LETTERS TO THE EDITOR

- The British Heart Journal welcomes letters commenting on papers that it has published within the past six months.
- All letters must be typed with double spacing and signed by all authors.
- No letter should be more than 600 words.
- In general, no letter should contain more than six references (also typed with double spacing).

Endothelium in control

SIR,—In his St Cyres Lecture (*British Heart Journal* 1991;65:116-25) Professor AH Henderson states that the half life of endothelium derived relaxing factor (EDRF) nitric oxide is "likely to be less than a second in vivo" and that "each millimetre of endothelium controls its little bit of the vascular system." I believe it is possible to place these suggestions on a more precise basis. In the picomolar concentrations of nitric oxide secreted by endothelium the half life of the reaction between nitric oxide and oxygen is far too slow to account for this proposed rapid removal.¹ By contrast the rate of removal by haem groups is exceedingly rapid and the second order rate constant for reaction of nitric oxide with the red cell is 167 l mmol⁻¹ s⁻¹ in vitro.² There is growing evidence that this value may be applied to blood nitric oxide uptake in vivo at least in human pulmonary capillary.³ Though the rate of reaction of nitric oxide with oxyhaemoglobin in vitro⁴ is about 250 times as fast as the reaction with the red cell, because less than 0.03% of the haemoglobin in blood is in the free form, the reaction with the red cell is quantitatively more important. Similar arguments apply to tissue haem groups and also superoxide ion. The half life of nitric oxide in blood is obtained as follows:

$t_{1/2} = 0.693 / (167 \times 9) = 4.6 \times 10^{-4}$ seconds where 0.693 is $\ln 2$, 167 is the second order rate constant (see above), and 9 is the concentration of Hb in mmol/l corresponding to a haemoglobin concentration of 14.6 g/dl. The distance travelled in one half life is obtained as the velocity of blood flow (1 m/s in the aorta during systole, 0.3 m/s in the vena cava, $5 \cdot 10^{-4}$ m/s in a capillary⁵) multiplied by $t_{1/2}$: that is, 4.6×10^{-4} m in the aorta, 1.4×10^{-4} m in the vena cava, and 2×10^{-7} m in capillary. The half life of nitric oxide in vivo in blood can be seen to be exceedingly short and the distance "downstream" over which a section of endothelium can have an influence extremely small especially in the capillary.

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- produced spherocytes. *J Gen Physiol* 1958; 42:83-107.
- 3 Borland C. NO and CO transfer. *Eur Respir J* 1990;3:977-8.
 - 4 Doyle MP, Hoekstra JW. Oxidation of nitrogen oxides by bound dioxygen in haemoproteins. *J Inorg Biochem* 1981;14:351-8.
 - 5 Horrobin DF. *Medical physiology and biochemistry*. London: Edward Arnold, 1968: 307-9.

This letter was shown to the author, who replies as follows:

SIR,—I am grateful to Dr Borland for his interest and for the quantitative pyramid he builds on my point which was simply to emphasise the very localised nature of vasodilatation mediated by EDRF.

The half life of EDRF was documented as being in the order of seconds by bioassay of oxygenated effluent buffer from endothelialised artery segments.¹ Nitric oxide oxidation in aqueous buffer is related to PO_2 but is some 30 times faster in transit through perfused hearts where its half life was shown to be about 0.1 s.² This is consistent with previously reported evidence that the intraluminal dilator signal in the anaesthetised dog femoral artery is localised to within 1 cm.³ Clearly the presence of haemoglobin (which has about 1500 times greater affinity for nitric oxide than for carbon monoxide) will further reduce the half life of nitric oxide within the vascular compartment in vivo. Small amounts of "free" haemoglobin are in fact complexed to haptoglobin in circulating blood (<0.03% of total haemoglobin in the blood, as Dr Borland states), accounting for the variable EDRF-inhibiting activity of plasma (the inhibitory activity in samples from some of our human volunteers was unusually high, and after appropriate experimentation, this was attributed to alcohol intake).⁴ The largest sink of haemoglobin is indeed within the erythrocytes, whose EDRF inhibitory activity we found to be that of similar concentrations of free haemoglobin,⁵ as expected from the lipid solubility of nitric oxide and its ready passage through red cell membranes. Superoxide anions, widely present in biological systems, further shorten the half life of nitric oxide⁶ (rate constant of the reaction $NO + O_2^-$ at physiological pH, $k = 3.7 \times 10^7$ M⁻¹s⁻¹). The concentration of oxygen free radicals is likely to vary widely, particularly under pathological conditions. Recent evidence suggests, for example, that the reduced EDRF activity shown in experimental hypercholesterolaemia may be due to EDRF inactivation rather than decreased production, on the basis of a decrease in bioassayed dilator activity but an increase in nitric oxide production when measured by chemiluminescence,⁷ the decrease in activity being attributable possibly to increased superoxide anion production beneath atheromatous plaques.⁸

