Br. J. Pharmac. (1986), **89**, 149–155

The protective action of allopurinol in an experimental model of haemorrhagic shock and reperfusion

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1 Haemorrhagic shock was induced in anaesthetized, open-chest dogs by controlled arterial bleeding, sufficient to reduce and maintain mean arterial blood pressure at 40 mmHg for 30 min. The blood volume was then restored to the pre-shock level by rapid, intravenous reinfusion of the blood shed during the shock period.

2 Haemorrhagic shock produced significant haemodynamic changes, characterized by a marked depression of myocardial function. Cardiac output (1226 ± 57 ml min^{-1}), peak aortic blood flow (6030 ± 383 ml min^{-1}) and maximum rate of rise of left ventricular pressure (2708 ± 264 mmHg s^{-1}) were all reduced by more than 50%. The haemodynamic profile was markedly improved by reinfusion of shed blood but this improvement was not sustained. There was a gradual decline such that 50% of the untreated animals suffered complete circulatory collapse and death between 60 and 120 min following reinfusion.

3 Neither haemorrhagic shock, nor reinfusion of shed blood produced any consistent or significant changes in the myocardial adenine nucleotide pool. The ATP, ADP and AMP levels were, respectively, 25.9 ± 4.2; 15.6 ± 1.0; 4.3 ± 1.9 nmol g^{-1} protein, before haemorrhagic shock; 21.6 ± 3.4; 21.5 ± 2.5; 10.2 ± 2.7 nmol g^{-1} protein, after 30 min haemorrhagic shock; and 29.9 ± 3.9; 16.5 ± 1.2; 4.2 ± 1.1 nmol g^{-1} protein, 60 min following reinfusion of shed blood.

4 Pretreatment with allopurinol (50.0 mg kg^{-1} i.v.), 60 min before inducing haemorrhagic shock, had no significant effect upon the haemodynamic response to shock, but did prevent the gradual decline seen following reinfusion in the untreated animals. All of the allopurinol-treated animals displayed significantly better haemodynamic profiles than the untreated animals, furthermore, there was a 100% survival rate in this group.

5 Allopurinol had no significant effect upon the myocardial adenine nucleotide pool either during haemorrhagic shock or following reinfusion of shed blood.

Introduction

The protective effects of the xanthine oxidase inhibitor allopurinol, in ischaemia, have been ascribed to its ability to preserve the tissue levels of high energy adenine nucleotides, such as ATP, during the ischaemia, and therefore prevent tissue damage, and loss of function (De Wall *et al.*, 1971; Hopkins *et al.*, 1975; Cunningham & Keaveny, 1978). More recently it has been suggested that the inhibition of xanthine oxidase can also reduce the formation of free radicals when molecular oxygen is abruptly restored to previously ischaemic tissues (i.e. post-ischaemic reperfusion) and thereby prevent reperfusion damage and loss of function (Gardner *et al.*, 1983; Chambers *et al.*, 1984; Akizuki *et al.*, 1985; McCord, 1985). Hence the protective effects of allopurinol may not be confined to the period of ischaemia, but also extend into the post-ischaemic reperfusion period.

It would be interesting to determine whether the protective effects of allopurinol are dependent upon both the preservation of tissue adenine nucleotides during ischaemia and the prevention of free-radical formation during post-ischaemic reperfusion. We have therefore used an experimental model of myocardial ischaemia (haemorrhagic shock) and reperfusion (reinfusion of shed blood) in which there is a marked reduction in myocardial function during the ischaemia, which is transiently improved following

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reperfusion but then deteriorates further (Wiggers, 1945; Sarnoff *et al.*, 1954; Downing, 1979), to re-examine the protective effects of allopurinol during the ischaemia and reperfusion phases.

Methods

Twelve age- and sex-matched Beagle dogs (body wt. 10–13 kg) were initially anaesthetized with intravenous pentobarbitone (Sagatal, May and Baker, 30 mg kg^{-1}). Subsequent anaesthesia was maintained as necessary, by additional doses of pentobarbitone.

