electron microscope. In most cases, 4–6 random sections – separated from one another by 2–3 micron intervals – were examined from each of two cylindrical segments per artery.

It should be emphasized that *all* arteries were infused by the various solutions of this study in exactly the same manner (same volume of infused solution, same infusion time and same rate of flow) and were sampled, fixed, dehydrated and embedded under identical conditions. Special care was taken to avoid the entry of air into the arterial lumen at all times since we became aware 12 years ago that even a few seconds' air exposure causes destructive drying of the endothelium.

The endothelial effects of the following solutions were tested by intraarterial infusion as described above: (1) *Millonig's phosphate buffer* (Dawes, 1971) alone; (2) *citrated rat plasma* obtained by centrifugation from a 1:10 v/v mixture of 0.1 Molar sodium citrate (dissolved in Millonig's phosphate buffer) with blood and stored overnight at 5° C before infusion; (3) *0.1 Molar sodium citrate* (dissolved in phosphate buffer) alone; (4) *1.8% saline;* (5) *heparinised rat plasma* obtained by centrifugation of whole blood that was drawn into clinical vacutainer heparinised specimen tubes (143 I.U. heparin per 7–10 ml whole blood) and was stored overnight at 5° C before infusion; (6) *rat serum* obtained by centrifugation from whole blood that was drawn into 15 ml conical centrifuge tubes, allowed to clot at room temperature without agitation for 4 h before spinning, and stored overnight at 5° C infusion. In every instance, whole blood was obtained by aortic puncture from ether-anesthetized donor rats of the same species, sex and weight as those receiving the arterial infusions. All solutions were infused at room temperature (22–25° C). Their pH and osmolarity (in mosmole/kg) were as follows: (1) Phosphate buffer: pH 7.3, 325 mOsm; (2) citrated plasma: pH 7.4, 332 mOsm; (3) 0.1 M sodium citrate in buffer: pH 7.2, 602 mOsm; (4) 1.8% saline: pH 6.9, 590 mOsm; (5) heparinised plasma: pH 7.4, 290 mOsm; (6) serum: pH 7.4, 292 mOsm.

Results

The results are summarized semiquantitatively in Table 1 and illustrated in Figs. 1–7.

It was found that *heparinised plasma* had no injurious effect whatsoever on the endothelium of the arteries into which it was infused (see Table 1 and

Table 1. Arterial endothelial injury induced by various intra-arterial infusions

No. of rats per group	Treatment group	Degree of endothelial injury					
		Rat No. 1	Rat No. 2	Rat No. 3	Rat No. 4	Rat No. 5	Rat No. 6
6	Plasma from heparinised blood	0	0	0	0	0	0
6	Plasma from citrated blood	++	+	++	0	+	++
6	Citrate in phosphate buffer	+++	++	+	+++	+++	+
6	Phosphate buffer	0	0	0	0	0	0
6	1.8% saline	0	0	0	0	0	0
6	Serum	++	+	+++	+	++	+++

The degree of injury encountered in each animal is assessed roughly (semiquantitatively) in this table as follows: (0)= no significant injury seen in any of the sections examined; (+)=significant injury in not more than 25% of endothelial cells examined; (++)=significant injury in more than 25% but not more than 50% of endothelial cells examined; (+++)=significant injury in more than 50% of endothelial cells examined

