Lipid peroxidation as a measure of oxygen free radical damage in the very low birthweight infant

EDITOR,—We read with interest the paper by Inder et al on lipid peroxidation as a measure of oxygen free radical damage in preterm infants. They showed a rise in malondialdehyde detected by the thiobarbituric acid (TBA) test over the first week which was significantly greater in those infants developing chronic lung disease. We have also used the TBA test to detect lipid peroxidation in 131 very preterm infants during the first seven days after birth. Concentrations rose from a median of 2·13 μmol/l (1·63–2·77 range) on day 1 to 3·27 μmol/l (2·49–4·48) on day 7 in those not developing chronic lung disease and from 2·07 μmol/l (1·16–9·28) to 3·77 μmol/l (2·6–4·21) in the 40 infants who developed chronic lung disease. No significant difference was observed. It is of interest that our values for the TBA test were about 30 times lower than those of Inder et al, in keeping with other published values for the test. We used a fluorimetric method, but the HPLC technique used by Inder et al generally gives lower values than the fluorimetric method. Until these differences are explained, we cannot accept the authors’ findings as evidence for lipid peroxidation in very preterm infants.

R W I COOKE
A DRURY
G A RUSSELL
Department of Child Health,
Institute of Child Health,
Royal Liverpool Children’s Hospital,
Alder Hey, Eaton Road,
Liverpool L12 2AP

Dr McIntosh comments:
In our studies using EMLA cream we were attempting to reduce pain and distress (apparently unsuccessfully) in newborn infants receiving heel pricks. The parents were informed that EMLA cream had not been used other than in our own study on neonates, but that it was commonly used and with no problem in older children. We knew about the possibility of methaemoglobinemia but at the time of starting the study there was only one report of this problem in a child who was also receiving a sulphonamide, so we did not believe that we ought to inform the parents of this specific but theoretical hazard.

Dr Inder and coauthors comment:
We are grateful for the query from Professor Cooke and colleagues which identified an unfortunate calculation error in our malondialdehyde-thiobarbituric acid (MDA-TBA) values that occurred during the conversion of our standard values in ng/ml to μmol/l. Due to this error the published MDA-TBA values were too high by a factor of 60, and should read for cord blood in full term infants (n=48) mean (SD) 1·05 μmol/l (0·16); preterm infants without chronic lung disease (n=6) from cord blood concentrations of 1·19 (0·1) μmol/l to 1·72 (0·1) μmol/l at 7 days and in premature infants with chronic lung disease (n=16) from cord blood concentrations of 1·42 (0·1) μmol/l to 2·66 (0·2) μmol/l at 7 days. These values are approximately half those found by Cooke et al in his premature infants. However, the significance of the raised MDA-TBA values in premature infants with chronic lung disease is unchanged. Why did our assay detect a significant difference in MDA-TBA concentrations? The key issue relates to the specificity of thiobarbituric assays for malondialdehyde as indicators of lipid peroxidation. Both the method we used1 and the method of Wong et al,2 use HPLC to measure MDA-TBA, which eliminates inaccuracies due to interfering chromatograms. However, there are several important differences. In our assay, no EDTA is added, plasma lipids are extracted before analysis, and ferric chloride (FeCl3) plus butylated hydroxyanisole are added before heating with TBA. The rationale for adding FeCl3 was to promote efficient breakdown of lipid hydroperoxides to MDA, but we are not sure that this is its only mode of action. The Wong method uses whole plasma and is thought to measure primarily protein bound MDA. Thus, although both are considered to be indicators of lipid peroxidation the two methods may clearly be measuring two different parameters. Further ongoing research in our premature infants continues to support the findings we have published. However, to understand the true nature of the MDA-TBA product measured, more specific analytical measures of lipid peroxidation products are awaited.