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ULTRASTRUCTURAL OBSERVATIONS ON THE PATHOGENESIS OF ASPIRIN-INDUCED GASTRIC EROSIONS

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Summary.—It is shown by transmission electron-microscopy that the first structural change in rats, following a dose of aspirin, is to the basement membrane of the endothelial cell of the capillary and post-capillary venule. This leads to the breakdown of small blood vessels before any other cytolytic effect. This is a focal effect and it is proposed that the erosion develops as an ischaemic infarct.

THE PATHOGENESIS of gastric erosions caused by acidic anti-inflammatory drugs has been the subject of some debate. Effects on mucus production (Menguy and Desbaillets, 1967), back diffusion of hydrogen ions (Davenport, 1964) and inhibition of prostaglandin synthetase (Vane, 1971; Carmichael *et al.*, 1976) are major, but not the only, theories accounting for the development of the acute mucosal damage that is the erosion. An examination of the literature shows that there are flaws in many of the proposed theories. For example, an effect on gastric acid secretion either by altering acid production or by changes in mucosal permeability to ion flow is considered to be a strong possibility even though it has been shown that aspirin causes blood loss in the presence of achlorhydria (St John and MacDermott, 1970; Gottschalk and Menguy, 1970; Raforth and Silvis, 1976). There are other discrepancies and contradictions in the various proposals accounting for the gastric damage caused by aspirin and similar drugs. An ultrastructural examination of the stomach, following aspirin administration, was therefore carried out to examine the degenerative changes which must occur before a recognizable erosion can be seen.

MATERIALS AND METHODS

Female rats weighing between 120–160 g were used and except for one control group they were all fasted for 16 h before dosing. The aspirin treatment for any given time interval was administered to 3–6 animals, and each experiment was accompanied by untreated controls. Groups of rats were killed at 5, 15, 30, 60, 120 and 240 min after a single treatment of aspirin. Aspirin was given orally at a dose of 50 mg/kg. This is a relatively low level for rats but was used because it was felt that a marginal effect would be more likely to reveal the primary degeneration. After deeply anaesthetising the animals with chloroform the tissue was perfused with an intraventricular injection of 2·5% glutaraldehyde fixative. The stomach was removed, opened along the lesser curvature and the contents were washed out with fixative and chilled saline. The stomach was saturated with glutaraldehyde fixative, examined under a dissecting microscope and several 2 × 1 mm rectangles of glandular mucosa, representing both rugal crests and folds, were excised and placed into ice cold fixative. Rectangles were made so that orientated sections could be made. Tissue was fixed for 2 h at 4° in the fixative (5 ml of 25% glutaraldehyde, 8·2 ml of 0·2M sodium cacodylate made up to a pH of 7·2–7·4). Tissues were then transferred to a 0·2M cacodylate sucrose buffer (0·215% w/v sodium cacodylate and 0·85 w/v sucrose in aqueous solution).

Tissue was dehydrated and then embedded in an orientated fashion in araldite resin. Sections were cut on an LKB ultramicrotome. Sections of about 60 nm thickness were stained with uranyl acetate and Reynolds lead citrate. A minimum of three grids from each block of tissue were examined on an AEI 6B electron microscope.

RESULTS

Ultrastructural change was noted 5 min after a 50 mg/kg dose. The first effect was

on small blood vessels near the mucosal surface. Thick (1 μ) sections had shown that in places these were more apparent than in control animals and that a dilatation had taken place. Electron microscopical examination showed that in certain areas capillaries and venules had increased numbers of fenestrations (Fig. 1). These were not quantitated because the increase in fenestrations was in localized concentrations and not uniform, and there were no other indications of damage. That this was almost certainly a drug induced effect was confirmed by the finding, in animals exposed to aspirin for 5 to 15 min, that focal concentrations of capillaries and post-capillary venules were frankly damaged. The affected vessels had breaks in