Biology, let alone pathology (and perhaps alcohol) clearly introduces complexity. I think we agree though that locally released EDRF has little downstream activity.

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cular tone by nitric oxide. *Circ Res* 1990; 66:1561-75.

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- 4 Edwards DH, Griffith TM, Ryley HC, Henderson AH. Haptoglobin-haemoglobin complex in human plasma inhibits endothelium-dependent relaxation: evidence that endothelium derived relaxing factor acts as a local autotoxin. *Cardiovasc Res* 1986;20:549-56.
- 5 Evans HG, Ryley HC, Hallett I, Lewis MJ. Human red blood cells inhibit endothelium-derived relaxing factor (EDRF) activity. *Eur J Pharmacol* 1989;163:361-4.
- 6 Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 1986;320:454-6.
- 7 Minor RL, Myers PR, Guerra R, et al. Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *J Clin Invest* 1990;86:2109-2116.
- 8 Nakamura M, Tagawa H, Tomoike H. Reduced release of EDRF and significant inactivation of EDRF at the tunica media beneath the atheromatous plaque in aortas of WHHL rabbits [abstract]. *Arch Int Pharmacodyn* 1990; 305:209.

Novel exercise protocol suitable for use on a treadmill or a bicycle ergometer

SIR,—The standardisation of exercise tests is now a major issue and the Working Group on Exercise Testing of the European Society of Cardiology has organised a committee to examine this problem. In November 1990 Dr Northridge and colleagues (*British Heart Journal* 1990;64:313-6) presented data on a new exercise protocol that is based on exponential (rather than linear) increments in workload. In their experience the rise in oxygen consumption (ml/kg/min) is very similar whether the test is performed on a bicycle or on a treadmill; also they mention that the highest stage required to test even relatively fit patients is reached after 15 minutes.

We have tested prospectively and randomly these new protocols in 13 healthy men (mean age 33, range 25-49; mean weight 80, range 62-115 kg) and our data (fig 1) indicate that oxygen consumption was significantly greater (unpaired t test; $p < 0.05$) during the last nine minutes of the bicycle standardised exponential exercise protocol (STEEP). The heart rates were also greater ($p < 0.05$) with the bicycle protocol during the last six minutes of the exercise test (fig 2). All the subjects were able to perform the 15 minutes of the STEEP treadmill test while only six were able to reach the fifteenth minute of the bicycle protocol; at the fifteenth minute of the STEEP bicycle test, all patients complained of pain in the legs, a symptom that was almost absent at the fifteenth minute of the STEEP treadmill test. Also the reason for interrupting the STEEP bicycle test was always leg muscle fatigue or pain. These observations about heart rate and exercise duration are very similar to those made by Northridge *et al.*

The explanation for our different results remains unclear. In our experience, we think that for the heaviest subjects the final increases in workload of the bicycle protocol are too large (from 25 to 40 w/min) to be tolerated by the leg muscles and that muscle soreness becomes the major limiting factor. Because of the differences in oxygen consumption and in exercise duration, we think that these new STEEP tests are not the answer to our growing need for standardised exercise tests that can be used on both the treadmill

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1 Griffith TM, Henderson AH, Hughes Edwards D, Lewis MJ. Isolated perfused rabbit coronary artery and aortic strip preparations: the role of endothelium-derived relaxant factor. *J Physiol (Lond)* 1984;351:13-24.

