Metabolic damage to human saphenous vein during preparation for coronary artery bypass grafting

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SUMMARY Segments of saphenous vein from patients undergoing coronary artery by-pass graft surgery were frozen in liquid nitrogen immediately on dissection (control), after stripping of the adventitia and side branch ligation (manipulation), after distention with blood (distention), or at completion of the last proximal anastomosis (prepared vein). Vein was stored during the operation in patient’s heparinised arterial blood at room temperature. Frozen vein was extracted with perchloric acid. ATP, ADP, AMP, adenosine, inosine and hypoxanthine concentrations were measured by high pressure liquid chromatography. Prepared vein had ca 50% lower ATP concentration and ATP/ADP ratio than control vein, higher concentrations of inosine and hypoxanthine and lower concentrations of AMP and adenosine. ATP concentration and ATP/ADP ratio did not correlate with the time elapsed between dissection and freezing of the prepared vein. The characteristic changes seen in prepared vein were not seen when control vein was simply stored in arterial blood at 23°C, in normal saline at 23°C or 4°C, in Krebs-Ringer bicarbonate buffer at 37°C or in St Thomas’s Hospital cardioplegic solution at 4°C. Distension with unlimited pressure but not distension at <300 mmHg gave rise to the same changes in ATP concentration and ATP/ADP ratio as in the prepared vein. These results show that vein suffered metabolic changes during preparation for bypass grafting and suggest that uncontrolled distention may contribute to these changes. Such biochemical measurements provide a quantitative estimate of tissue damage and allow objective comparison of different preparative techniques.

Autologous saphenous vein is the graft material most commonly used for coronary artery bypass graft surgery (CABG). An unresolved problem of CABG is the occurrence of graft thrombosis in 10 to 20% of cases during the first year and an attrition rate of ca. 2% per year thereafter. There is extensive evidence that morphological changes in grafted saphenous vein associated with early and late thrombotic occlusion are critically influenced by the intraoperative procedures used to prepare the vein. Dissection of suitable segments of vein, handling of the vein with surgical instruments, adventitial stripping and distension to overcome spasm and to check for competence of side-branch ligatures are all potentially traumatic. Tissue damage may result either from cell breakage or from more subtle changes such as interference with intermediary metabolism.

A number of metabolites involved in cellular energy metabolism have been used as markers of tissue injury. Myocardial hypoxia or ischaemia, for example, is associated with a large and early fall in the concentrations of creatine phosphate and ATP while the concentration of degradative products AMP, adenosine, inosine and hypoxanthine rapidly rise. It has been suggested that adenosine, in particular, functions as a signal to initiate responses to ameliorate or reverse the energy imbalance. In the context of CABG its vasodilator and anti-thrombotic properties may be particularly relevant.

In this study we have established that surgical preparation of saphenous vein for CABG does alter the concentrations of adenine nucleotides and their metabolites. The measurement of metabolic concentrations formed the basis for comparing the effect of different preparative techniques including the influence of manipulation, of media used for...
intra-operative preservation, of storage time and of distension pressure.

**Methods**

**PATIENTS**

Material was obtained from 30 males (age, 54±2 yr) and 4 females (age, 53±4 yr) who were undergoing a coronary artery by-pass graft operation. Premedication was with 2 to 2.5 mglorazepam 2 h preoperatively and 10 to 20 mg papaveretum 1 h preoperatively. Anaesthesia was induced with a combination of fentanyl (40 mg·kg⁻¹) and pancuronium bromide (0.125 mg·kg⁻¹) and was maintained with nitrogen oxide and oxygen with occasional addition of halothane. Heparin (3 mg·kg⁻¹) was administered intravenously before the first incision.