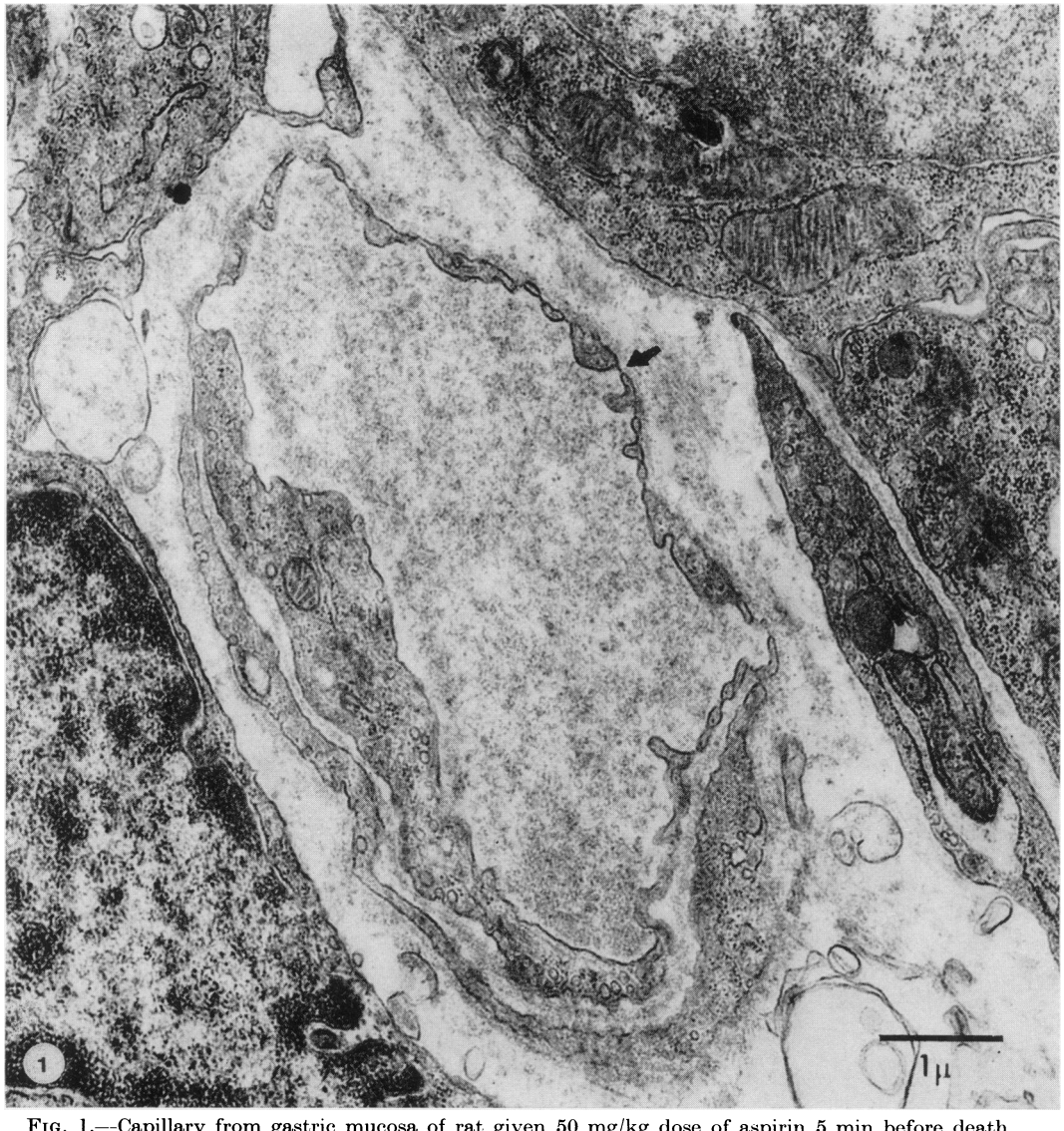

FIG. 1.—Capillary from gastric mucosa of rat given 50 mg/kg dose of aspirin 5 min before death. There are more reductions to the basement membrane (arrow) than are seen in endothelial cells in control animals. This is a focal effect, apparent in some blocks.