In each animal, a tracheotomy was performed, and all animals artificially ventilated (18 breaths min^{-1}; 200 ml tidal volume) with room air via a large animal respirator (Palmer).

Polythene cannulae were inserted into the right femoral artery, and vein for the measurement of arterial blood pressure, and injection of substances, respectively. The arterial cannula was advanced into the aorta, and connected to a blood pressure transducer (Statham). Further cannulae were placed in the brachiocephalic artery, via the left carotid artery, and into the left external jugular vein. These cannulae were used for the haemorrhagic and reinfusion procedures respectively. A pressure tip transducer cannula (Millar 5F) was also introduced into the right atria, to measure right atrial pressure, via the right external jugular vein.

The chest was then opened at the 4th intercostal space, and the ribs retracted so as to expose the heart. The pericardium was then opened, and the root of the ascending aorta cleared of surrounding fat and connective tissue, in preparation for the placement of an electromagnetic flow probe (10–14 mm) connected to a flowmeter (Statham SP2202) to measure aortic flow. A left ventricular cannula was then inserted into the left ventricular chamber, via the apex of the heart, and secured with a purse-string suture. This cannula was attached to a blood pressure transducer (Statham) to measure left ventricular pressure. The electromagnetic flow probe was then placed on the aorta, as described above.

A small polythene cannula was also placed in the main pulmonary artery via the most accessible of its secondary branches and connected to a blood pressure transducer (Statham) for the measurement of pulmonary artery pressure.

All arterial cannulae were filled with heparinized saline (100 i.u. ml^{-1} Pularin: Duncan, Flockhart and Co. Ltd) and flushed as necessary to prevent the formation of blood clots.

On completion of these surgical procedures, the chest opening was covered with polythene film to prevent heat and moisture loss. Body temperature was maintained at 37–38°C, by the use of a heating underblanket. All animals were then allowed to equilibrate for at least 30 min, and haemodynamic measurements made continuously using a polygraph (Grass Model 7D).

The protocol used for these experiments was as follows: the 12 animals were randomly divided into two groups of 6 animals. The first group were treated with allopurinol (Zyloric: Wellcome, 50 mg kg^{-1}), administered intravenously at the end of the equilibrium period. The second group received only saline. Sixty minutes after treatment all animals were bled, via the brachiocephalic artery cannula, into a jacketed glass reservoir (38°C) containing 100 ml of heparinized-saline (10 i.u. ml^{-1}), until a mean arterial blood pressure of 40 mmHg was achieved. This arterial blood pressure was maintained for 30 min with additional bleeding as necessary. At the end of this 30 min period, the volume of shed blood was measured, (in untreated animals this volume was 377 ± 18 ml; in allopurinol-treated animals it was 350 ± 40 ml), and the shed blood then reinfused via the left external jugular vein cannula. This reinfusion procedure lasted for 5–10 min, and all animals were then observed for a further 120 min from the end of the reinfusion period.

Small arterial blood samples (0.5 ml) were taken and assayed (Radiometer Blood Gas Analyser) for blood gases, blood pH and base excess. In addition small cardiac biopsy samples were rapidly removed from the left ventricular wall (Biopsy drill) and immediately frozen in liquid nitrogen. These biopsy samples were subsequently assayed for adenosine 5'-triphosphate (ATP), adenosine 5'-pyrophosphate (ADP) adenosine 5'-phosphate (AMP) and inosine content, using an h.p.l.c.-method (Ingebretsen *et al.*, 1982).

