*Br. J. Pharmac.* (1969), **36**, 219–224.

# ACTH and decubitus ulceration: an experimental study

A. A. BARTON AND MARY BARTON

*John Curtin School of Medical Research, Australian National University, Canberra, Australia*

1. Ulcers due to neurectomy with excision of the sciatic nerve are more severe than those due to tenotomy and can be inhibited by a single dose of ACTH.

2. Timing and mode of administration of ACTH to prevent decubitus ulceration is critical and depends on its ability to prevent the separation of endothelial cells.

Avulsion of a segment of the sciatic nerve or tenotomy of the tendo calcaneus causes decubitus ulcers to appear on the heels of mice. Following nerve avulsion there is extensive retraction of endothelial cells in the base of ulcers. The lumen of the vessels becomes occluded by platelets and red cells and there is anoxic necrosis of the skin (Barton & Barton, 1968a). A single dose of lente ACTH inhibits ulceration due to nerve avulsion by preventing retraction of endothelial cells, and limiting the extent of platelet thrombosis of vessels (Barton & Barton, 1968b).

In the present paper, changes in the capillaries of the base of ulcers due to tenotomy of the tendo calcaneus are described. A comparison is made of the extent of ulceration due to tenotomy and that due to excision of 0·5 cm of the sciatic nerve. The effect of ACTH on ulceration due to tenotomy has also been investigated. The latter causes direct damage to capillaries and little retraction of endothelial cells, on which ACTH has no significant inhibitory effect.

A preliminary study showed that the period during which ACTH exerted an inhibitory effect on decubitus ulcers due to excision of the sciatic nerve was short and limited to that during which endothelial cell retraction was taking place. Once this had occurred ACTH had no inhibitory effect. In the earlier experiments (Barton & Barton, 1968b) the drug was administered into the thigh of the side opposite to operation at the time of nerve avulsion. Absorption was rapid and the inhibitory effect on ulceration marked. In the present experiments we tested the effects of giving the lente ACTH into the calf of the operated side at the time of operation, and 4 hr before its performance.

## Methods

Two hundred male mice weighing approximately 35 g each were anaesthetized using ether, followed by nitrous oxide, oxygen, and trilene, and 0·5 cm of the sciatic nerve was excised from the thigh. The wounds were closed with aseptic precautions. Recovery was quick and uneventful. The mice were kept in groups of

twelve in cages 18 in. × 12 in. × 6 in. on a layer of clean springy wood shavings, which provided a 1 in. lining to the bottom of each cage. Each cage contained a treadmill and the mice made continual use of this before and almost immediately after the operation. Corticotrophin (ACTH) 0·002 ml. in gelatine solvent (20 u./ml.) was injected into the calf muscles of the operated leg 4 hr before the operation in one group of animals. In a second group the drug was given at the time of operation and in a third group it was given 24 hr after the operation.

In a further two hundred anaesthetized mice the tendo calcaneus was divided at the ankle and corticotrophin administered as previously described.

The method used to assess the extent of ulceration was to give one mark if the skin was broken and a further mark when the area of ulceration exceeded 3 mm in diameter. In addition a single mark was added if oedema of the foot could be detected and a further mark if this was excessive and extended up the calf. These marks were added up so that the maximum number of marks for an individual mouse was four when the ulcer was large and the foot grossly oedematous. The mice were examined once daily and the total markings assessed by dividing the total number of marks by the number of mice. It may be seen that this method of marking provides an index of inflammation caused by the operation.

Material for electron microscopy was fixed at pH 7·2 in 1% osmium tetroxide in 0·1M phosphate buffer and embedded in Araldite. Sections were cut using a diamond knife with an L.K.B. microtome, stained in uranyl acetate and then with lead citrate (Fiske, 1966). They were examined in a Seimens Elmiskop 1 electron microscope.

