Free Rad. Res. Comms., Vol. 16, No. 5, pp. 285–294
Reprints available directly from the publisher
Photocopying permitted by license only

© 1992 Harwood Academic Publishers GmbH
Printed in the United Kingdom

LIMITED PROTECTION AGAINST IRON-INDUCED LIPID PEROXIDATION BY CORD BLOOD PLASMA

JAN H.N. LINDEMAN[1], ERNST HOUDKAMP[1], EEF G.W.M. LENTJES[2], BEN J.H.M. POORTHUIS[1] and HOWARD M. BERGER[1]

[1]*Department of Pediatrics,* [2]*Department of Clinical Chemistry, University Hospital, Leiden, The Netherlands*

(Received January 20, 1992; in revised form February 17, 1992)

The ability of plasma from newborn babies (cord blood) and adults to inhibit iron-induced lipid peroxidation was compared. The caeruloplasmin and transferrin concentrations, and latent iron-binding capacity were lower in the babies ($p < 0.001$). The plasma of many of the babies had no latent iron-binding capacity and contained non-protein-bound iron (measured by the bleomycin assay). The *in vitro* ability of plasma to inhibit iron-induced liposome peroxidation by either ferroxidase antioxidant activity (caeruloplasmin) or iron-binding antioxidant activity (transferrin) was measured. The antioxidant activity in both assays was decreased in the babies ($p < 0.001$). The percentage inhibiton of peroxidation in the iron-binding antioxidant assay correlated positively with the latent iron-binding capacity ($p < 0.001$) and negatively with the presence of bleomycin-detectable iron ($p < 0.02$) in the babies. This assay produced stimulation of peroxidation in 42% of the babies but none of the adults. The diminished capacity of cord blood plasma to prevent iron-induced lipid peroxidation may predispose the newborn baby to the toxic effects of oxygen.

KEY WORDS: newborn, transferrin, caeruloplasmin, bleomycin-detectable iron, lipid peroxidation.

INTRODUCTION

Reactive oxygen species play a role in the pathogenesis of bronchopulmonary dysplasia, retinopathy of prematurity, and intracerebral haemorrhage in the newborn.[1,2] Increasing the removal of reactive oxygen species, e.g. by vitamin E therapy, in order to limit the incidence and severity of these diseases, has produced conflicting results.[3] Decreasing the production of reactive oxygen species may offer an alternative means of therapy.[4] Production of reactive oxygen species is enhanced by non-protein-bound iron,[4] and in plasma iron-induced formation of reactive oxygen species is inhibited by the synergistic action of caeruloplasmin's ferroxidase activity and transferrin's ferric-ion binding capacity.[5,6] Concentrations of these primary (preventive) antioxidants are low in babies compared to adults, and iron-induced oxygen damage may occur.[7] We therefore compared, *in vitro*, the ability of plasma from newborn babies and adults to inhibit iron-induced peroxidation of liposomes.[8]

Address for correspondence: H.M. Berger MRCP, Neonatal Unit, Department of Pediatrics, University Hospital, PO Box 9600, 2300 RC Leiden, The Netherlands, Tel **-3171262909.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK

TABLE I
Clinical details of the newborn babies

	Preterm	Term
Number	20	21
Gestational age, (completed weeks)†	31 (23–35)	39 (37–41)
Weight percentile‡	39 (17)	48 (31)
Female	10	12
APGAR score 1 min†	7 (1–9)	9 (7–10)
APGAR score 5 min†	9 (7–10)	10 (9–10)
Cord blood pH‡	7.36 (0.08)	7.34 (0.06)

†Median (range).
‡Mean (sd).

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

MATERIALS AND METHODS

Patients

All patients were Caucasian. Healthy adults: 10 males, and 10 females who were not pregnant or taking contraceptives,[9] aged 24–50 yrs were studied. The babies, born to healthy mothers, were well nourished (weight 10th–90th percentile[10]) (Table I). The term babies (gestational age > 37 wks) did not show signs of birth asphyxia (Apgar score† > 7 at 1 min, cord blood pH > 7.2). Ten of the preterm babies showed no signs of birth asphyxia; the other 10, had a normal cord blood pH and their Apgar scores were $\geqslant 7$ at 5 min.[11] Babies with clincial evidence of haemolysis, e.g. rhesus haemolytic disease were excluded from the study.