Throughout these procedures continuous recordings of haemodynamic parameters were made. These were arterial blood pressure, pulmonary artery blood pressure, right atrial pressure (RAP), left ventricular pressure (LVP) and peak aortic blood flow (PAF). From these were derived mean arterial blood pressure (MAP), mean pulmonary artery blood pressure (MPAP), maximum rate of rise of left ventricular pressure ($LV dP/dt_{max}$), stroke volume (SV), cardiac output (CO) and heart rate (HR). Left ventricular stroke work index (LVSWI) was calculated as follows:

$$\text{LVSWI} = \frac{\text{SV} \times \text{MAP}}{\text{Body surface area}}$$

Results

In the allopurinol-treated animals, the administration of allopurinol produced an immediate haemodynamic response which was primarily a fall in blood pressure and an increase in heart rate. Although this immediate response was not sustained at this same level, 60 min

Table 1 The haemodynamic response to haemorrhagic shock and reinfusion in open-chest, anaesthetized dogs

Haemodynamic variable	*Shock period* (min)		*Post-reinfusion period* (min)				
	0	*30*	*0*	*30*	*60*	*90* ($n=4$)	*120* ($n=3$)
MAP (mmHg)	91 ± 5	45 ± 4	106 ± 9	76 ± 5	57 ± 9	54 ± 11	61 ± 10
MPAP (mmHg)	15.5 ± 1.5	14.2 ± 2.3	26.7 ± 4.1	18.8 ± 2.3	16.0 ± 2.0	13.3 ± 1.0	16.0 ± 3.1
HR (beats min^{-1})	181 ± 11	191 ± 10	174 ± 11	223 ± 9	218 ± 5	214 ± 11	220 ± 17
PAF (ml min^{-1})	6030 ± 383	2678 ± 310	9468 ± 746	4529 ± 229	3067 ± 389	2755 ± 629	2429 ± 414
CO (ml min^{-1})	1226 ± 57	468 ± 114	2250 ± 310	695 ± 55	503 ± 69	424 ± 62	325 ± 31
SV (ml)	6.98 ± 0.69	2.45 ± 0.55	13.50 ± 2.70	2.78 ± 0.27	2.31 ± 0.31	1.97 ± 0.22	1.48 ± 0.09
LVSWI (g m $beat^{-1}$ m^{-2})	14.2 ± 1.5	2.6 ± 0.7	28.2 ± 3.6	5.5 ± 0.9	3.1 ± 0.7	2.5 ± 0.7	2.0 ± 0.3
$LVdP/dt_{max}$ (mmHg s^{-1})	2708 ± 264	2133 ± 252	3858 ± 284	2746 ± 376	2233 ± 390	1750 ± 418	1733 ± 289
SBE (mmol l^{-1})	−6.7 ± 0.9	−13.9 ± 0.9	−11.7 ± 0.4	−9.6 ± 0.6	−11.8 ± 1.3	−13.7 ± 2.0	−12.5 ± 2.7

Results shown are mean ± s.e.mean; $n = 6$ unless otherwise indicated. Abbreviations used: MAP, mean arterial blood pressure; MPAP, mean pulmonary artery blood pressure; HR, heart rate; PAF, peak aortic blood flow; CO, cardiac output; SV, stroke volume; LVSWI, left ventricular stroke work index. $LVdP/dt_{max}$, maximum rate of rise of left ventricular pressure; SBE, standard base excess.

after the administration of allopurinol (just before haemorrhagic shock), MAP (92 ± 4 to 77 ± 5 mmHg) was still significantly reduced, whilst HR (181 ± 8 to 218 ± 5 beats min^{-1}) and $LVdP/dt_{max}$ (2638 ± 370 to 3679 ± 551 mmHg s^{-1}) were significantly elevated, when compared to pretreatment values. In the untreated animals at the same point in the protocol, these same haemodynamic parameters showed only minor changes over the 60 min, but were not significantly different when compared to the allopurinol-treated group of animals (see Tables 1 and 2).

At the end of the 30 min haemorrhagic shock during which MAP was maintained at 40 mmHg changes were similar in both groups. Left ventricular performance was depressed, as indicated by the falls in SV, CO, LVSWI and $LVdP/dt_{max}$, but there were no consistent changes in MPAP or HR in either group (Tables 1 and 2).

