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# Haemodynamically-induced intimal tears in experimental U-shaped arterial loops as seen by scanning electron microscopy

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**Summary.** Experimentally-induced U-shaped carotid loops, simulating arterial tortuosities and kinks were examined by scanning electron microscopy to seek flow-induced changes in the intimal surface. In 14 New Zealand white rabbits carotid arterial transplants were fashioned into U-shaped loops by microvascular surgery. The rabbits were sacrificed from 4 to 226 days post-operatively. Tears in the internal elastic lamina occurred in all loops from 5 days post-operatively and were predominantly transverse and localised about the greater curvature of the bends of each loop. Though initially denuded, all tears appeared endothelialised after 6 days, coalescing later as they increased in size and extent. In older animals only islands of wrinkled internal elastic lamina remained at those sites. Endothelial cells in the tears were small, numerous and polyhedral with raised nuclei. The lesser curvature of the three bends in the loops displayed some irregular wrinkling of the internal elastic lamina. In the three oldest animals a few longitudinal tears were observed on the lesser curvature of the main bend. The specific localization of intimal tears supports the concept that they were hemodynamically induced.

Keywords: U-shaped arterial loops, intimal tears.

## Introduction

Atherosclerosis is accentuated at bends or curvatures in the course of the distributing arteries (Glagov 1965; Texon 1957) and it has been demonstrated that the inner surface of blood vessels can be profoundly altered by flow disturbances associated with experimental coarctation (Legg & Gow, 1982), arteriovenous fistulae (Greenhill & Stehbens 1981, 1983), and aneurysms (Greenhill & Stehbens 1982). Therefore the inner surface of experimental U-shaped loops simulating spontaneous carotid tortuosities and kinks in man (Weibel & Fields 1965) was investigated by scanning electron microscopy.

## Materials and methods

In 14 New Zealand white rabbits (Group A) aged from 3 to 6 months, and of either sex, U-shaped loops were fashioned under sterile conditions on the right common carotid

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artery as follows. Approximately 3 cm of the left common carotid artery was dissected, mobilized and ligated proximally and distally. It was excised and flushed with isotonic saline after ligation of the branches. The right common carotid artery was dissected and mobilized in the mid-cervical region and then clamped proximally and distally. After oblique transection, the stumps were flushed with isotonic saline and the left common carotid artery was transplanted to the right common carotid by oblique end to end anastomoses using 8-0 monofilament nylon microvascular sutures. Two stay sutures were used at the extremity of each anastomosis which was slanted obliquely to reduce the possibility of stenosis to a minimum. The corresponding stay sutures on either side of each anastomosis were then approximated and tied to form a U-shaped loop which was fixed by additional sutures through loose adventitia to the lateral side of the trachea to prevent twisting and coil formation.

In an additional seven rabbits (Group B) the transplants were left *in situ* without tying the stay sutures to form a U-loop. In five rabbits a short segment of the right common carotid artery (approximately 1.5 cm) was removed before transplanting the left common carotid artery. In the remaining two rabbits, the transplants were anastomosed without removal of a segment of the right common carotid artery.

All animals were given a standard pellet diet and water *ad libitum*. They were killed at varying times from 4 to 254 days postoperatively by an overdose of sodium pentobarbital preceded by intravenous heparin (1000 iu). The carotid arteries of each group of animals were perfused via an intracardiac catheter and fixed at a pressure of 100 mm Hg. The right common carotid artery was then dissected and prepared for scanning electron microscopy as previously described (Greenhill & Stehbens 1983). Each artery was bisected longitudinally and cut into numbered blocks for scanning electron microscopy, with the location and orientation of each block being recorded.

The nomenclature used in describing the changes observed is indicated in Fig. 1.

## Results

### *Macroscopic Observations*

At the time of surgery augmented pulsation was noticed in the loop in many animals. In carotid transplants with no loop formation, some tortuosity appeared with each systolic pulsation.