Collection of Samples

Venous blood from the adults and cord blood (from the separated placenta within 15 min of the babies birth), was slowly drawn through a 1.1 mm diameter needle to prevent haemolysis. The heparinized blood was immediately centrifuged (750 g, 10 min) and the plasma stored at $-70°C$ under argon until analysis (none of the plasma samples showed haemolysis assessed visually as pink discoloration). Preliminary studies showed that plasma values did not change during storage under these conditions.

Measurements

C-Reactive protein was measured by fluorescent polarization on the TDx® (Abbott, Amstelveen, The Netherlands). Iron and the latent iron-binding capacity were measured by spectroscopy using ferrozine complexation on the Cobas Mira® (Roche, Mijdrecht, The Netherlands). Caeruloplasmin and transferrin were determined by radial immunodiffusion (Behring, Amsterdam, The Netherlands). All methods were performed following the manufacturers recommendations.

Plasma antioxidant activities (due to ferroxidase activity or iron-binding) were measured in assays 1, 2, and 3 (see below) which were based on the methods developed by Gutteridge.[8] These *in vitro* assays assess the ability of plasma to prevent iron-induced lipid peroxidation of phospholipid liposomes. Peroxidation is prevented by

†A clinical score to assess the babies condition at birth.[11]

RIGHTSLINK

either oxidation or binding of iron by plasma. Small unilamellar vesicles[12] were prepared from fresh ox brain phospholipid.[13] The phospholipids were dissolved (100 mg/mL) in dichloro-methane : methanol (2 : 1) and stored in capped glass vials at −70°C (no lipid peroxidation occurred during storage). The use of multilamellar liposomes,[8] showed a large interassay variation in our experiments, but small unilamellar vesicles gave reproducible results (i.e., coefficients of variation for assay 1, 2, and 3 were 9%, 4%, 4% respectively). Small unilamellar vesicles were prepared as follows: phospholipids in cold PBS (5 mg/mL), were vigorously vortexed (3 min), then sonicated (titanium probe, 10 min in ice water) under nitrogen. The resulting suspension was centrifuged (30 000 *g*, 1 h, 4°C) and the supernatant containing the small unilamellar vesicles, was used.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

Plasma Antioxidant Activity

Assay 1 measures mainly plasma ferroxidase activity. Lipid peroxidation is stimulated by added ferrous ions, and inhibited when these are oxidized to ferric ions. 200 μL phosphate buffer (0.1 M, pH 7.4), 200 μL liposome suspension and 50 μL plasma were mixed, and peroxidation was started with the addition of 100 μL ferrous ammonium sulphate (1 mM) (prepared in deoxygenated water). The high ascorbate levels in cord blood compared to adult blood[14] may antagonize caeruloplasmin ferroxidase activity.[15] Therefore, the influence of ascorbate on ferroxidase antioxidant activity was assessed. In cord blood ($n = 4$), ascorbate was removed by an ascorbate oxidase spatula (Boehringer Mannheim, Amsterdam, The Netherlands) and in adult plasma ($n = 2$) ascorbate was increased to cord blood levels[14] by adding 50 μL ascorbate (0.1 mM).

Assay 2 also measures mainly plasma ferroxidase antioxidant activity. Lipid peroxidation however, is stimulated by ferrous ions formed by the reduction of ferric ions by ascorbate. 200 μL phosphate buffer (0.1 M, pH 6.5), 200 μL liposome suspension, 30 μL ascorbate (0.1 mM) and 20 μL plasma were mixed and peroxidation was started by the addition of 50 μL ferric ammonium sulphate (0.5 mM).

Assay 3 measures mainly plasma iron-binding antioxidant activity. Lipid peroxidation is stimulated by intrinsic iron (present in glassware and reagents), which will be reduced by added ascorbate, if it is not first bound by transferrin. 200 μL phosphate buffer (0.1 M, pH 7.4), 200 μL liposome suspension and 5 μL plasma were mixed and peroxidation started with 30 μL ascorbate (6.0 mM). Since the added ascorbate results in concentration many times greater than in normal plasma (*ca.* 150 fold), plasma caeruloplasmin activity is inhibited and the plasma ascorbate levels of the babies do not interfere with the results.