Table 2 The effects of allopurinol on the haemodynamic response to haemorrhagic shock and reinfusion in open-chest, anaesthetized dogs

Haemodynamic variable	*Shock period* (min)		*Post-reinfusion period* (min)				
	0	*30*	*0*	*30*	*60*	*90*	*120*
MAP (mmHg)	77 ± 5	41 ± 1	*78 ± 5	70 ± 4	66 ± 4	69 ± 5	62 ± 3
MPAP (mmHg)	20 ± 2	19 ± 2	29 ± 2	24 ± 2	*23 ± 2	22 ± 3	23 ± 2
HR (beats min^{-1})	218 ± 5	221 ± 9	207 ± 11	227 ± 9	220 ± 8	*242 ± 6	245 ± 6
PAF (ml min^{-1})	7718 ± 855	3977 ± 520	9295 ± 530	**6979 ± 721	**6438 ± 883	*5966 ± 796	*5753 ± 850
CO (ml min^{-1})	1421 ± 197	700 ± 162	1965 ± 153	*1275 ± 196	*1148 ± 226	1052 ± 231	1037 ± 239
SV (ml)	6.56 ± 0.92	3.14 ± 0.67	9.82 ± 1.2	*5.71 ± 0.93	*5.28 ± 1.04	4.39 ± 0.95	4.26 ± 0.98
LVSWI (g m $beat^{-1}$ m^{-2})	11.4 ± 1.8	2.9 ± 0.7	*16.6 ± 2.6	8.7 ± 1.3	*7.5 ± 1.5	*6.6 ± 1.5	5.8 ± 1.3
$LVdP/dt_{max}$ (mmHg s^{-1})	3679 ± 551	2083 ± 355	3850 ± 605	3558 ± 370	3346 ± 385	**3926 ± 325	**3583 ± 228
SBE (mmol l^{-1})	−7.3 ± 1.2	*−10.5 ± 1.2	−9.7 ± 1.2	−6.9 ± 0.9	*−6.4 ± 1.6	*−7.9 ± 0.8	−9.7 ± 1.2

Results shown are mean ± s.e.mean; $n = 6$. For abbreviations see Table 1 legend. *$P<0.05$, **$P<0.01$, significantly different from untreated animals (Student's t test).

Immediately after reinfusion of the shed blood, both groups of animals showed a marked improvement in left ventricular performance although the rise in LVSWI and MAP in the untreated animals was significantly greater compared with the allopurinol-treated animals (2.5 ± 0.7 to 28.2 ± 0.7 compared with 2.9 ± 0.7 to 16.6 ± 2.6 g m beat^{-1} m^{-2}; 45 ± 4 to 106 ± 9 compared with 41 ± 5 to 78 ± 5 mmHg, respectively). However, 15 min after reinfusion the untreated animals were showing signs of a deterioration in left ventricular performance. This continued to develop and at 60 min after reinfusion, there were significant differences between the two groups. Left venticular performance was markedly depressed in the untreated animals when compared with the allopurinol-treated animals. SV, CO, LVSWI and LV$dP/dt_{max}$ were all lower in the untreated animals (2.73 ± 0.3 vs 5.28 ± 1.04 ml; 503 ± 69 vs 1148 ± 226 ml min^{-1}; 3.1 ± 0.7 vs 7.5 ± 1.5 g m beat^{-1} m^{-2}; 2233 ± 390 vs 3346 ± 385 mmHg s^{-1}, respectively). MPAP and MAP were also lower in the untreated animals (16 ± 2 vs 23 ± 2 mmHg; 57 ± 9 vs 66 ± 4 mmHg, respectively) (see Tables 1 and 2).

Between 60 and 120 min after reinfusion, three of the six untreated animals died, and those that survived continued to deteriorate. This contrasted with the six allopurinol-treated animals, all of which survived beyond 120 min after reinfusion and possessed a markedly better haemodynamic profile (Tables 1 and 2).