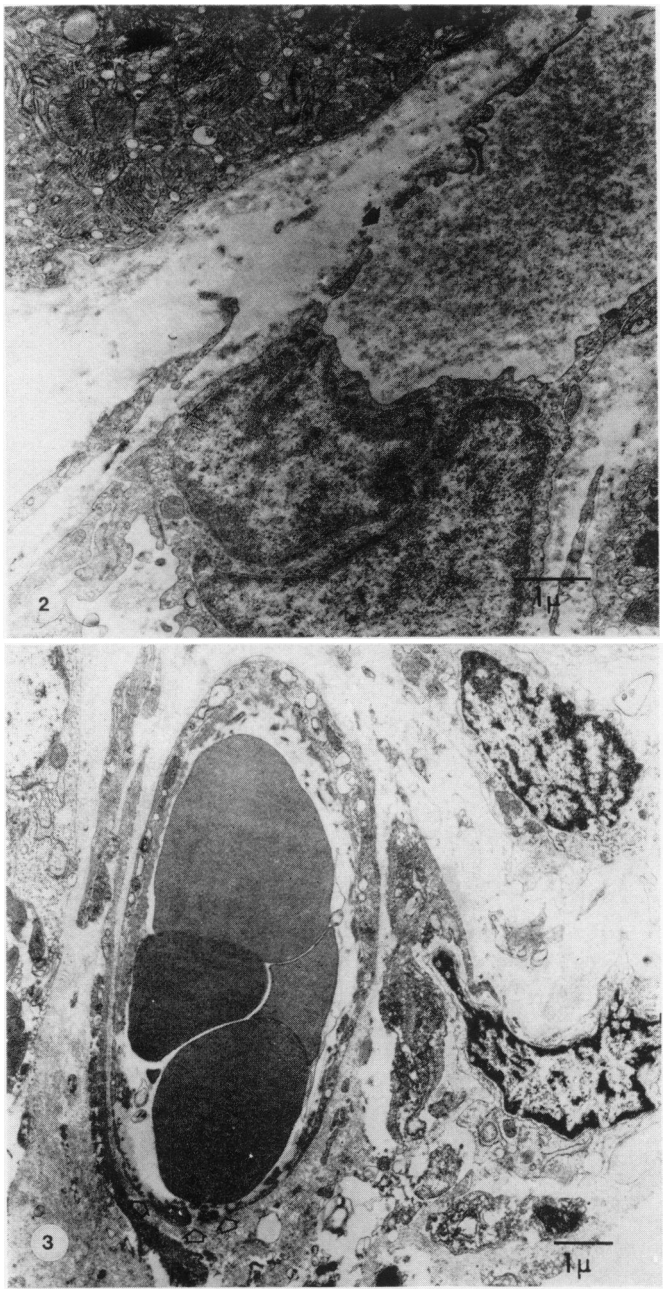

FIG. 2.—Part of a capillary 5 min after a 50 mg/kg dose of aspirin. The endothelial cell is very attenuated. There are several fenestrations and the cell is broken at two points (arrowed). In other respects the cell is normal; pinocytic vesicles can be seen and the nucleus is normal. Mitochondria in the adjacent cell are normal.

FIG. 3.—A venule from the gastric mucosa of a rat 5 min after receiving a 50 mg/kg dose of aspirin. The endothelial cell is broken at three points at the bottom of the figure (arrowed).

the endothelial cell cytoplasm which seem likely to have been caused by broken fenestrations (Figs 2–5). Normal pinocytic vesicles and other organelles were present in the cytoplasm which makes a generalized endothelial cell cytolysis unlikely. Normal undamaged cells were seen alongside damaged vessels.

Damage to mucosal cells was seen in animals exposed to aspirin for 15 min or longer, and although considerable overlap occurred in the timing of these developments, a progressive change was noted. At 15 min the only change involving mucosal cells was to the mitochondria of parietal cells. Damage consisted of disrupted cristae and sometimes a swelling of the mitochondria. This was a focal finding