## Results

### *Macroscopic*

Figure 1 compares ulceration of the heel where loss of plantar flexion has been caused by (1) neurectomy and (2) tenotomy at the ankle. Ulcers formed most

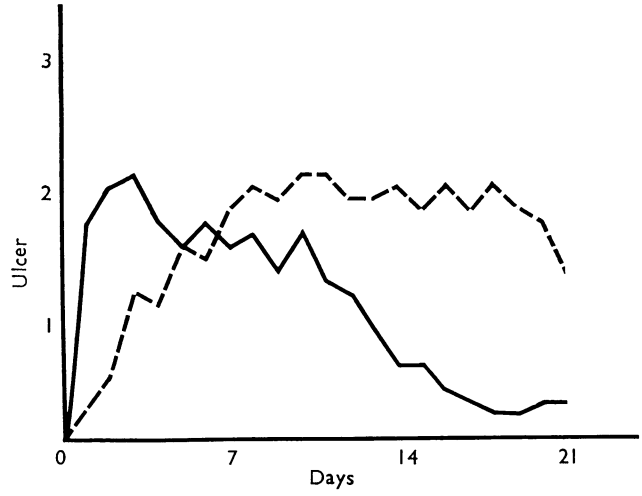


FIG. 1. Extent of ulceration due to tenotomy (——) and neurectomy by excision of a segment of the sciatic nerve (- - -). Ulcers due to neurectomy take longer to develop and heal less readily than those due to tenotomy.

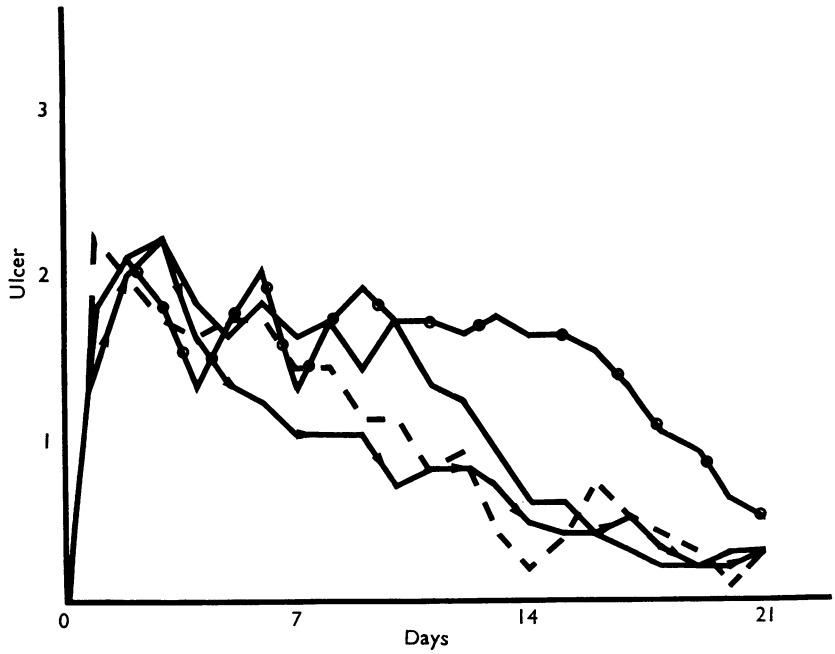


FIG. 2. Ulceration due to tenotomy (——). When a single dose of ACTH is given there is a slight inhibition when given at (- - -) or 4 hr before (→—) the time of operation, with an increase in length of ulceration when given 24 hours after (-⊖-).

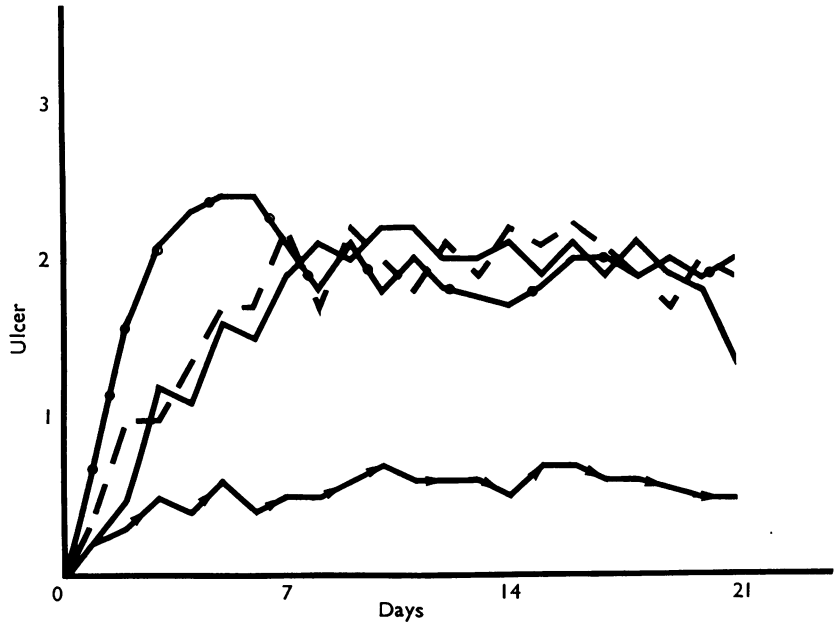


FIG. 3. Ulceration due to neurectomy (——). When a single dose of ACTH is given there is inhibition of ulceration when the drug is given 4 hr before operation (→—). ACTH given at time of neurectomy (- - -); ACTH given 24 hr after neurectomy (-⊖-).