Throughout these experiments there was very little difference between the two groups of animals in the response of RAP or LVP to haemorrhagic shock or reinfusion. Similarly the arterial blood samples revealed few differences with respect to blood gases. There was, however, a decrease in blood pH in both groups of animals, during the haemorrhagic shock period, which was sustained despite the reinfusion. This pH change was also accompanied by an increase in standard base excess (SBE), but this increase appeared to be more marked and sustained in the untreated animals compared with the allopurinol-treated animals (-6.7 ± 0.9 to -13.9 ± 0.9 vs -7.3 ± 1.2 to -10.5 ± 1.2) (see Tables 1 and 2). These observed pH and SBE changes presumably reflect metabolic changes (i.e. increased lactate production) induced by tissue ischaemia during the shock period.

The biochemical assay of the myocardial biopsy samples showed that there was no consistent change in adenine nucleotide content during haemorrhagic shock or reinfusion in the untreated animals and the allopurinol-treated animals. The ATP:ADP:AMP ratio was unchanged, as was the total pool, and there was no significant difference between the untreated and allopurinol-treated animals (see Table 3). Although the assay method was able to measure inosine, the amounts of inosine detected in each sample were too small to merit inclusion in the data presented here.

A representative summary of the time course for the changes in myocardial function (i.e. CO and PAF) and myocardial adenine nucleotides (i.e. ATP) during these experimental procedures, are shown in Figures 1 and 2.

Discussion

In the experimental model of haemorrhagic shock and reperfusion used in these studies, the reduction in myocardial function, and the overall haemodynamic depression, observed during haemorrhagic shock was

Table 3 The effects of allopurinol on the response of the myocardial adenine nucleotide pool to haemorrhagic shock and reinfusion in open-chest, anaesthetized dogs

	Shock period (min)		*Post-reinfusion period* (min)		
Adenine nucleotide	*0*	*30*	*0*	*30*	*60*
ATP (nmol g^{-1}protein)					
Untreated	25.9 ± 4.2	21.6 ± 3.4	24.8 ± 3.5	25.9 ± 4.9	29.9 ± 3.9
Allopurinol-treated	27.0 ± 2.5	27.8 ± 0.9	27.4 ± 2.1	22.9 ± 2.0	27.2 ± 2.6
ADP (nmol g^{-1}protein)					
Untreated	15.6 ± 1.0	21.5 ± 2.5	18.8 ± 1.8	18.3 ± 1.5	16.5 ± 1.2
Allopurinol-treated	18.9 ± 2.7	16.5 ± 1.4	18.2 ± 3.2	17.6 ± 2.5	16.3 ± 1.1
AMP (nmol g^{-1}protein)					
Untreated	4.3 ± 1.9	10.2 ± 2.7	7.3 ± 2.8	6.8 ± 2.4	4.2 ± 1.1
Allopurinol-treated	8.1 ± 3.1	4.3 ± 1.3	6.1 ± 3.4	6.5 ± 3.1	4.2 ± 0.7
Total (ATP + ADP + AMP)					
Untreated	45.8 ± 3.2	53.3 ± 5.3	50.1 ± 2.3	50.9 ± 2.8	50.6 ± 4.3
Allopurinol-treated	53.9 ± 5.5	48.6 ± 2.7	51.6 ± 5.4	47.0 ± 4.4	47.6 ± 3.0

Results shown are mean ± s.e.mean; $n = 6$ in untreated and allopurinol-treated groups.