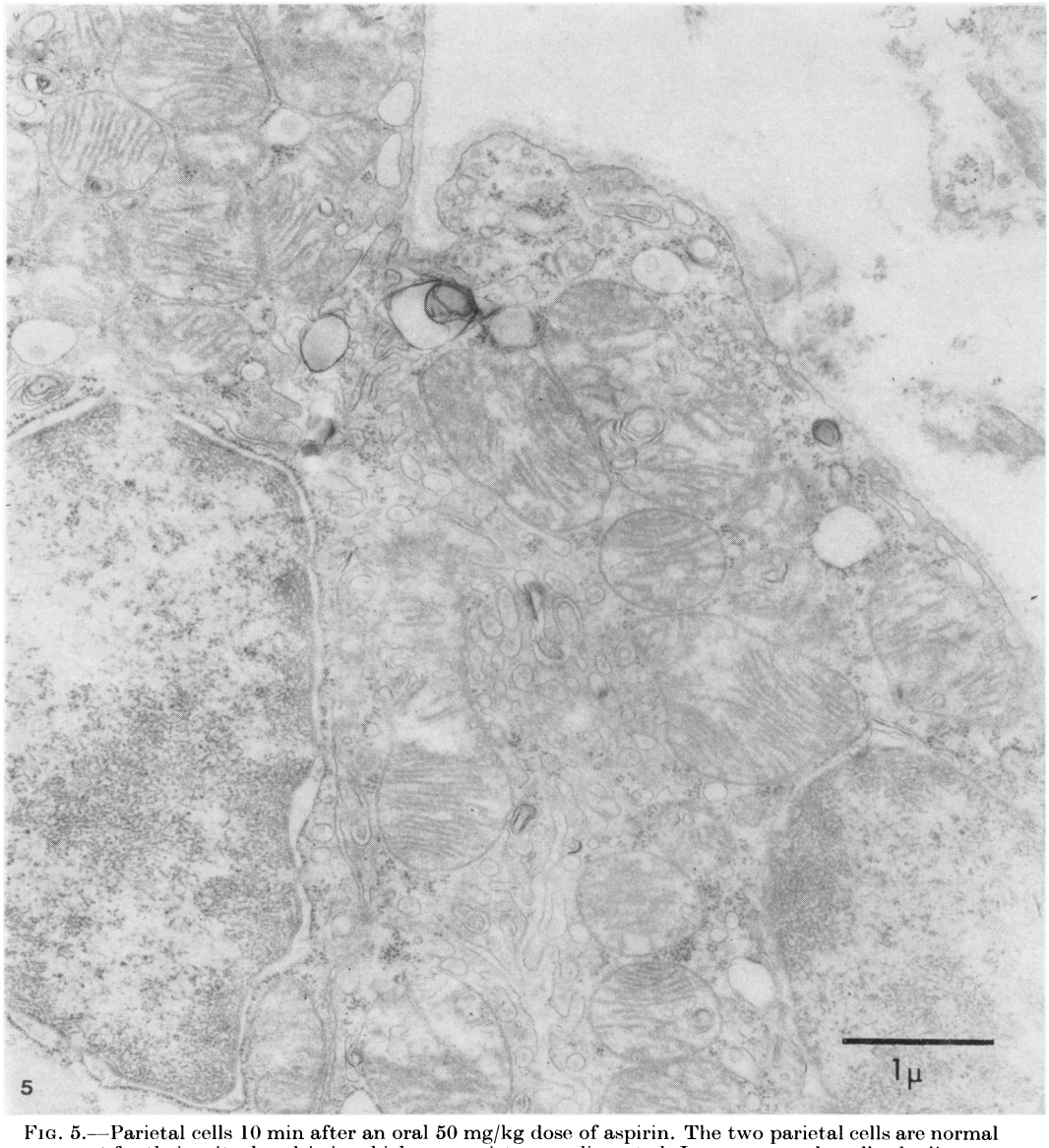

FIG. 5.—Parietal cells 10 min after an oral 50 mg/kg dose of aspirin. The two parietal cells are normal except for their mitochondria in which some cristae are disrupted. In some cases lamellar bodies can be seen in the mitochondria. The nuclei are normal.

