**Short reports**

**Serum antioxidant activity in neonates**

J L SULLIVAN* AND R B NEWTON†

*Veterans Administration Medical Center, and †Medical University of South Carolina, Charleston, South Carolina, USA

**SUMMARY** Antioxidant activity was measured in cord sera from 25 infants whose birth weights ranged from 830 to 3700 g. Antioxidant activity showed a significant positive association with birth weight. Deficiency in serum antioxidant activity may be important in the pathogenesis of certain neonatal disorders such as retinopathy of prematurity.

The plasma proteins ceruloplasmin and apotransferrin have strong antioxidant activities, as measured by their ability to prevent autoxidation in brain homogenates. The antioxidant activity of both proteins involves interactions with iron; the antioxidant activity of apotransferrin depends on its ability to bind two ferric ions and that of ceruloplasmin depends on its ferroxidase activity. The antioxidant potency of these two proteins in the brain homogenate assay has been estimated to be 200–500 times that of α-tocopherol.

In babies born at term the plasma concentrations of ceruloplasmin and apotransferrin are low by adult standards, and they are even lower in premature infants. These findings imply that serum antioxidant activity is low in premature infants and increases with gestational age. Such a pattern might help to explain the apparently increased susceptibility of some premature infants to the adverse effects of increased oxygen tensions.

**Methods**

Antioxidant activity was measured in cord sera from 25 infants by a modification of the method of Stocks et al. Birth weights ranged from 830 to 3700 g. Antioxidant activity was also measured in sera from a group of 21 adult blood donors, 12 men and nine women with a mean age of 31-5 years.

To measure serum antioxidant activity, 40 μl of serum was added to 2 ml of ice cold bovine brain homogenate (about 1:10 w/v in phosphate buffered saline, pH 7.4). The mixture of serum and homogenate was then serially diluted with ice cold homogenate and incubated at 37°C for one hour. Thiobarbituric acid reacting substances generated by spontaneous autoxidation of the brain homogenate were determined by measuring the absorbance in the supernatants at 535 nm after addition of 2 ml of thiobarbituric acid reagent mixture, heating, and centrifugation. Controls contained either homogenate alone or homogenate with butylated hydroxytoluene at a final concentration of 60 μM. Residual autoxidation was calculated by dividing the absorbance at 535 nm of the cord serum with homogenate (minus the absorbance at 535 nm, of the homogenate with butylated hydroxytoluene) by the absorbance at 535 nm of the homogenate control (minus the absorbance at 535 nm of the homogenate with butylated hydroxytoluene). Residual autoxidation was plotted against the amount of serum present in six serial twofold dilutions. The x intercept of the resulting straight line, derived by linear regression, was the serum dose in μl (Dmax) required for maximal inhibition of autoxidation. A large value for Dmax indicates a small antioxidant activity.

A wide range of serum doses in each case was used to give a better estimate of extreme values of antioxidant activity. Use of a single serum dose, and expression of antioxidant activity as per cent inhibition of the formation of thiobarbituric acid reacting substances would compress the range of observed antioxidant activities. Antioxidant activity, defined as per cent inhibition, is proportional to reciprocal Dmax.

To show the corrective effects of various substances on antioxidant activity, 20 μl samples of a selected infant's cord serum were added to 5 μl of selected adult sera, or to 5 nmol of d-penicillamine, dl-α-tocopherol, deferoxamine mesylate, or human apotransferrin. The infant weighed 1480 g and had extremely low antioxidant activity with an indeterminately large Dmax. The final concentration of each substance was 125 μM in the resulting mixture,
before the addition of homogenate. Triplicate 1 ml samples of the mixtures were incubated with homogenate for 1 hour at 37°C. Formation of thiobarbituric acid reacting substances was expressed as net absorbance at 535 nm after subtraction of the absorbance at 535 nm in homogenates treated with butylated hydroxytoluene; dl-α-tocopherol was added in ethanol. Correction for the effect of ethanol was not made because the ethanol control was not significantly different from the infant serum alone (unpublished). The adult sera were from a 34 year old man who had not donated blood and a 20 year old woman who was a frequent blood donor.

Results

In this group of 25 infants, Dmax was inversely related to birth weight (fig 1) and this was significant by Spearman’s rank correlation (p<0-05). A previous study found that antioxidant activity in infants born at term measured as per cent inhibition of formation of thiobarbituric acid reacting substances was only about 20% of the normal adult value. In the present study the 12 infants who weighed more than 2500 g at birth had a mean (SEM) Dmax of 41-1 μl (4-52 μl) compared with a mean (SEM) for the adult donor group of 12-2 μl (0-955 μl), (p<0-001). These findings are comparable with those previously reported.