2 Kelm M, Schrader J. Control of coronary vas-

and the bicycle ergometer. Also we feel that the very slow rise in oxygen consumption seen during the first six minutes of both STEEP tests make them an unsatisfactory basis for the extrapolation of maximal oxygen uptake from maximal workload and for measuring the subtle changes observed after a given treatment. Before being tested in patients, these new protocols should be extensively tested in healthy volunteers and compared with other exercise protocols.

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This letter was shown to the authors, who reply as follows:

SIR,—The data presented by Dr Essamri and colleagues in 13 male volunteers confirm many of our findings with the STEEP exercise test. There are, however, some important differences that may be explained, at least in part, by methodological considerations. It is

likely that young men living in Belgium are more accustomed to cycling than our Glaswegian volunteers and this may explain why they are able to perform more aerobic work on a bicycle ergometer. Furthermore, the significance levels quoted were obtained using unpaired *t* tests, such that for the final stages the six fittest subjects who were able to complete the bicycle protocol were compared with the whole group who completed the treadmill protocol. It is not surprising that the six fittest subjects had a higher mean oxygen consumption than the group as a whole. It would be more appropriate to use a paired test, such that data from subjects completing a given stage of one protocol are compared only with data from the same subjects during the other protocol.

We are encouraged that Dr Essamri and colleagues agree with the need for standardisation of exercise testing within Europe—but which protocol are we to use as a standard? The STEEP protocol has several advantages over existing protocols, including short stages (as recommended by Buchfuhrer *et al*¹), suitability for both treadmill and bicycle testing, adjustment for body weight during

the bicycle protocol so that subjects of different size exercise at similar relative intensity at each stage, and exponential increments in workload making the test applicable to a very wide range of patients. None of these improvements over existing protocols is contested in the letter from Essamri *et al*. They have, however, demonstrated a difference in oxygen consumption during the later stages of the treadmill and bicycle protocols, which may indicate a need for a minor modification of one or other protocol. However, we did not suggest that the two tests were identical, only that they were comparable. Bicycle and treadmill testing have certain fundamental differences—which were discussed in our original report. The STEEP tests merely offer a pragmatic solution to the problem of standardisation when some laboratories use treadmills while others use bicycle ergometers.

Their assertion that the final workload increments are too large in heavy subjects is clearly unwarranted because the whole point of adjustment for body weight is that all subjects experience the same relative workloads and increments. In fact the data of Essamri *et al* confirm this principle because the standard deviations of the mean oxygen consumption for each stage of the bicycle protocol (assuming that their figure shows standard deviations rather than standard errors) are very small even though the study included a remarkable range of body weights—from 62 kg to 115 kg. We fully agree that the STEEP protocol is not suitable for inferring maximal oxygen uptake from maximal workload in patients with cardiovascular disease—but this is not recommended for any protocol.^{2,3}

Finally, we think that firm conclusions cannot be reached until new standard exercise protocols, such as the STEEP test, are validated in suitable patient populations.

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- 1 Buchfuhrer MJ, Hansen JE, Robinson TE, Sue DY, Wasserman K, Whipp BJ. Optimising the exercise protocol for cardiopulmonary assessment. *J Appl Physiol* 1983;55:1558–64.
- 2 Ragg KE, Murray TF, Karbonit LM, Jump DA. Errors in predicting functional capacity from a treadmill exercise stress test. *Am Heart J* 1980;100:581–3.
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Fatal aortic rupture during balloon dilatation of recoarctation

SIR,—I read the paper by Balaji (*British Heart Journal* 1991;65:100–1) with interest. They reported aortic rupture and death in an eight year old child after balloon angioplasty for aortic recoarctation that developed after patch angioplasty procedure. We have had extensive experience with balloon angioplasty of aortic coarctations, both native and postoperative,^{1–9} and have not observed a similar complication. We do not agree with Balaji *et al* that balloon angioplasty should be avoided in cases of recoarctations after patch angioplasty. The complication reported in Balaji *et al* is a problem related to the technique of angioplasty that they adopted.

Firstly, I do not believe that balloon angioplasty should be performed without monitoring the pressure in the balloon. The purpose of monitoring the pressure is not to prevent

Figure 1 Oxygen consumption.

Figure 2 Heart rate.