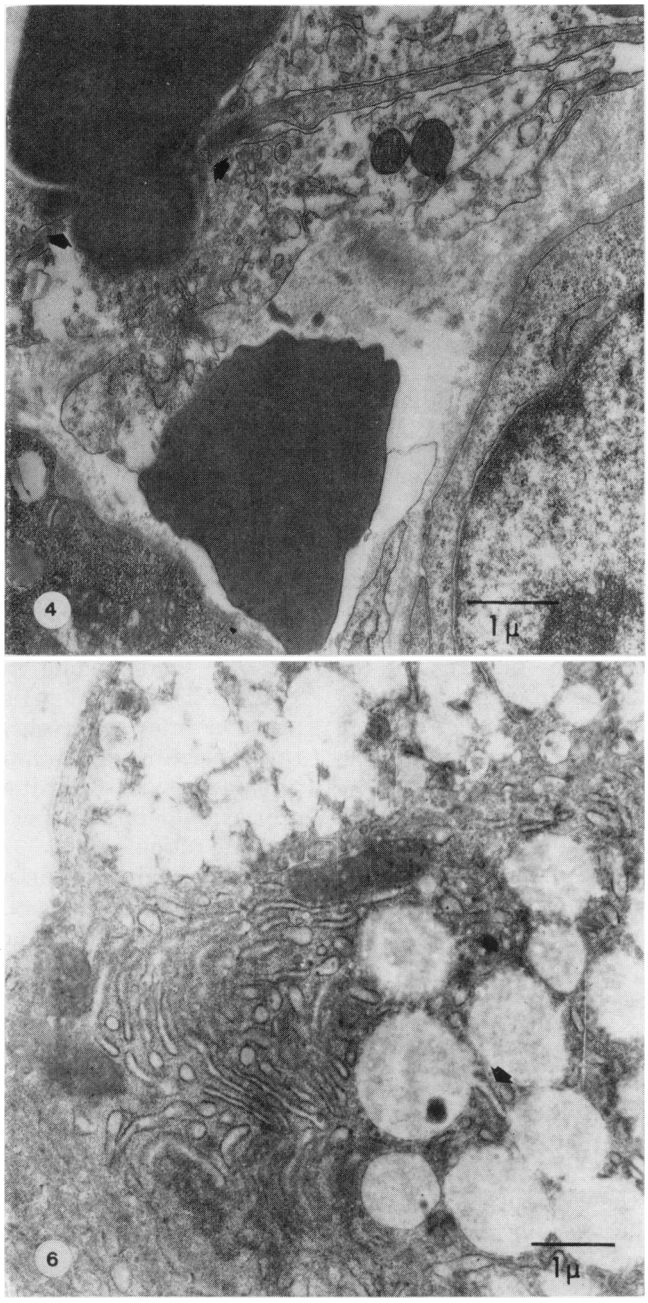

FIG. 4.—Part of a venule from the rat gastric mucosa 15 min after receiving a 50 mg/kg dose of aspirin. The endothelial cell is broken. An erythrocyte can be seen free in the interstitium of the gastric mucosa and another is protruding through the break in the venule wall (arrowed). Though mitochrondria are damaged in the cell at the bottom left the nucleus (bottom right) is normal. Blood loss can be seen occurring before cytolysis has developed.

FIG. 6.—A cell 30 min after an oral 50 mg/kg dose of aspirin. A superficial cell showing several large vacuoles. The vacuoles can be seen to be arising from endoplasmic reticulum. In one place two vacuoles are still connected by endoplasmic reticulum (arrow).

with a number of adjacent cells affected and other parietal cells from nearby tissue remained normal.

The mitochondrial damage was followed by an increase in the numbers of intracellular vacuoles. These small sac-like bodies appeared in parietal cells in considerable numbers, sometimes when the cell was otherwise not particularly affected. Later in the development of the lesions (generally at the margin of an established erosion) larger vacuoles developed in some cells. These occurred particularly in zymogen cells and developed as swellings of endoplasmic reticulum. Damaged zymogen cells were not found except at the margin of established erosions.

Cell death marked the potential beginnings of a recognizable erosion. Aggregation of chromatin material occurred in the nuclei involved, with an almost complete loss of nuclear structure. Cytoplasmic disorganization became bizarre to the extent that it could be difficult to identify the cell type. By this stage neighbouring cells would be undergoing a similar cytolytic pattern.

No changes were seen in the attachments between individual mucosal cell membranes. Indeed clumps of cells could become exfoliate and/or undergo cytolysis while adhering one to the other.