**OPERATIVE PROCEDURES**

A “control” (approx 1 cm) segment of vein was taken from the lower portion of the long saphenous vein as quickly as possible after performing the first incision. The vein was touched as little as possible and dissection was kept to the minimum necessary to free the vein from surrounding tissue. Within 2 to 5 s of resection, the vein segment was drained of blood and frozen in liquid N₂. The remaining vein was then dissected free of surrounding tissue and all side branches secured with a 4-0 silk ligature. Partial stripping of the adventitia was carried out to release any apparent narrowing of the vein lumen. Following this the vein was tied to a Tibbs cannula and distended in segments of 30 to 100 cm in length after occluding the distal end with an atraumatic bulldog clamp. In one group (n = 13) vein was distended using a 20 ml syringe containing the heparinised patient’s arterial blood at room temperature and with gentle but controlled manual pressure. In the second group a Bonchek-Shiley vein distension system was interposed between the syringe and Tibbs cannula and applied. The distended vein was then transected into segments appropriate in length for a particular aorto-coronary graft and then stored in heparinised patient’s arterial blood at room temperature and with gentle but uncontrolled manual pressure.

In a separate series of experiments pairs of 1.5 to 2 cm segments of vein were removed, before the whole vein was distended, and these segments were distended for 1 min each with either controlled or uncontrolled pressure. The ends of the vein injured by the bulldog-clamp and by tying to the Tibbs cannula were discarded and the remaining part was immediately blotted and blotted and frozen in liquid N₂.

**LABORATORY PROCEDURES**

**Incubations**

In some experiments a 5 cm segment of control vein was brought from the operating theatre to the laboratory in patient’s heparinised arterial blood at 26°C, dissected free from adhering fat and cut with a scalpel into 1 cm pieces. This complete process took approx 10 min. Incubations were conducted in tightly-closed plastic universal containers (diameter 22 mm, capacity 25 ml) for 120 min in 20 ml of various media, with gentle agitation either at room temperature (20 to 26°C) or on ice (2 to 4°C). Media used were heparinised arterial blood, 0.9 g·litre⁻¹ of NaCl solution and St Thomas’s Hospital cardioplegic solution number 2. The pH, pCO₂ and PO₂ values for blood at the start of incubation were 7.44±0.02, 31.0±0.1 and 212±12 at 37°C or 7.60±0.02, 18.2±0.1 and 154±11 at 26°C. At the end of incubations the values were 7.42±0.02, 29.1±2.3 and 208±11 at 37°C or 7.59±0.02, 17.3±1.0 and 147±15 at 26°C. Incubations were also conducted at 37°C in a water-jacketed vessel containing 20 ml of Krebs-Ringer bicarbonate buffer supplemented with 10 mmol·litre⁻¹ glucose and equilibrated with O₂:CO₂ 95:5 v/v, pH 7.4. After the incubation veins were gently blotted and then snap frozen in liquid N₂. Veins incubated in blood were briefly rinsed in normal saline, blotted and then frozen.

**Measurement of metabolites**

Veins frozen in liquid N₂ for up to a month were crushed under liquid N₂ and added to preweighed tubes containing 1.2 ml of 5g·100 ml⁻¹ of perchloric acid dissolved in water: methanol 4 : 1 (v/v) and maintained at −15°C. Tubes were reweighed and then the slurry contained within was sonicated twice for 15 s using an MSE (Crawley, Sussex, model PG100) sonicator at 20 μm tip amplitude with 5 min rests on ice between bursts. The suspension was then centrifuged at 1500 g for 10 min at 4°C and the supernatant removed. This method has been shown to effectively extract adenine nucleotides without causing their degradation. Supernatants were neutralised with 2 ml of 0.5 mol·litre⁻¹ tri-n-octylamine dissolved in 1,2,2-trichlorotrifluoromethane. The aqueous phase was collected...
Acid extracts from human saphenous vein were subjected to HPLC in aqueous phosphate buffer (28) (system I) or in buffer with 10% (v/v) methanol (29) (system II). Samples were incubated for 5 min at 37°C as follows: for ATP + 5 mmol·litre⁻¹ glucose + 1 U hexokinase; for ADP + 5 mmol·litre⁻¹ phosphoenolpyruvate + 1 U pyruvate kinase; for hypoxanthine + 0.1 U xanthine oxidase; for AMP + 1 U AMP deaminase; for inosine + 0.1 U of nucleoside phosphorylase + 0.1 U of xanthine oxidase; for adenosine + 1 U adenosine deaminase. Extracts were also supplemented with the amounts of standard compounds shown and the recovery determined. No increase in any metabolite other than that added in the standard was noted. Values are means of duplicate observations.