All assays were performed in duplicate using freshly prepared reagents in ultra clean water (Elgastat®, ELGA , High Wycombe, Bucks, England). After incubation of the liposomes (1 h, 37°C), 0.5 mL HCl (3 M) and 0.5 mL thiobarbituric acid (1% (w/v)) in NaOH (0.01 M) were added, the mixture heated (15 min, 100°C) and then cooled on ice. Thiobarbituric-acid reactive substances were extracted in 1.5 mL butan-1-ol and centrifuged (10 min, 1500 *g*). The butan-1-ol fraction was removed and centrifuged (2 min, 10 000 *g*) and the absorption measured at 532 nm. The percentage inhibition of lipid peroxidation was calculated as follows:

((absorbance 100% peroxidation − absorbance 100% blank) − (absorbance plasma inhibited peroxidation − absorbance plasma blank))/(absorbance 100% peroxidation − absorbance 100% blank)

RIGHTSLINK

TABLE II
The concentrations of plasma proteins and iron

	Preterm $n = 20$	Term $n = 21$	Adult $n = 20$
Caeruloplasmin mg/L	75 (41)[†‡]	132 (61)[§]	313 (44)
Transferrin g/L	1.98 (0.40)[†‡]	2.70 (0.34)[§]	3.49 (0.36)
Iron μmol/L	14.4 (9.7)[†]	25.3 (5.9)[§]	15.3 (6.9)

Results are shown as mean (sd).
[†]Preterm different from term group.
[‡]Preterm group different from adult group.
[§]Term group different from adult group (all p values < 0.002).

100% peroxidation was derived from a sample to which no plasma was added.

After completion of the above studies the bleomycin assay for the presence of non-protein-bound iron[16,17] became available in our department. As a result, retrospective studies were performed on the 17 babies and 9 adults for whom plasma samples were still available. Preliminary studies showed that storage did not affect the results of this assay.

Statistics

The differences between the groups were tested by analysis of variance (ANOVA). When significant, pairwise comparisons were carried out using the independent t test or Mann Whitney as indicated. Correlations were analyzed by Pearson's or Spearman's rank method as appropriate. P values less than 0.05 were regarded as significant.

RESULTS

Caeruloplasmin and transferrin are acute phase proteins, and C-reactive protein was measured to exclude elevated levels due to infection. All C-reactive protein values were normal ($\leqslant 15$ mg/L).[18]

Table II shows the concentrations of proteins and iron in plasma. Caeruloplasmin transferrin and iron levels were lower in preterm than in the term babies and correlated positively with gestational age (Table III). The adults had higher protein levels than the babies but their iron levels were lower than levels in term babies (Table II).

Figures 1 and 2 show that plasma ferroxidase antioxidant activities (assays 1 and 2) were similar in the preterm and term babies, and lower than the adults. The potentially inhibiting effect of high ascorbate concentrations on ferroxidase antioxidant activity was measured and the following results obtained: (a) ferroxidase antioxidant activity

TABLE III
The effect of gestational age on the concentrations of plasma proteins and iron ($n = 41$)

	Correlation[†]	Slope (increase per week)
Caeruloplasmin	0.50	24.6 mg/L
Transferrin	0.76	0.14 g/L
Iron	0.58	3.47 μmol/L

[†]All p values were < 0.001.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK

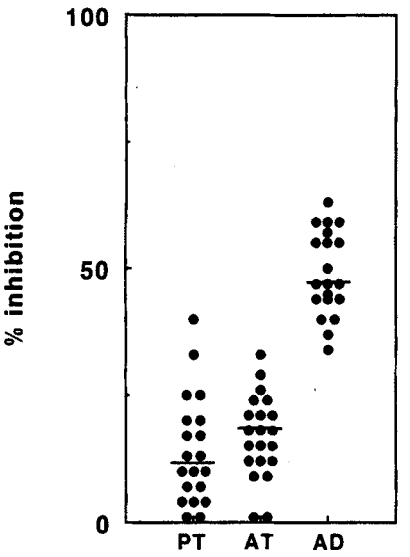

FIGURE 1 Plasma ferroxidase antioxidant activity (individual and mean values) as measured in assay 1 (ferrous ammonium sulphate added) in the 3 patient groups: preterm (PT) or term (AT) versus adult (AD): $p < 0.001$ (Mann Whitney).