At the time of slaughter all 21 transplants were patent and there was slight mural thickening about the suture lines. In six of the carotid loops (Group A), the axis was orientated at right angles to the axis of the parent carotid artery, in two it was inclined proximally and in the remaining six there was a variable inclination distally. A slight, though distinct dilatation in the loop from six days postoperatively (eleven rabbits), was most noticeable in the proximal limb but at times it involved the greater curvature of the main bend. Of the seven non-looped transplants (Group B), four displayed distinct S-shaped tortuosity in the fixed state and of

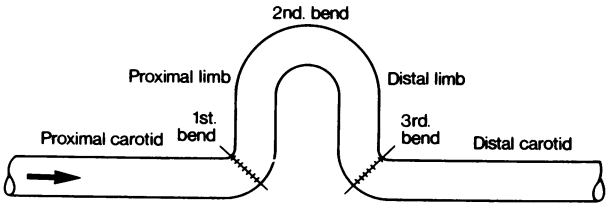


**Fig. 1.** Diagram of the U-shaped carotid loop, showing the nomenclature used. The crossed lines represent the oblique suture lines.

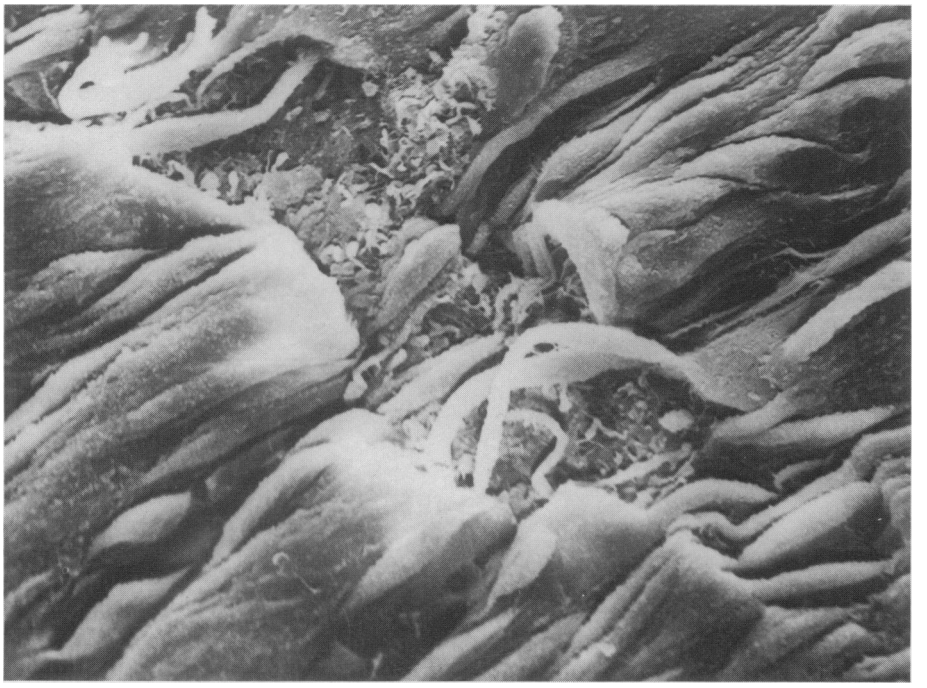

**Fig. 2.** A tear in a loop 5 days postoperatively showing thrombus on the floor with endothelial cell remnants bridging the gap. × 1150.

these one exhibited slight diffuse dilatation of the transplant. The remaining three appeared relatively straight.

*Ultrastructural Observation*

*Group A.* The common carotid artery proximal to the first suture exhibited no gross changes. Clamp sites healed rapidly and could not be detected after one week. The luminal surface exhibited longitudinal parallel corrugations due to the underlying internal elastic lamina. The elongated endothelial cells were arranged parallel to or slightly obliquely to the corrugations. Their cell borders were not prominent and there were few microvilli. The suture lines appeared to be endothelialized within 2 weeks.

In seven of nine animals from 4 to 28 days postoperatively there were areas in the proximal limb in which the endothelial cells had a swirling orientation.