After centrifuging at 300 g × 5 min at 4°C, filtered through a 0.45 µm Millipore filter (HAWP04500) and 25 µl subjected to HPLC for measurement of nucleotides and hypoxanthine, or 50 µl for measurement of adenosine and inosine. Chromatograms showed peaks with the same retention times as ATP, ADP, AMP, adenosine, inosine and hypoxanthine (table 1). Peaks were further characterised by the "enzyme shift" method. Extracts were incubated with appropriate enzymes which removed the relevant peak (table 1). Further control experiments established that standard compounds added to acid extracts of vein were >85% recovered during the subsequent procedures and did not suffer interconversion (table 1).

### Protein estimation
Blotted samples of vein were weighed (approx 100 mg) and then dissolved in 2 ml of 3M NaOH for 3 to 4 h at 100°C. The suspension was sampled (25 µl) and protein measured by the method of Lowry using bovine serum albumin (Sigma) as standard.

### Statistical analysis
Unless otherwise specified values are quoted as mean ± SEM with the number of observations in parenthesis. Statistical significance between groups was established with the Student's t test using unpaired values unless otherwise stated. The correlation coefficient was obtained from a least-squares linear regression.

### Results

**Metabolite concentrations in control and prepared vein**
Vein frozen immediately after dissection (control), had an ATP concentration of 480 ± 30 nmol·g⁻¹ wet wt after centrifuging at 300 g × 5 min at 4°C, filtered through a 0.45 µm Millipore filter (HAWP04500) and 25 µl subjected to HPLC for measurement of nucleotides and hypoxanthine, or 50 µl for measurement of adenosine and inosine. Chromatograms showed peaks with the same retention times as ATP, ADP, AMP, adenosine, inosine and hypoxanthine. Peaks were further characterised by the "enzyme shift" method. Extracts were incubated with appropriate enzymes which removed the relevant peak (table 1). Further control experiments established that standard compounds added to acid extracts of vein were >85% recovered during the subsequent procedures and did not suffer interconversion (table 1).

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### Results
Metabolic damage to saphenous vein (n = 28) with values ranging from 251 to 861 nmol·g⁻¹ wet wt. The mean ADP concentration was 200 ± 10 nmol·g⁻¹ wet wt with a range from 121 to 349 nmol·g⁻¹ wet wt. The mean values were not significantly altered by rinsing the vein in normal saline to remove adhering erythrocytes before freezing (data not shown). The ATP/ADP ratio, 2.41 ± 0.07, had a coefficient of variation of 16% which was smaller than the coefficients of variation of either ATP (26%) or ADP (26%) measurements. Moreover values of ATP and ADP concentration in the same sample of vein were significantly correlated (r = 0.78, p < 0.001). Concentrations of AMP adenosine, inosine and hypoxanthine in control vein are also shown in table 2.

