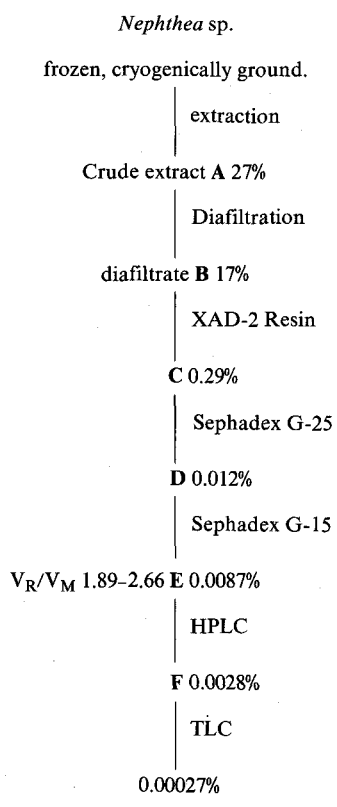

Separation scheme for isolation of the cardioactive constituent from *Nephthea* sp.

The tachycardia produced by **1** (1 mg kg^{-1}) was 40 ± 5 beats min^{-1} (mean $\pm$ SEM, 3 rats) whereas **2** (1 mg kg^{-1}) only raised heart rate by 15 ± 1 beats min^{-1}, the difference being highly statistically significant ($p < 0.05$). The rise in blood pressure produced by **2** (1 mg kg^{-1}; 34 ± 2 mm Hg) was not significantly greater than that produced by **1** (1 mg kg^{-1}; 29 ± 6 mm Hg). Administration (i.v. 1 mg kg^{-1}) of **1** and **2** to 3 normotensive rats produced analogous rises in heart rate (**1**, 37 ± 2 beats min^{-1}; **2**, 14 ± 2 beats min^{-1}) and blood pressure (**1**, 12 ± 2 mm Hg; **2**, 12 ± 1 mm Hg).

There are no previous reports of the occurrence of **1** in marine organisms. However, **1** is known to occur in cacti[4] and has been detected in human urine[5]. The cardiovascular properties of **1** are previously unreported but **1** has been implicated in central nervous system disorders such as Parkinsonism[6,7], hyperactivity[8], schizophrenia[5] and dyskinesias[9].

1 Principal author and from whom reprints may be requested.
2 RRIMP Museum specimen FN 1922, collected from North West Reef off Tryon Island, Queensland, Australia. We thank P.A. Alderslade for taxonomy.
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Increased vascular prostacyclin activity in rats after endotoxin administration[1]

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Summary. Endotoxin did not interact in vitro with prostacyclin activity but stimulated its release from vascular tissues when administered in single doses to rats 30 min before testing.

Over the last decade several investigators have found increased levels of prostaglandins (PG), particularly of the E and F type, in endotoxemia[3-6]. There is evidence that PGs mediate some in vivo effects of endotoxins such as fever[7], abortion[8] and early phases of shock[9]. More recently, elevated blood levels of respectively thromboxane B_2[10] and 6-keto-$PGF_{1\alpha}$ the stable derivative of PGI_2 (prostacyclin)[11], have been observed after administration of large amounts of endotoxin to rats and rabbits. Whether the high blood levels of 6-keto-$PGF_{1\alpha}$ are the consequence of increased vascular production of PGI_2 - a potent vasodilator and inhibitor of platelet aggregation[12] - has not yet been established.

We report here that vascular tissues from rats given low doses of endotoxin show increased PGI_2 activity. This may result from a complex in vivo interaction between endotoxin and vessel wall since it could not be demonstrated in vitro.

Materials and methods. Male CD-COBS rats (250–300 g b.wt) from Charles River, Calco, Italy, were used. PGI_2 activity was measured in arterial and venous tissues as platelet aggregation inhibitory potency[13]. Saline suspensions of the following endotoxins (lipopolysaccharides (LPS)), were prepared: *Salmonella minnesota* LPS, and *Escherichia coli* 0111: B4 LPS, W. (Difco Laboratories, Detroit, Michigan, USA).

For in vitro experiments, vascular rings from untreated rats[13] were incubated at room temperature for 5 min with different concentrations of endotoxins (100–500 µg/ml) or saline as a control. Afterwards, the supernatant was tested for PGI_2 activity[13]. Synthetic prostacyclin, sodium salt (Upjohn Co., Kalamazoo, USA) was dissolved in ethanol and diluted in 0.05 M Tris buffer pH 9.0 just before use. Mixtures of prostacyclin and endotoxin at different concentrations were incubated for 5 min at room temperature and tested for their antiaggregating activity.