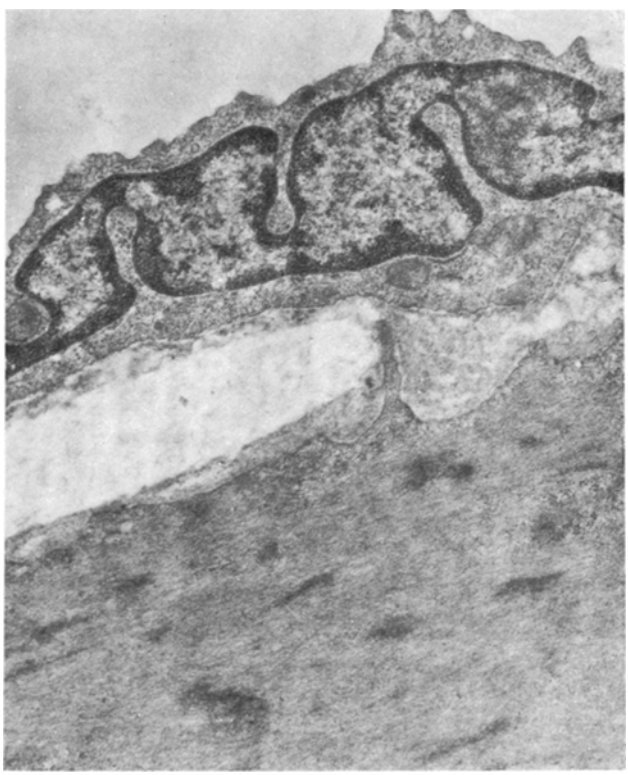

Fig. 1. Typical findings following heparinised plasma infusion. Normal endothelium, overlying a translucent internal elastic lamella which at this particular site exhibits a pore. ×12,000

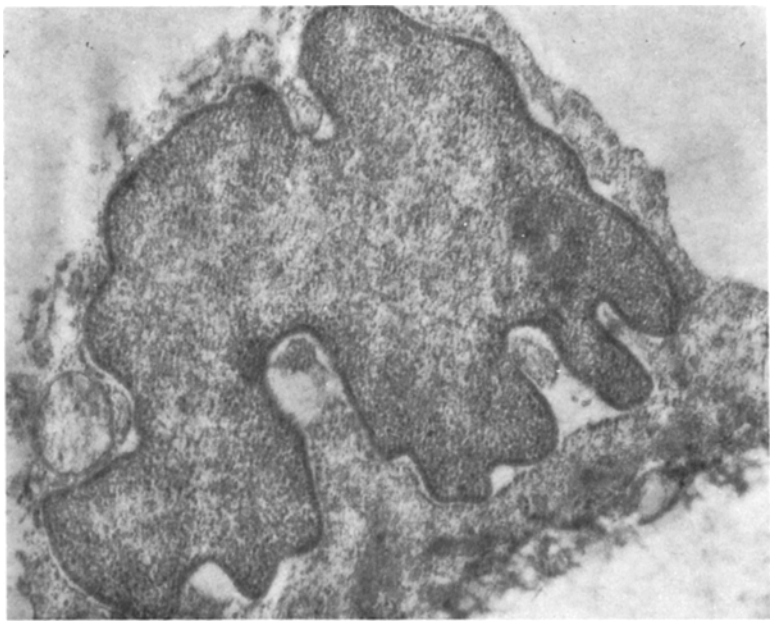

Fig. 2. Typical findings following citrated plasma infusion. Severely injured endothelial cell with extensive loss of plasma membrane and cytoplasm leading to partial exposure of nucleus to lumen. There is a swollen mitochondrion, the nucleus is very edematous, and the perinuclear envelope is focally dilated. ×15,000

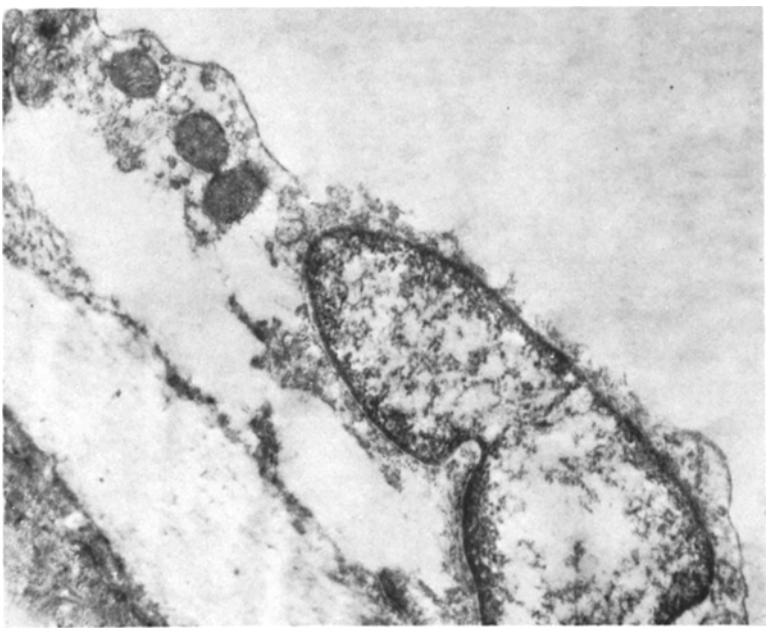

Fig. 3. Typical results of infusion of citrate alone. Disintegrating endothelial cell with extensive loss of plasma membrane on both sides and marked nuclear edema. It is in the process of dropping into the arterial lumen. ×12,000