Samples of vein in excess at the time of completing the last proximal anastomosis (prepared vein) had concentrations of ATP and ATP/ADP ratios significantly lower than those in control vein (table 2). Veins which had been distended at <300 mmHg with the Bonchek-Shilley vein distension system showed slightly but significantly more ATP and a higher ATP/ADP ratio (table 2) than veins distended at unlimited pressure. AMP and adenosine concentrations in vein prepared with either distension pressure were significantly lower than in control vein and were similar to those found in vein incubated in blood for a similar period (cf fig 1, table 2). Indeed the concentration of these metabolites in control vein declined rapidly on incubation suggesting that their initially high concentrations resulted from surgical

![FIG 1 Variation in the concentration of adenosine and AMP on incubation of human saphenous vein](image)

![FIG 2 Relationship between ATP concentration and ATP/ADP ratio in prepared vein and operation time](image)

The time interval between the first incision and the freezing of the sample of prepared vein was noted for 10 operations in which the vein was distended at <300 mmHg (○) and seven operations involving unlimited pressure distension (■). The relationship between ATP/ADP ratio and operation time (panel a) yielded a correlation coefficient of 0.34 (p > 0.1) for the <300 mmHg group (○) and 0.24 (p > 0.1) for the unlimited pressure group (■■). The relationship between ATP concentration and operation time (panel b) yielded a correlation coefficient of 0.29 (p > 0.1) for the <300 mmHg group (○) and 0.14 (p > 0.1) for the unlimited pressure group (■■■).
trauma at the time of dissection. The concentrations of inosine and hypoxanthine were significantly elevated in the prepared veins relative to control, although there was no significant difference between the controlled and uncontrolled pressure groups. Taken together these data demonstrated significant biochemical deterioration of the vein during preparation for CABG.

THE INFLUENCE OF STORAGE TIME AND PRESERVATION IN DIFFERENT MEDIA

The ATP/ADP ratio and ATP concentration were plotted (fig 2a, b) against the time interval between taking the control sample and the termination of the last proximal anastomosis, at which time the prepared sample was frozen. The average operation time for the group distended at limited pressure was 141±40 (SD) min and for the unlimited pressure group 112±42 (SD) min. No significant correlation with time was observed for either parameter when considering vein distended with controlled pressure, uncontrolled pressure or both groups pooled. This suggested that factors other than storage time were preponderant in determining the changes observed during routine preparation. Control vein incubated for 120 min in arterial blood at 23°C did show a significant fall in ATP concentration when compared to unincubated control vein (table 3) but the ATP/ADP ratio was only slightly and insignificantly reduced, suggesting that there was a leakage of total adenylates (ATP+ADP+AMP) on storage but that the metabolic disturbance which led to a change in ATP/ADP was not the result of storage per se.

The influence of different storage media on the metabolite concentrations in control vein was investigated (table 4). In all cases studied a significant fall in ATP concentration and total adenylates was observed on storage relative to unincubated control. Interestingly, a fall in ATP/ADP ratio was only observed on storage in cardioplegic solution or saline at 4°C. Storage in saline at 4°C yielded a significantly (p<0.05) lower ATP/ADP ratio than storage in saline at 23°C which gave a value similar to unincubated control. The two solutions tested at 4°C unlike the other solutions also led to a significant elevation of inosine concentration, again consistent with inhibition of ATP-generating reactions at low temperatures. Incubation in saline at 23°C or in oxygenated Krebs-Ringer bicarbonate buffer at 37°C was not...
Metabolic damage to saphenous vein

TABLE 5 Effect of different surgical procedures on human saphenous vein

<table>
<thead>
<tr>
<th>Condition</th>
<th>Metabolite concentration nmol.g⁻¹ wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP +ADP</td>
</tr>
<tr>
<td>A Control</td>
<td>810±70</td>
</tr>
<tr>
<td>B Manipulation, adventitial</td>
<td>760±50</td>
</tr>
<tr>
<td>C Plus distension at 300 mmHg</td>
<td>740±70</td>
</tr>
<tr>
<td>D Plus distension at unlimited</td>
<td>540±50*</td>
</tr>
<tr>
<td>E Prepared</td>
<td>490±30</td>
</tr>
</tbody>
</table>

a p<0.01 vs control
b p<0.05 vs manipulated
c p<0.005 vs manipulated
d p<0.05 vs limited pressure distension
e p<0.005 vs limited pressure distension
f p<0.05 vs unlimited pressure distension

Segments of vein were frozen in liquid N₂ immediately after the treatments shown. Crushed tissue was subsequently extracted with perchloric acid and metabolites measured by high pressure liquid chromatography. Values are means ± SEM of eight observations.

significantly different from incubation in blood at 23°C, though the number of replicate samples was small.