rapidly after tenotomy. Every one of these mice had a sore on its heel 6 hr after operation. A maximum mark for ulceration combined with oedema was reached at three days. After tenotomy the animal's weight is supported by a small area of skin covering the os calcis. This takes the full weight in running, walking and leaping. The ulcers healed rapidly.

Ulcers following avulsion of the sciatic nerve take longer to develop. A peak for ulceration combined with oedema was reached at 7 days. Once established, these ulcers either continued to get worse, so that animals had to be killed, or they became chronic perforating ulcers.

Following neurectomy there was paralysis of muscles. The animal's weight was distributed over a wide area of the foot. The toes were clawed and the foot could be raised by the thigh muscles so that the animal could shuffle along. In movement, the dorsal surface of the toes was used as well as the heel. They were also used to support the animal while at rest. The dorsal surface of the digits was often ulcerated.

*Electron microscopy*

Two hours after tenotomy the lumen of vessels below the dermo-epidermal junction contains amorphous material believed to be coagulated protein with thrombi consisting of distorted red cells with a few platelets. The endothelial cells remain adherent at their junctions. Many were vacuolated or appeared dead.

Appearances were similar to those of direct damage described by Cotran & Majno (1964) and Cotran (1965) and are not illustrated. Many of the vessels in the deeper layers of the dermis show direct damage to endothelial cells. The lumen of the capillaries remains patent.

The fine structure of the blood vessels associated with ulcers due to nerve avulsion, where there is extensive retraction of endothelial cells, has been described elsewhere (Barton & Barton, 1968b).

### *Effects of ACTH*

Figure 2 shows the effect of a single dose of ACTH given both before and after tenotomy. The drug has a slight inhibitory effect when given at the time of operation or 4 hr before. There is a transitory increase in bleeding from the wound consistent with the action of ACTH in depressing platelet aggregation. Given 24 hr after operation, the acute phase of ulceration lasts for approximately 2 weeks, whereas untreated ulcers begin healing after 1 week. The extent of the final degree of healing is not changed by treatment.

Figure 3 shows the effect of giving lente ACTH on ulceration due to excision of the sciatic nerve. When given at the time of operation or 24 hr afterwards, the severity of the ulceration is increased. As in the case of tenotomy the acute phase of ulceration is prolonged. Given in single doses ACTH has no effect in reducing oedema due to the operation. The effect of administering 0·002 ml. into the calf muscle 4 hours before operation is markedly to inhibit ulceration.

## Discussion

After division of the tendo calcaneus ulcers form within 6 hr. The animal's

weight is supported by a relatively small area of skin over the os calcis, which possesses none of the characteristics of a weight-bearing surface (Elson, 1965) and ulcerates rapidly. With the electron microscope it can be seen that damage to the vessels is direct (Cotran & Majno, 1964) and that while many of the endothelial cells of superficial vessels in the dermis are damaged, or even appear dead, they remain attached to one another. Many vessels remain patent, continuing to provide an adequate microcirculation to deeper tissues in the pressure area, limiting the extent of ulceration. We concur with Ashford & Freiman's (1967) view that an important function of the endothelium, even when damaged, is to act as a passive barrier separating the procoagulants of the blood from the subendothelial tissues of which collagen is particularly important.

Following excision of the sciatic nerve the endothelial cells of capillaries in the area of presumptive ulceration separate from one another, the cell junctions are fractured and become incorporated into the cytoplasm. Large gaps are formed in the wall of vessels, which become plugged by platelet thrombi. In the centre of the ulcer these fuse and enclose red cells, obliterating the vascular lumen. At the same time partial separation of endothelial cells occurs over a wide area of the surrounding tissues. Gaps are plugged with platelets which are not sufficient to occlude the lumen (Barton & Barton, 1968b). It is thought that once the cell junctions have been broken the vessels are sensitized so that further retraction of the cells is facilitated. Additional trauma, which in itself cannot cause complete separation, may then cause the complete and rapid retraction of cells. Contact between the contents of the vessel and the subendothelial tissues takes place and is followed by occlusion of vessels over a wide area of the dermis.