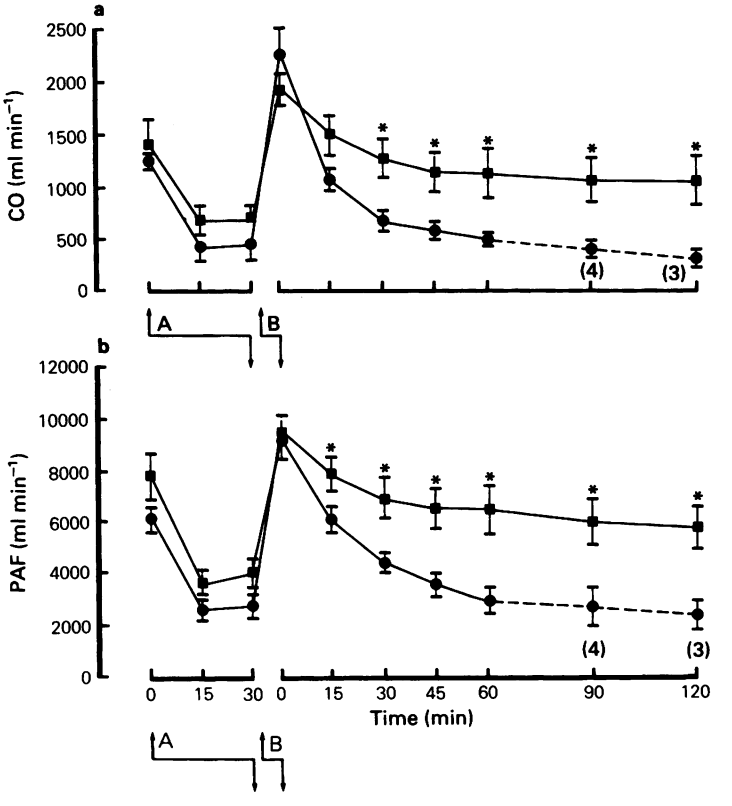

Figure 1 Changes in myocardial function (a) cardiac output (CO) and (b) peak aortic blood flow (PAF), in anaesthetized, open-chest dogs after haemorrhagic shock (A) and reinfusion of shed blood (B), and the effect of allopurinol pretreatment. Each point is the mean, with vertical lines indicating s.e.mean of 6 animals except where indicated by number in parentheses. *Statistically significant difference ($P < 0.5$, Student's t test) between untreated (●) and allopurinil-treated (■) groups.

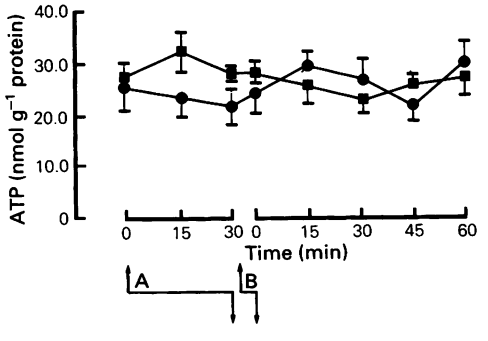

Figure 2 Changes in myocardial ATP in anaesthetized, open-chest dogs after haemorrhagic shock (A) and reinfusion of shed blood (B), and the effects of allopurinol pretreatment. Each point is the mean, with vertical lines indicating s.e.mean, of 6 animals. (●) Untreated group, (■) allopurinol-treated group.

not accompanied by any consistent change in myocardial adenine nucleotides. This response to haemorrhagic shock was not modified by pretreatment with allopurinol. Immediately following reperfusion, myocardial function and the overall haemodynamic profile, were restored to pre-shock levels and above, but subsequently deteriorated, resulting in complete circulatory collapse in approximately 50% of the preparations. As before, these changes were not accompained by any consistent change in myocardial adenine nucleotides. Although pretreatment with allopurinol had no effect upon the myocardial adenine nucleotides, it did prevent the deterioration in myocardial function and the overall haemódynamic profile following reperfusion, and maintained a 100% survival rate.