The lowered concentrations of ATP and total adenylates could have resulted from water retention which would have distorted the wet weight measurements. In a separate series of experiments the protein/wet wt ratio was measured in veins stored under various conditions. This ratio was significantly reduced when veins were incubated in aqueous buffers but not when incubation was performed in blood (table 4). Hence the fall in ATP concentration and total adenylates seen in vein stored in blood appeared to reflect a genuine leakage from the adenylate pool. There was no difference in protein/wet wt ratio between controls and prepared veins (table 4).

The influence of different preparative steps in the surgical procedure

Table 5 directly compares the results from segments of the same veins (A) under control conditions immediately after careful dissection, (B) after routine manipulation including adventitial stripping, (C) and (D) after the additional step of distension of the segments either at limited pressure (<300 mmHg) or at unlimited pressure, and (E) "prepared" samples in which the whole residual vein had been subjected to routine distention at limited pressure and to intra-operative storage in blood at 23°C until the end of the operative procedure (135±47 (SD) min).

Manipulation alone slightly but not significantly lowered the ATP concentration and ATP/ADP ratio. It significantly raised the adenosine and inosine concentration, possibly as a direct consequence of trauma. Manipulation followed by distension at limited pressure (<300 mmHg) led to a small but significant fall in ATP/ADP ratio relative to control vein accompanied by elevation of adenosine, inosine and hypoxanthine concentrations; the fall in ATP level failed to reach significance; inosine and hypoxanthine concentrations were significantly elevated relative to manipulation alone. Manipulation followed by distension with unlimited pressure led to significantly lower ATP concentrations and ATP/ADP ratios than control vein, manipulated vein or vein distended with limited pressure; the adenosine concentration was significantly higher than in each other group; inosine and hypoxanthine concentrations were significantly higher only than control. The ATP concentration in these distended veins was 300 nmol·g⁻¹ wet wt lower than control veins while the concentration of AMP was increased by 30 nmol·g⁻¹ wet wt; adenosine by 110 nmol·g⁻¹ wet wt; inosine by 110 nmol·g⁻¹ wet wt and hypoxanthine by 40 nmol·g⁻¹ wet wt. Thus the accumulation of degradative products accounted for the loss of ATP. Veins distended at unlimited pressure showed ATP concentrations and ATP/ADP ratios insignificantly different from those in "prepared" veins, though the concentrations of AMP, adenosine and inosine were significantly less in "prepared" veins.

Discussion

Damage to human saphenous vein during intraoperative preparation has been reported previously, largely on the basis of morphological evidence. Separation and desquamation of the
vascular endothelium has been observed by several groups,3-15 32-34 associated with platelet adhesion, fibrin deposition and reduced fibrinolytic activity.35 Alterations in the tensile properties of vein have also been noted.36 Factors which have been identified as giving rise to damage are devascularisation, distension pressure, the substitution of saline for blood during distension and storage, the presence of blood elements during distension and storage, the temperature during distension and storage, and the occurrence of vasospasm. Hasse and colleagues10 have shown that intraluminal pressures of >600 mmHg are readily generated even by gentle uncontrolled distension, as is often employed during CABG operations including the series described here. Several workers have suggested that such pressures are deleterious.6 8 10 35 36 However, there is no general agreement on the relative importance of these factors and different groups have reached contradictory conclusions, especially when different morphological criteria (eg deendothelialisation or fibrin attachment) have been used.

Little appears to be known about the biochemical status of veins. In this study, we have measured the concentrations of adenine nucleotides and their catabolites and have examined how these are altered during routine surgical preparation and by its component steps. Such data may be subjected to statistical analysis to compare alternative procedures. Furthermore, biochemical studies may eventually be used to elucidate the mechanisms underlying functional and morphological changes.