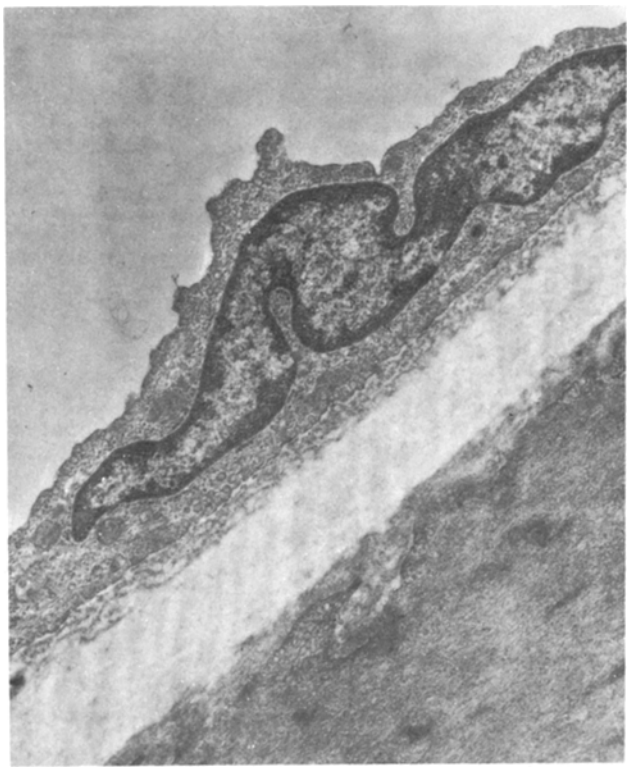

Fig. 4. Typical findings after phosphate buffer infusion. Normal endothelial cell, attached to translucent internal elastic membrane which overlies a normal media myocyte. Identical results were also obtained after 1.8% saline infusion. × 12,000

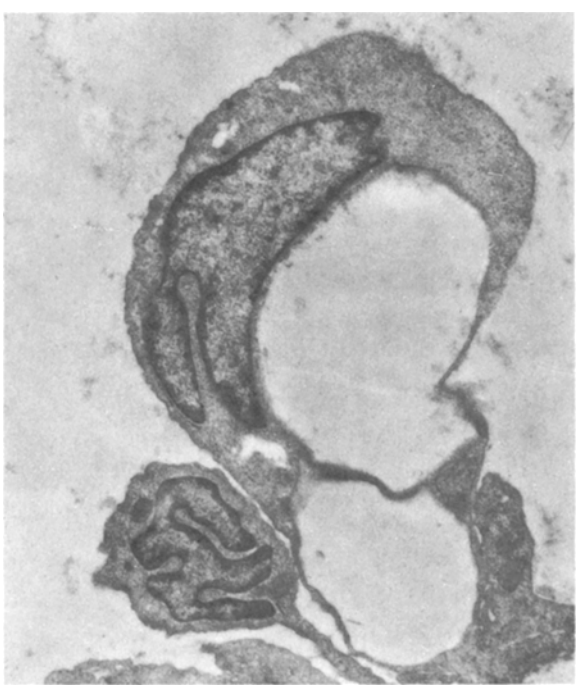

Fig. 5. Serum infusion. Formation of two huge cavities within an endothelial cell. These giant vacuoles have pushed the nucleus against one side of the cell and have stretched wide areas of peripheral cytoplasm to an extremely thin film so that the cell is ready to rupture. Another smaller, highly contracted, endothelial cell is also visible. × 6,000