Separation of endothelial cells appears to be due to prolonged trauma and is most severe in decubitus ulcers due to excision of the sciatic nerve. These take 7 days to attain maximum severity, while ulcers due to tenotomy take 3 days, but are less severe. The importance of the time factor in the development of anoxic changes was stressed by Husain (1953). He showed that relatively light pressure over a long period of time caused more tissue damage than a more intense pressure for a short time. Loss of vasomotor control (see Chapman & Goodell, 1964), debility, intercurrent illness, nutritional inadequacy, infection and endotoxins (McKay, Margaretten & Csavossy, 1966) may facilitate the separation of endothelial cells. This could provide an explanation of the exceptional severity of bed sores where these factors are present and where even pressure from the bedclothes or weight of a hand can cause severe ulceration.

Bensch, Gordon & Miller (1964) compared filaments in the cytoplasm of endothelial cells to leiomyofibrils. Contraction of these filaments may cause the separation of cells in decubitus ulcers. Separation must involve the breakage of cell junctions. Since endothelial cells contract in decubitus ulcers treated with ACTH but do not become separated from one another, we have suggested that this drug acts by stabilizing the protein polysaccharide at cell junctions. This is thought to form an important part of the antiphlogistic effect of ACTH.

When ACTH is given either before or at the time of operation into the operated leg of tenotomized mice, there is little effect. In this case ulceration is not associated with endothelial cell retraction and damage to the capillaries is direct. ACTH given 24 hr after tenotomy appears to prolong the intensity of ulceration.

In animals, sex, time and route of administration are of great importance in

determining the pharmacological effects of corticoids (Frenkel & Havenhill, 1963). It seems likely that optimum methods of administering single doses of ACTH in man so as to inhibit decubitus ulceration are also critical because the period during which ACTH has an inhibitory effect on endothelial cell separation is short. Timing is important. If there is a delay in absorption due to the loss of vasomotor control, such as that which occurs following neurectomy in the mouse, there is no effect. In this case, however, when the drug was administered 4 hr beforehand, a single dose of lente ACTH markedly inhibits decubitus ulceration.

## REFERENCES


ASHFORD, T. P. & FREIMAN, D. G. (1967). The role of the endothelium in the initial phases of thrombosis. An electron microscope study. *Am. J. Path.*, **50**, 257–273.

BARTON, A. A. & BARTON, MARY (1968a). Plantar ulcers occurring after neurectomy: a light and electron microscope study. *Aust. J. exp. Biol. med. Sci.*, **46**, 155–163.

BARTON, A. A. & BARTON, MARY (1968b). The inhibition of decubitus ulceration with ACTH *J. Path. Bact.*, **96**, 345–351.

BENSCH, K. G., GORDON, G. B. & MILLER, L. (1964). Fibrillar structures resembling leiomyofibrils in endothelial cells of mammalian pulmonary blood vessels. *Z. Zellforsch. mikrosk. Anat.*, **63**, 759–766.

CHAPMAN, L. F. & GOODELL, HELEN (1964). The participation of the nervous system in the inflammatory reaction. *Ann. N.Y. Acad. Sci.*, **116**, 990–1017.

COTRAN, R. S. (1965). On the presence of an amorphous layer lining vascular endothelium under abnormal conditions. *Lab. Invest.*, **14**, 1826–1833.

COTRAN, R. S. & MAJNO, G. (1964). A light and electron microscopic analysis of vascular injury. *Ann. N.Y. Acad. Sci.*, **116**, 750–764.

ELSON, R. A. (1965). Anatomical aspects of pressure sores and their treatment. *Lancet*, **1**, 884–887.

FISKE, SUSAN (1966). An adaptation of Reynolds' lead citrate stain for high resolution autoradiography. *J. Microsc.*, **5**, 355–360.

FRENKEL, J. K. & HAVENHILL, M. A. (1963). The corticoid sensitivity of golden hamsters, rats and mice. Effects of dose, time and route of administration. *Lab. Invest.*, **12**, 1204–1220.

HUSAIN, T. (1953). An experimental study of some pressure effects on tissues, with reference to the bed-sores problem. *J. Path. Bact.*, **66**, 347–358.

MCKAY, D. G., MARGARETTEN, W. & CSAVOSSY, I. (1966). An electron microscope study of the effects of bacterial endotoxin on the blood-vascular system. *Lab. Invest.*, **15**, 1815–1829.


(*Received July* 25, 1968)