The most prominent finding was the occurrence of tears in the internal elastic lamina in the region of the greater curvature of the three bends. These tears were predominantly transversely orientated. No tears were seen in two animals examined 4 days postoperatively. Few tears were present at 5 days. They lacked endothelium and were coated with thrombus (Fig. 2). From 6 days onwards, all tears were endothelialized. They increased in number and size (up to 600 $\mu$m), often becoming confluent with intervening islands of internal elastic lamina, which, after 14 days were often irregularly ridged and wrinkled (Fig. 3). The endothelium overlying these islands of elastic lamina was of fairly conventional appearance whereas endothelial cells on the floor of the tears were small, numerous, and often polyhedral with prominent nuclei (Fig. 4). Endothelial cells in the floor of the tears and over the islands of elastic lamina were often aligned obliquely

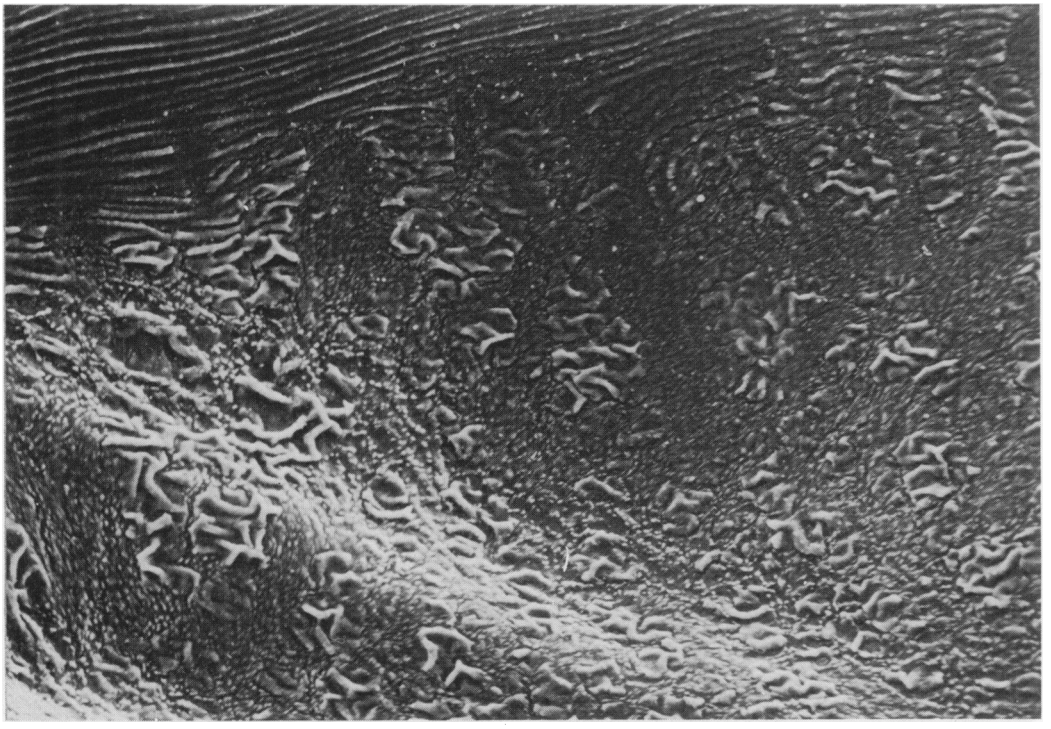

**Fig. 3.** Multiple transverse tears from the greater curvature of the first bend in a loop 14 days postoperatively. The tears have coalesced. Note the normal longitudinal corrugations of the underlying elastic lamina at the top, and the irregular wrinkling of the intervening remnants of elastic lamina. × 63.