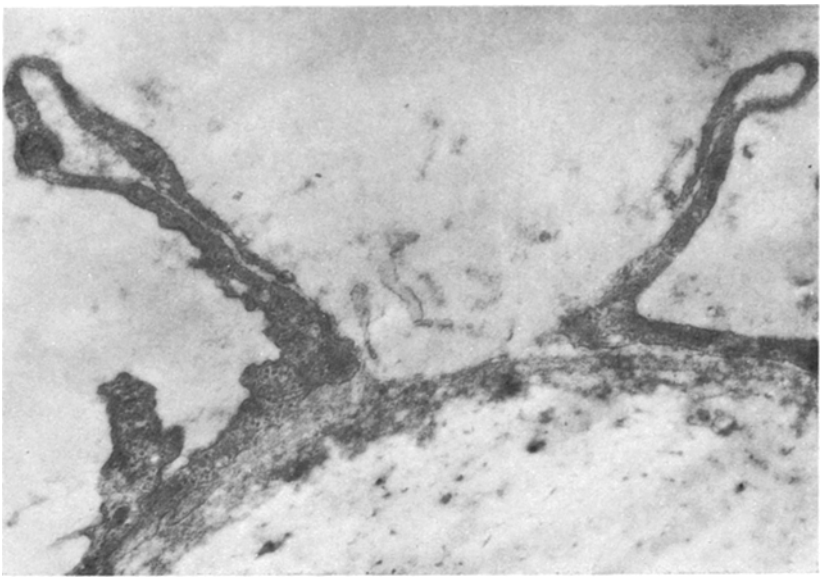

Fig. 6. Serum infusion. Break in continuity of the endothelium exposing a segment of the underlying internal elastic membrane to the arterial lumen. × 12,000

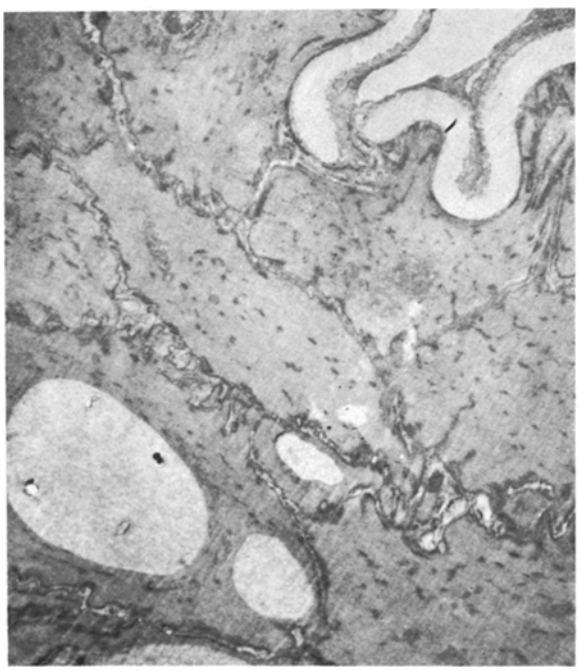

Fig. 7. Serum infusion. Development of large cavities within the cytoplasm of myocytes in the media. The cavities seem to be filled with a coagulated proteinaceous fluid. × 2,400

Fig. 1). By contrast, *citrated plasma* produced significant endothelial injury, ranging from cytoplasmic and nuclear edema to breaks and loss of plasma membrane and cytoplasm in many endothelial cells of most animals to which it was given (see Table 1 and Fig. 2).

Citrate alone (dissolved in phosphate buffer) produced similar and more intensive endothelial injury than citrated plasma (see Table 1 and Fig. 3), while *phosphate buffer* and hypertonic *1.8% saline* (which possessed the same osmolarity as citrate dissolved in buffer) had no deleterious effect on the arterial lining (see Table 1 and Fig. 4).

Unlike the harmless heparinised plasma, the infusion of *serum* caused significant injury to the arterial lining of all animals receiving it, producing large intracytoplasmic cavities and breaks of plasma membranes in many endothelial cells (see Table 1 and Figs. 5 and 6); it also caused huge vacuoles in myocytes of the arterial wall of two of the rats that received it (see Fig. 7). Myocyte vacuolisation was not observed in any other group of this study besides the one receiving serum infusions.

Discussion

The first clear conclusion from our results was that plasma derived from heparinised blood is harmless to the arterial endothelium – and, therefore, eminently suitable as a vehicle for ultrastructural studies of intra-arterially infused drugs or other chemicals – whereas plasma from citrated blood is toxic to the endothelium.

Our data strongly suggest that the endotheliotoxic effect of citrated plasma was caused by its citrate content, because citrate alone (dissolved in phosphate buffer) produced similar and more intensive injury, whereas plasma prepared with heparin instead of citrate was innocuous.