For ex vivo experiments, endotoxin suspensions (0.25–1.0 mg/kg b.wt) were given i.v. and the animals were killed at intervals thereafter. In 1 group of rats 2 endotoxin injections were given 24 h apart and the animals were killed

1 h after the 2nd injection. In some experiments, sodium heparin (Liquemin® Roche, Basle, Switzerland, 100 IU/kg b.wt) was given i.v. 10 min before endotoxin.

Vascular rings were removed from all animals under ether anesthesia, and prostacyclin activity was measured as described[13].

Results and discussion. The figure reports a representative in vitro experiment showing no effect of *E. coli* endotoxin on prostacyclin activity released from arterial or venous tissues. Endotoxin did not change the platelet inhibitory activity of synthetic prostacyclin. Similar results were obtained with *S. minnesota* LPS. These data are at variance with those obtained by Bult and Herman with *E. coli* 0127:B8 LPS[14].

The table summarizes the results of ex vivo experiments. Supernatants from vascular rings removed 30 min after *S. minnesota* endotoxin injection had higher prostacyclin activity than control specimens (as indicated by a reduction of at least 50% in the slope of the aggregation tracings). At the endotoxin dose of 250 µg/kg b.wt, the effect was seen only in venous specimens, whereas at 1 mg/kg b.wt arterial tissues too appeared more active. Pretreatment with heparin did not prevent the stimulating effect of endotoxin on vascular prostacyclin activity in all 6 animals studied (data not shown). A double injection of *S. minnesota* endotoxin (250 µg/kg, 24 h apart) did not result in any change of vascular prostacyclin activity in the majority of the animals (table). In a few experiments, 1 mg/kg *E. coli* LPS (given 30 min before testing) also appeared to stimulate prostacyclin activity in venous tissues (5 out of 5 rats).

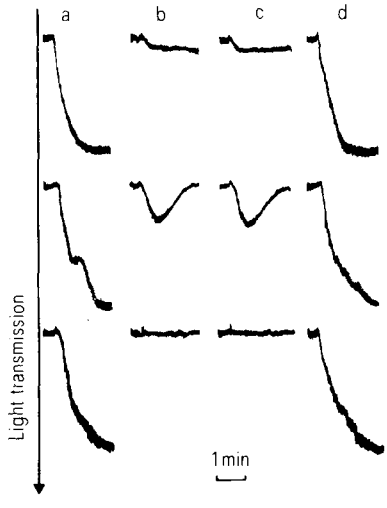
Increased blood levels of 6-keto-$PGF_{1\alpha}$, the stable derivative of prostacyclin, have been reported by Bult et al.[11] in rabbits shortly after the administration of 5 mg/kg b.wt of *E. coli* 0111.B4 endotoxin. This finding and those presented here in rats, suggest that endotoxin may stimulate vascular prostacyclin production in vivo.

The mechanism by which endotoxin produces this effect is not yet known. Failure of heparin to prevent the stimulating effect of endotoxin on prostacyclin activity makes the involvement of blood clotting activation in this phenomenon unlikely. On the other hand, no direct interaction between endotoxin and prostacyclin was found in vitro, thus pointing to a complex interplay between endotoxin and the vessel wall in vivo.

Several investigators have observed lesions in the vessel wall of rats and other animals shortly after endotoxin administration[15]. The relationship between morphological changes and the biological effect at vascular level described here remains to be established. Increased prostacyclin activity has been reported in rats in mechanically-injured endothelial tissues[16] and in rings of some thrombosed veins[17] or vascular tissues from spontaneously hypertensive rats[18]. It has been suggested that in these conditions increased PGI_2 activity could be a general vascular defence mechanism. Prostaglandins seem to be primarily involved in the expression of some detrimental endotoxin effects[4-10]. However, infusion of arachidonic acid has been reported to increase the survival of rabbits in endotoxin shock[9]. The present study indicates that endotoxin administration results in increased vascular prostacyclin activity. The physiopathological implications of this finding remain to be defined.

Effect of *S. minnesota* LPS (given 30 min before testing) on vascular prostacyclin activity. The number of animals with more than 50% increase of activity is reported for each experimental condition studied

Endotoxin (µg/kg)	Aorta	V. cava
S. minnesota		
250	1/6	6/6
1000	5/6	5/6
250 (×2)	1/6	2/6
E. coli		
1000	-	5/5



Representative platelet aggregation tracings obtained with 0.8 µM ADP (curves a). Inhibitory effect of prostacyclin (curves b), prostacyclin + *E. coli* LPS (500 µg/ml) (curves c) or *E. coli* (curves d). The upper panel represents experimens with abdominal aorta (5 µl supernatant), the middle panel those with inferior vena cava (25 µl supernatant) and the bottom panel those with synthetic prostacyclin (10 nM).

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