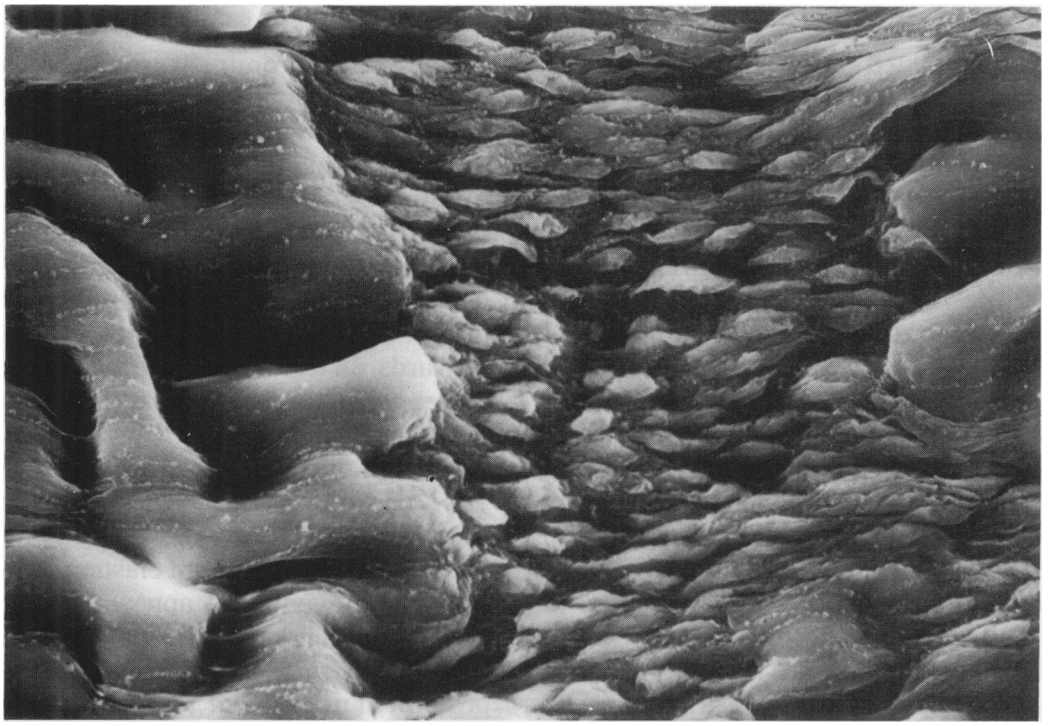

**Fig. 4.** Higher magnification of disrupted internal elastic lamina 21 days postoperatively with numerous endothelial cells in the floor of the tear in contrast with those over the elastic lamina. × 735.

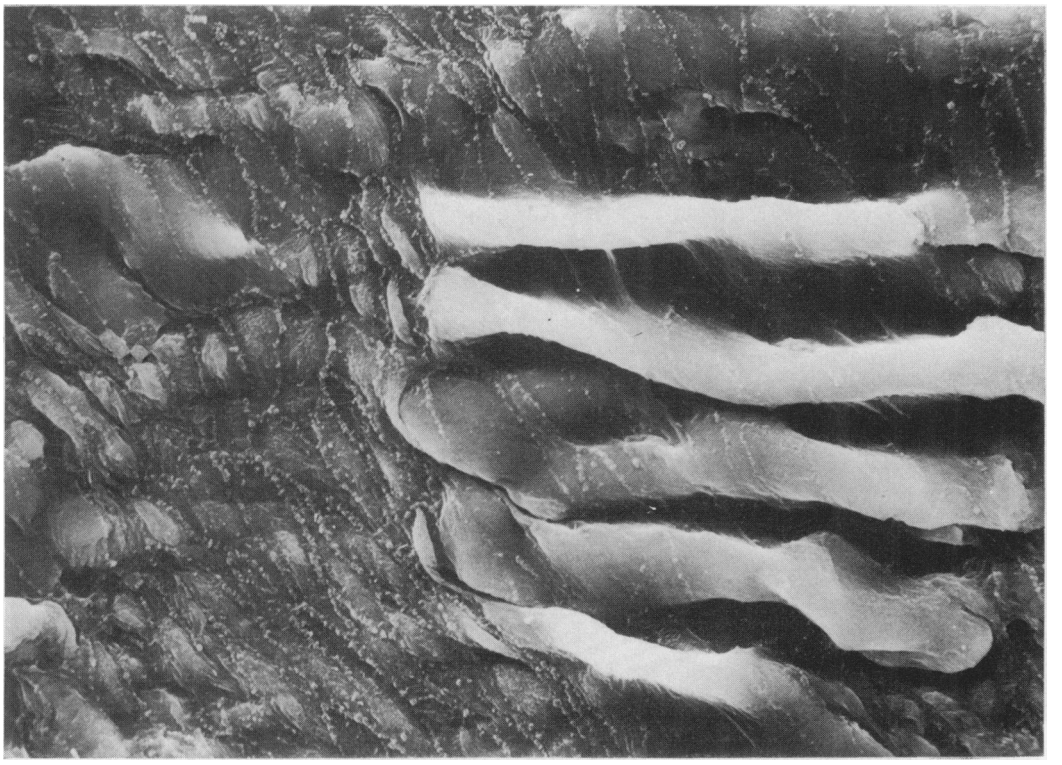

**Fig. 5.** Endothelial cells are more numerous in the floor of the confluent tears than over the residual elastic lamina (37 days postoperatively). Note the oblique orientation of the endothelial cells. × 725.

and sometimes transversely to the vessel axis as if participating in the swirling orientation (Fig. 5).