Lefer *et al.* (1969) were also unable to show any changes in myocardial adenine nucleotides using a similar experimental model of haemorrhagic shock

and reperfusion. They also found no modifying effect of allopurinol pretreatment. The lack of change in myocardial adenine nucleotides, in response to these procedures does, however, contrast with that of other tissues. Although no other measurements of non-myocardial adenine nucleotides were made in the studies described in this paper, in the liver, kidney and intestine, under similar conditions, the adenine nucleotide content is markedly reduced, and this is prevented by allopurinol pretreatment (Chaudry *et al.*, 1974; Hopkins *et al.*, 1975; Cunningham & Keaveny, 1978). It therefore appears that there is some heterogeneity in the response of different tissues to haemorrhagic shock and reperfusion, and in the myocardium, at least, this does not involve changes in adenine nucleotide.

It has long been known that the myocardial response to haemorrhagic shock is characterized by a marked depression of myocardial function (Wiggers, 1945; Sarnoff *et al.*, 1954; Downing, 1979) which is only transiently improved by the restoration of the blood volume (Wiggers, 1945; Downing, 1979). In those studies which have examined the survival rate of experimental animals subjected to haemorrhagic shock and reperfusion, it is evident that the survival rate is low (Crowell *et al.*, 1969; Lefer *et al.*, 1969; Baker, 1972; Salles *et al.*, 1972). However, there is some evidence that pretreatment with allopurinol increases the survival time (Crowell *et al.*, 1969; Salles *et al.*, 1972). More recently, allopurinol has been found to prevent the damage and loss of function, associated with post-ischaemic reperfusion in a number of different tissues, including the myocardium (Chambers *et al.*, 1984; Akizuki *et al.*, 1985). Hence the protective effects of allopurinol in haemorrhagic shock and reperfusion, specifically in the myocardium, may have more relevance to the events associated with the reperfusion.

The results of our studies, in conjunction with those described above, indicate that the protective effects of allopurinol, in the myocardium does not involve changes in myocardial adenine nucleotides. However, they do not preclude the possibility that the inhibition of xanthine oxidase is the primary mechanism by which allopurinol provides protection to the ischaemic myocardium. Despite the recent negative observations of Reimer & Jennings (1985), those findings which implicate xanthine oxidase in the formation of free-radicals in post-ischaemic reperfusion in the myocardium (Gardner *et al.*, 1983; Chambers *et al.*, 1984; Akizuki *et al.*, 1985; McCord, 1985) remain extremely relevant to the myocardial response to haemorrhagic shock and reperfusion. Additional indirect support for this thesis can be found in the protective effects of other free-radical scavenging treatments in the post-ischaemic, reperfused myocardium (Shlafer *et al.*, 1982; Jolly *et al.*, 1984). It must, however, be borne in mind that a prerequisite for myocardial free radical formation is a preceding period of ischaemia followed by reintroduction of molecular oxygen (McCord, 1985). Myocardial ischaemia is invariably associated with a reduction in myocardial adenine nucleotides (Braunwald & Kloner, 1982). Since in our studies myocardial adenine nucleotides were unchanged, this questions whether our model of haemorrhagic shock exhibits an appropriate degree of myocardial ischaemia to evoke free radical formation.

In conclusion, it is evident that the cardiovascular response to haemorrhagic shock and reperfusion is a complex one, but the results of our studies demonstrate that pretreatment with allopurinol is able to protect the myocardium against the deleterious effects of this treatment. Although allopurinol does not affect the marked reduction in myocardial function, and haemodynamic depression observed during haemorrhagic shock, it does prevent the subsequent deterioration following reperfusion. Our results also demonstrate that the loss of myocardial function cannot be ascribed to a loss of myocardial adenine nucleotides, nor does this seem important when reperfusion takes place. It therefore seems possible that the protection provided by allopurinol in this experimental model is related to its ability to prevent the formation of free-radicals following reperfusion, and the probability remains that this is still a consequence of the inhibition of xanthine oxidase. However, a protective mechanism implicating the inhibition of free-radical formation must remain tentative until further studies are undertaken to support this conclusion.

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(Received February 1, 1986.
Revised April 11, 1986.
Accepted April 30, 1986.)