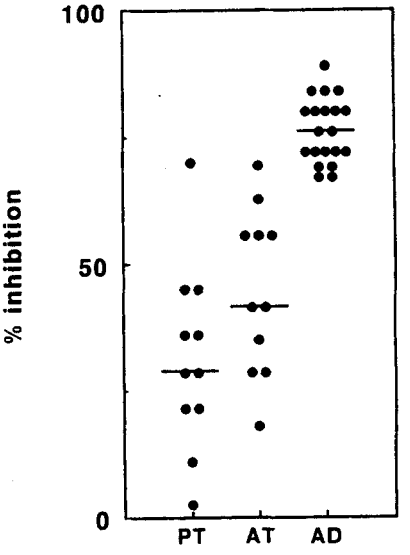

FIGURE 2 Plasma ferroxidase antioxidant activity (individual and mean values) as measured in assay 2 (ferric ammonium sulphate and low concentrations of ascorbate added) in the 3 patient groups: preterm (PT) or term (AT) versus adult (AD): $p < 0.001$ (Mann Whitney). In this assay 11 preterm and term babies were studied.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK

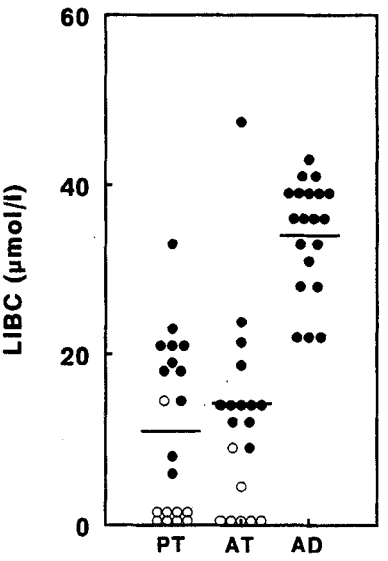

FIGURE 3 Latent iron-binding capacity (individual and mean values) in the 3 patient groups: preterm (PT) or term (AT) versus adult (AD): $p < 0.001$ (Mann Whitney). Open dots indicate samples which stimulated lipid peroxidation in assay 3 (see text and Figure 4). In the term group 1 sample was lost.

rose after removal of ascorbate from neonatal plasma: inhibition of peroxidation increased from 16% to 40%, while (b) addition of ascorbate to adult plasma diminished ferroxidase antioxidant activity from 50% to 34%.

Figure 3 shows that latent iron-binding capacity was similar in the preterm and term babies, and that they had a lower iron-binding capacity than the adults. In 9 babies there was no latent iron-binding capacity at all.

Figure 4 shows the influence of plasma iron-binding antioxidant activity on lipid peroxidation in assay 3. Antioxidant activity was significantly higher in adults than in babies. In all adult plasmas peroxidation was inhibited, but in 17 (42%) of the babies peroxidation was actually stimulated i.e., negative inhibition is indicated in Figure 4. All of these babies had a low or absent latent iron-binding capacity (see legend, Figure 3). The babies' plasma iron-binding antioxidant activity, expressed as % inhibition of peroxidation, correlated positively with their latent iron-binding capacity ($n = 40, r = 0.79, p < 0.001$) and negatively with their bleomycin-detectable iron concentration ($n = 17, r = -0.55, p < 0.02$). When lipid peroxidation was stimulated by plasma, peroxidation could be completely inhibited by addition of conalbumin (ovo-transferrin) (Figure 5). The use of desferrioxamine and human apotransferrin, instead of conalbumin gave similar results (data not shown).