The tears were localized predominantly to the greater curvatures of the bends and with time extended distally particularly beyond the first and third bends. These changes did not tend to spread circumferentially.

From 4 days postoperatively, the lesser curvature of the second (main) bend exhibited a variable degree of irregular wrinkling of the internal elastic lamina. This change, though observed to some extent at the first and third bends, was complicated by the presence of suture lines. The overlying endothelium in these regions was normal in appearance with the cells aligned mostly in the direction of flow. A few longitudinally orientated tears (100–300 $\mu$m in length) in the internal elastic lamina were observed around the lesser curvature of the main bend in the four oldest animals (66, 158, 203, 226 days postoperatively) (Fig. 6). The endothelial cells on the floor of the tears were small, numerous and similar to those in the tears elsewhere.

*Group B.* Repair at the suture lines was similar to that in Group A. Tears were found in each of the four specimens exhibiting obvious tortuosity. In appearance and orientation these tears were similar to those in Group A and were located about the greater curvature of the bends where blood flow might be expected to impinge on the vessel wall. A whorling orientation of endothelial cells was observed in three specimens (5, 12, and 62 days postoperatively) in proximity to the suture lines.

The three relatively straight specimens exhibited tears. In the 5-day-old specimen there was some swirling of the endothelium near the sutures and two small tears beyond the distal suture. In the 254 day specimen

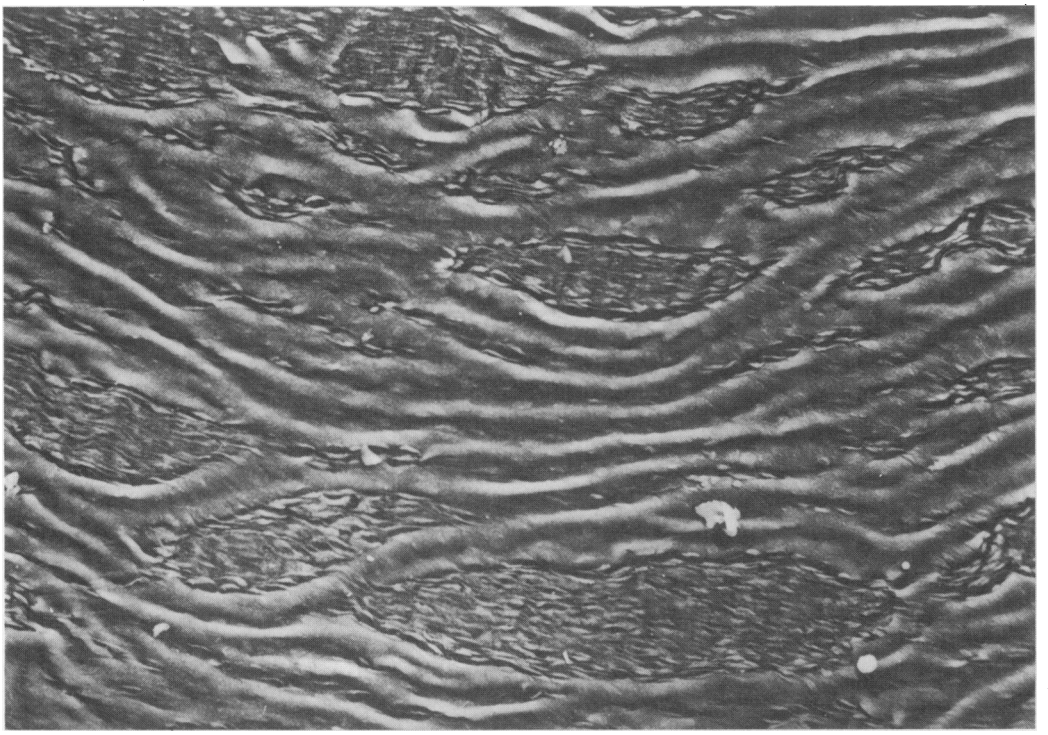

**Fig. 6.** Longitudinal tears from the lesser curvature of the second bend of a loop 158 days postoperatively. Note the numerous small endothelial cells in the floor of the tears. × 175.

tears were restricted to an area just distal to each suture line. Tears were more extensive in the remaining specimen (122 days postoperatively) being located on one side of the distal half of the transplant and just beyond the distal suture.