The damage caused to the arterial lining by citrated plasma and by a citrate-buffer solution was apparently due to a specific interaction between citric acid and endothelium and not due to hyperosmolarity or buffer effects. It should be pointed out that (a) citrated plasma was isotonic, (b) the phosphate buffer used as a solvent for citrate proved harmless when infused into arteries alone, in confirmation of earlier findings (Constantinides and Robinson, 1969a) and (c) a 1.8% saline solution that was approximately as hypertonic as the citrate-buffer solution did not damage the endothelium, in agreement with previous observations (Constantinides and Robinson, 1969a).

The mechanism of the action of citrate is unknown at the moment and could be due, theoretically, to a number of factors. If we consider that citrate is well known to bind calcium ions – and by removing them from the blood to prevent coagulation – it is quite possible that it acts (a) by pulling calcium ions and perhaps other bivalent cations from the plasma membrane and peripheral cytoplasm of the endothelial cells, if such ions contribute to the structural integrity of these cells, (b) by removing calcium and other similar cations that may be bound to the anionic acid polysaccharide coat of the endothelial plasma membrane, i.e., its glycocalyx, or (c) by inhibiting certain calcium requiring

enzymes in the endothelial plasma membrane (e.g. ATP-ase) through removal of the calcium they need for their operation. These and other possibilities will have to be the subject of future studies.

The *second clear conclusion* of this study was that, unlike plasma from heparinised blood, a single infusion of serum damages the endothelium (and in some cases the myocytes) of the arteries into which it is infused. This finding may explain the results of Friedman et al. (1975) who found that repeated infusions of human serum (both lymphocytotoxic and non-lymphocytotoxic) into carotid artery segments of rabbits produced myo-intimal hyperplasia and variable thrombosis in these vessels. Perhaps the thrombosis and the intimal hyperplasia represented responses of the blood and the arterial wall, respectively, to the repeated injury of endothelium and myocytes caused by serum.

Our ultrastructural serum findings may also relate to the previously reported ability of diluted serum to increase capillary permeability and promote inflammation, as evidenced by the dye leakage test (Spector, 1958). However, it will be up to future research to decide whether the above "diluted serum factor" that increases the permeability of capillaries is identical with the factor in undiluted serum that injured the endothelium and myocytes of arteries in the present study.

Finally, our results with serum may have some relevance to the intriguing observations made several years ago by Wessler and his coworkers (Wessler and Reimer, 1960) that systemic infusion of serum – but not plasma – produces massive blood coagulation in vascular segments containing stagnant blood. While these investigators hypothesized that the procoagulant effect of serum in their experiments was due to the activated coagulation-promoting factors it contains (activated factors that are absent from plasma), our present ultrastructural findings suggest that this effect of serum could also have been due, at least in part, to the endothelial injury it produces.

The mechanism through which serum injures arterial endothelium in the acute, single-shot experiments of this study is not yet known, but it seems unlikely to be of an immunological nature, for the following reasons: (a) serum donors and recipients belonged to the same species, (b) the recipients were not previously "sensitized" to serum, (c) the injury materialized within an extremely short time, and (d) plasma, which was as antigen-rich as serum, proved harmless.

Perhaps the endotheliotoxic effect of serum is due to the well-known proteolytic activity of some of the activated clotting factors it contains, all the more since it has been found that the intra-arterial infusion of certain proteolytic enzymes, such as ficin, papain, or elastase, will cause injury to the arterial endothelium (Constantinides and Robinson, 1969b). Other serum contents, such as fibrinopeptides (generated by the activated fibrinolysin within the clot prior to removal of the supernatant serum) could also add to the proteolytic injury by virtue of their recently established ability to greatly increase endothelial permeability and thus promote inflammation (Ryan and Majno, 1977).

As to the giant vacuoles produced in the arterial myocytes of some of our serum-infused animals, we can only speculate at the present time about their origin and their possible relationship to the herniation-induced intramyo-

cytic vacuoles recently reported by Joris and Majno in small muscular arteries (1977). Evidence available so far indicates that the myocytic vacuoles of the present study are different from those reported by Joris and Majno, since they seem to lack the double-membrane wall described by the latter authors. Evidently, additional studies will be required to elucidate the mechanism of both the endothelial and myocytic serum effects we observed.

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