The ability of various substances to correct the deficient antioxidant activity of one infant’s serum was investigated, d-penicillamine, dl-α-tocopherol, deferoxamine mesylate, and human apotransferrin being added in eqimolar amounts to give final concentrations that roughly approximated to those that are achievable in an infant’s plasma. The dl-α-tocopherol concentration was near the upper limit achieved by pharmacological doses in a recent study of retinopathy of prematurity.

As shown in fig 2, dl-α-tocopherol, deferoxamine mesylate, human apotransferrin and adult sera were able to decrease thiobarbituric acid reacting substances formation significantly. Deferoxamine mesylate and human apotransferrin were significantly superior (p<0-001) to dl-α-tocopherol in decreasing thiobarbituric acid reacting substances formation. The addition of α-pencillamine did not result in a significant decrease. Human apotransferrin, deferoxamine mesylate, and the adult female serum were by far the most effective at correcting the infant’s antioxidant activity.

Discussion

These data confirm the report of Gutteridge and Stocks that antioxidant activity in the serum of term neonates is lower than that of adults. The present study extends the applicability of their findings to premature infants. Antioxidant activity in the lower birthweight infants was generally lower than that in high birthweight infants. In some cases at the lower end of the birthweight range, extremely high values for Dmax were found. This pattern is comparable
with the low concentrations of human apotransferrin and ceruloplasmin in premature infants found by other workers.2 3

The possible antioxidant role of plasma dl-

α-tocopherol in neonatal disorders such as retinopathy of prematurity has received much attention.5 The present findings raise the possibility that deficiencies in the potent human apotransferrin/ceruloplasmin dependent antioxidant system may be important in the pathogenesis of retinopathy of prematurity and similar disorders.

These findings are relevant to the suggestion6 that increased plasma concentrations of non-haem iron in the neonatal period are of central importance in the pathogenesis of retinopathy of prematurity. Large increases in serum ferritin and transferrin iron saturation are usually seen in premature infants and infants born at full term shortly after birth. Additional sources of exogenous iron in the neonatal period include dietary iron supplementation and blood transfusion.6 The present data suggest that one important effect of increased non-haem iron may be to neutralise much of the premature infant’s scarce antioxidant activity, thereby sharply increasing vulnerability to oxygen.

We thank Dr DL Phelps and Dr WA Silverman for their helpful suggestions. Dr DM Purohit for help in obtaining samples of cord serum, and American Red Cross Blood Services, Carolina Low Country Region for samples of serum from blood donors. RBN had a postdoctoral fellowship from the College of Graduate Studies of the Medical University of South Carolina, and the work was supported by the Veterans Administration.

References

Correspondence and requests for reprints to Dr JL Sullivan, VAMC (113), 109 Bee Street, Charleston, SC 29403, USA.

Accepted 18 December 1987

Simple method for securing umbilical catheters

M SOUTH* AND A MAGNAY†

*Department of Paediatrics, University of Cambridge, and †Birmingham Maternity Hospital

SUMMARY A method for securing umbilical catheters is described. It has been used successfully in over 350 babies. It is a simple and rapid method that allows for easy repositioning and, unlike some established techniques, has no potential for damage to the preterm baby’s skin.

Since their introduction in the early 1960s the techniques of umbilical arterial and venous catheterisation have been accepted into routine clinical practice. Adequate fixation of catheters is important: displacement can lead to malposition of the catheter tip, with consequent poor sampling and increased risk of vascular thrombosis; catastrophic haemorrhage can occur if the catheter ‘falls out.’

Most recommended methods for catheter fixation involve a combination of typing loops of suture material around the base of the catheter, and the use of a ‘goalpost’ bridge of adhesive tape attached to the catheter and to the skin of the abdominal wall.1 Having used such methods for a number of years we became aware of several problems: tying the loops of suture material is fiddly, and it can be difficult to tie them sufficiently tight to grip the catheter without partially occluding its lumen. The adhesive tape is often difficult to attach to the baby’s moist, vernix covered skin. The tape reduces the area available for the use of transcutaneous electrodes. The bridge of tape can be caught accidentally during handling, and it has even provided an effective footstrap for an active baby to kick against. Removal of adhesive tape from the often extremely fragile skin of a very preterm baby can be damaging and can lead to ooz of plasma, increased fluid loss, and risk of sepsis. Once fixation is complete it can be difficult to reposition the catheter if a radiograph shows an unsatisfactory placement of the tip.