## Discussion

In the formation of the loops, oblique suture lines were used to reduce the likelihood of stenotic lesions although the scar tissue may have resulted in less compliance during systolic distension than in the adjoining wall and some functional stenosis possibly with kinking cannot be excluded. In experimental aortic coarctations in rabbits Legg & Gow (1982) observed swirling endothelial patterns as well as a dilatation in the poststenotic region. The swirling pattern of the endothelial cells in the present experiment may have been due to a stenotic effect or to disturbed flow in the loop.

When it was apparent after the first two control transplants in Group B. that redundant vessel wall produced tortuosity which varied with arterial pulsations, a segment of the right common carotid artery was excised thereafter before transplanting a segment of the left carotid. In two of the five animals, despite this procedure, the artery was obviously tortuous after fixation. In the remaining three there could well have been tortuosity during each pulse, which might have accounted for the few tears in these specimens. In view of the retraction of both the transplant and the stumps of the right carotid artery after transection, it is essential that the transplant is longer than the excised segment to facilitate suturing with minimal trauma because the sutures easily tear through the arterial wall. It is probable that some tortuosity was present in each animal of Group B. It would appear therefore that to obtain the ideal control would be extremely difficult although the specific localization of

the intimal tears in the U-shaped loops does not support surgical trauma as an aetiological agent in their production. This view is supported by the control material of a previous paper by Greenhill & Stehbens (1983).

The corrugations in the carotid arteries are believed to be caused by muscular contraction of the vessel wall with resultant longitudinal folding of the internal elastic lamina. As in the present experiments, corrugations may still occur even following perfusion fixation at physiological pressures, (Gertz *et al.* 1975; Hirsch *et al.* 1981; Kawamura *et al.* 1974). The significance of the altered corrugations at the lesser curvatures is uncertain. It could be an artefact of fixation but in view of the consistent localization it may indicate some alteration in the physical properties of the internal elastic lamina.

The most significant observation in this study was the occurrence of tears in the internal elastic lamina. These were morphologically similar to those in the artery feeding a sizable experimental arteriovenous shunt (Greenhill & Stehbens 1983). Significantly the arteries developed tears at 5 days in both studies, indicating that this is a critical time period for the stress associated with these flow models. In the present model the augmented flow and increased pulsation may be instrumental in causing the tears but the localization suggests some other haemodynamic factor. These observations could explain the prevalence of circumferential tears on the greater curvature of bends in the splenic artery (Meyer & Weber 1968), and contribute to the thinning of the arterial wall on the greater curvature of the carotid siphon (Stehbens 1979).

Transversely orientated depressions and ridging associated with tears in the internal elastic lamina can be readily observed in iliac and carotid arteries of human infants (Meyer & Lind 1972). Their occurrence has been attributed to the large blood flow to the placenta and brain (Meyer 1975). These splits had closely related calcific deposits arranged in a similar transverse pattern.

Texon *et al.* (1966) induced tortuosities in canine carotid arteries and produced intimal thickening along the lesser curvature of the bends. Matsuda *et al.* (1978) fashioned U-bends on the side of the intact artery and also stenosed the recipient artery midway between the end to side anastomoses of the U-bends. This too resulted in intimal thickening at specific anatomical sites (along the lesser curvatures), but there was no mention in either study of any degenerative changes about the greater curvature of the U-bend.

Campbell & Campbell (1980) reported the occurrence of tears in the internal elastic lamina in the renal arteries of hypertensive and normotensive rats. Intimal proliferation associated with such tears was more pronounced in the hypertensive rats. They also looped the renal artery of normotensive rabbits around polyethylene tubing without occlusion. Within 2 weeks they found tears in the internal elastic lamina with intimal thickening but their findings are not necessarily flow related because of the need to stretch and fix the renal artery. There was no mention of control material to exclude trauma. In the present study such traumatic stretching was obviated by using transplants. The specific localization of the tears also suggests that trauma was not the initiating factor in the development of tears in the present study.

Meairs *et al.* (1983) found longitudinally orientated intimal tears (200–300 $\mu$m in length) in coronary arteries of dogs with hypertension of one years duration.

The U-shaped loop used in this experiment could prove a useful model for the haemodynamic production of intimal tears and facilitate investigation of the underlying mechanism of their development.

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