DISCUSSION

In common with most other approaches to the pathogenesis of drug induced gastric erosions, previous ultrastructural studies have yielded contradictory data. Agreed observations include the time course of the events leading to the erosion. Hingson and Ito (1971) found damage in the mouse 8–10 min after drug administration. Pfieffer and Weibel (1973) using the ferret saw changes at 10 min, and Rainsford (1975) using rats also saw damage in 10 min. All were using aspirin. It is also generally agreed that there is no primary damage to cell membranes or junctions, a change which might have been expected if Davenport's (1964) theory of damage by back diffusion of hydrogen ions were correct. The confirmation of intact intercellular membranes is made by Baskin *et al.* (1976) in man, Hingson and Ito (1971) in the mouse and Pfeiffer and Wiebel (1973) in the ferret. The only report of rupture of intercellular junctions is made by Frenning and Obrink (1971) after giving aspirin to cats. Theirs was a scanning electron-microscopical study and Baskin *et al.* (1976) have suggested that the effect was an artefact.

Primary damage to the parietal cell was found by Hahn *et al.* (1975), Rainsford (1975) and Pfeiffer and Wiebel (1973). The nature of the damage seen in the parietal cell is different, however, in each case. Hahn, using guinea pigs, recorded an increase in secondary lysosomes with no effect on the mitochondria, while Rainsford thought that the selective effect was on the matrix of the mitochondria. Pfeiffer and Weibel thought that a swelling of smooth endoplasmic reticulata created vesicles in the parietal cells. The findings made here support Rainsford's conclusions that parietal mitochondria are preferentially damaged, with mitochondrial damage appearing before any swelling of the endoplasmic reticulata.

Parietal mitochondria are not however the first point of damage. The observation, illustrated here, that mucosal blood vessels are the primary point of damage is a novel one. Both Hingson and Ito and Rainsford have commented on capillary breakdown but only in a context of secondary damage as the erosion enlarges.

Harding and Morris (1976) have concluded that aspirin induced erosions are the result of a focal ischaemia. Theirs is the only finding that revolves around an ischaemic response to aspirin. Using scanning and transmission electron microscopy they found a generalized vasodilatation, a blanching response and focal ischaemia after exposing rat gastric mucosae to solutions of 16mM aspirin. This confirms the conclusion drawn here that there is a localized vascular response to absorbed aspirin which precedes the development of erosions.

It has been shown in this paper that in response to aspirin the small vessels of the gastric mucosa become dilated, that there is an increase in the number of fenestrations and that rupture of the endothelial cell occurs. It is reasonable to assume that these events are connected and possibly sequential. How the initial focal vasodilatation is induced is a matter of speculation. The effect is on membranes rather than intracellular organelles and this reduces the number of potential mechanisms. These include interference with the effect of a number of mediators on membranes or on the relaxation of smooth muscle. Prostaglandins, bradykinin, histamine and 5-hydroxytryptamine increase vascular permeability and leakage in the capillary and venular bed. While this is unlikely to occur as a direct consequence of inhibition of prostaglandin synthetase the acidic anti-inflammatories are known to also act as antagonists to the endoperoxidases and the final prostaglandins (Ferriera and Vane, 1973). While no such evidence yet exists it is possible that the antagonism could be specific or differential and alter the balance of prostaglandins so as to temporarily favour vasodilatation. Mepyramine, the histamine H_1-receptor antagonist has no effect on the incidence of aspirin induced erosions (Robins, 1978). This makes it unlikely that changes in histamine are involved in the effect on the blood vessels. It is just possible that this initial effect is independent of the later cytolytic observations, so perhaps an ultrastructural study should be carried out using antagonists of the possible mediators, while attempting to induce erosions.

The vascular effect is followed by changes in parietal cell mitochondria, eventual cytolysis and erosion formation. As all of the vessels and then cells appear to be affected in a small area it is proposed that the erosion develops as a functional focal ischaemia. Parietal cell mitochondria are the organelles most sensitive to the oxygen deprivation caused in this way. The erosion would, then, be produced as an ischaemic infarct and could be the underlying cause of many of the isolated observations previously reported as primary but unconnected events. These include the uncoupling of oxidative phosphorylation and reduction in ATP (Glarborg-Jorgensen *et al.*, 1976), increase in cAMP (Mengla *et al.*, 1974), decrease in acid secretion (Brodie and Chase, 1967) and changes in ionic gradients (O'Brien and Carter, 1975).

Why vessels in a small area are affected while others remain normal is unexplained. One possibility is that the effect is a threshold one such as might be obtained by a high local absorption of drug caused by the presence of particles. This, however, does not explain the fact that the initial damage is caused well before maximal absorption can have taken place.

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