The ATP content of vein/wet wt was only ca one tenth that of organs such as muscle and liver.37 This may be explained if the bulk of the tissue weight is made up by collagenous matrix which is consistent with the morphological appearance of veins. By the same criteria, the majority of vein ATP is probably contributed by the medial smooth muscle cells. The ATP/ADP ratio (2.4±0.07) of control vein was also lower than the values obtained in other tissues.37 This might in principle be due to deterioration of the vein during dissection or to an artefact of freezing or extraction. Since the vein ATP/ADP ratio was maintained constant for 2 h after excision (table 3), it seems unlikely that deterioration took place during dissection. Vein samples frozen in isopentane at the temperature of solid CO₂ showed an ATP concentration and ATP/ADP ratio indistinguishable from vein frozen in liquid N₂ (data not shown). Interconversion of ATP and ADP during extraction was ruled out (table 1). The values of ATP concentration and ATP/ADP ratio obtained may therefore be characteristic of vein tissue in vivo.

The control vein samples also had AMP and adenosine concentrations in excess of those found in other tissues.21 The concentrations of these metabolites rapidly declined on incubation of the vein in blood, suggesting that they may have arisen from surgical trauma at the moment of dissection. Since it is not feasible to obtain vein samples without dissection, some distortion of the in vivo pattern of metabolites seems unavoidable. The variability of individual measurement of adenine nucleotides most probably reflected variation in the cellularity of the vein segments, since the measurements of both ATP and ADP concentration were affected in parallel.

We obtained clear evidence of metabolic deterioration during the course of intra-operative preparation and storage. Not only were the ATP concentration and the sum of ATP+ADP+AMP concentrations reduced but also the ATP/ADP ratio. This combination of changes cannot be explained by the loss of a certain proportion of viable cells but suggests interference with intermediary metabolism. Storage alone caused some fall in total adenine nucleotide content but ATP/ADP ratios did not fall unless veins were stored at 4°C, consistent with inhibition of metabolic processes at this low temperature. Distention with uncontrolled pressure led to a rapid fall in ATP concentration and ATP/ADP ratio accompanied by the accumulation of adenosine, inosine and hypoxanthine. When distension pressure was limited to <300 mmHg these changes were almost completely avoided. Nonetheless, "prepared" veins at the end of the entire preparative procedure, including distension with the Bonchek-Shiley device, showed a similar reduction in ATP concentration and ATP/ADP ratio to vein segments subjected to unlimited pressure distension alone. The cumulative effects of manipulation, distension at limited pressure and intraoperative storage may be equivalent to the effect of the single step of distension at uncontrolled pressure alone. Adenosine content was lower in "prepared" veins than control, manipulated or distended samples, presumably reflecting washout and further metabolism21 of the nascent adenosine, since it occurred during storage in all media tested.

How these metabolic changes affect subsequent graft patency remains at present a matter for speculation. Smooth muscle and endothelial function are both relevant. Adenosine for example, is a potent vasodilator38 and anti-platelet aggregatory factor.39 Clinical trials suggest that dipyridamole may improve graft potency,2 40 in which case the mechanism is likely to be through increased interstitial adenosine concentration.41 In the present work a large proportion of the ATP degraded during distension of the vein was recovered as adenosine (table 5). An incidental consequence of ATP depletion may be a decreased capacity to generate adenosine, though this remains to be demonstrated. Whether the metabolic changes are
associated also with subsequent impairment of specific functions such as the production of prostaglandins and endothelium-derived vascular relaxant factor and how they might influence platelet-endothelium interactions are likewise matters for further investigation.

We thank Mrs Ann Williams for her excellent technical assistance. This work was supported by grants from the British Heart Foundation and the Heart Research Council for Wales. A.H., fund holder of the British Heart Foundation Sir Thomas Lewis Chair of Cardiology.

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