DISCUSSION

Non-protein-bound iron can greatly enhance formation of reactive oxygen species.[6,19] Ferrous ions stimulate formation of reactive oxygen species such as alkoxyl ($RO^{\cdot}$) and the hydroxyl radical ($OH^{\cdot}$) by Fenton-type reactions.[20] In plasma, iron-induced formation of reactive oxygen species is normally rigorously inhibited by the synergistic

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK

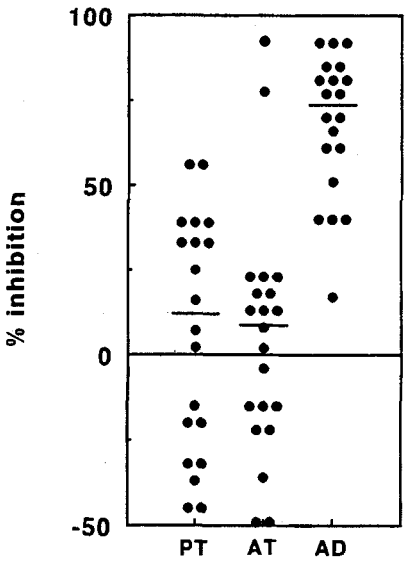

FIGURE 4 Plasma iron-binding antioxidant activity (individual and mean values) as measured in assay 3 (high amounts of ascorbate added, intrinsic iron only) in the 3 patient groups: preterm (PT) or term (AT) versus adult (AD): $p < 0.001$ (Mann Whitney). A negative inhibiton indicates stimulation of lipid peroxidation by plasma.

action of the preventative antioxidants caeruloplasmin and transferrin.[5,6] However this defence system can fail in iron-overload (e.g., haemochromatosis[21] and thalassaemia[22]), ischaemia-reperfusion injury,[23] rheumatoid arthritis[24] and in leukaemic patients after chemotherapy.[25]

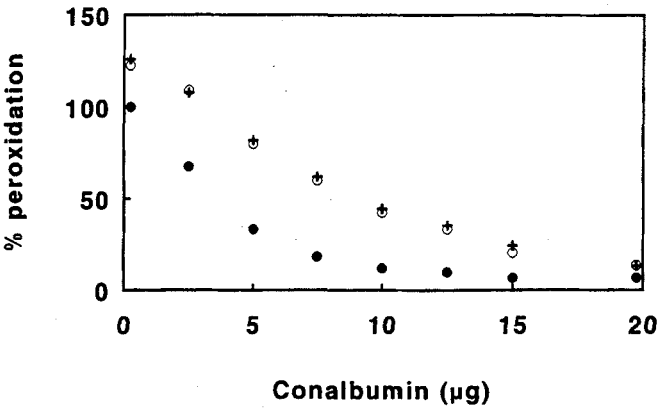

FIGURE 5 Inhibition of peroxidation in assay 3 by addition of conalbumin (ovo-transferrin). The relative amount (%) of peroxiation is shown on the Y axis. A 100% peroxidation value was obtained without iron or plasma addition (●). Addition of conalbumin (shown on the X axis) diminished peroxidation. Addition of 5 μl ferric ion (25 μM) (+) increased the initial peroxidation value (125%) and addition of conalbumin diminished peroxidation. Addition of 5 μL cord blood plasma sample (○), also increased the initial peroxidation value (124%), and addition of conalbumin also diminished peroxidation.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK

It has been postulated that iron-induced formation of reactive oxygen species also plays an important role in diseases of the newborn, e.g., bronchopulmonary dysplasia and retinopathy of prematurity.[7] We have shown that iron-overload in babies due to rhesus haemolytic disease is associated with disturbed plasma chain-breaking anti-oxidant activity and increased plasma peroxidation products.[26]

Sullivan and Newton, using a bovine brain homogenate assay concluded that neonatal plasma is less effective than adult plasma in preventing iron-induced peroxidation.[27] However, Gutteridge has pointed out that the intrinsic enzymatic and non-enzymatic pro- and anti-oxidant substances in brain homogenate, make it difficult to determine the specific effect of the primary plasma antioxidants.[8] The modified method we use here is not affected by intrinsic pro- and anti-oxidants derived from the brain homogenate, and discriminates between plasma ferroxidase anti-oxidant activity and iron-binding antioxidant activity.

As reported in previous studies, the concentration of caeruloplasmin was lower in newborns than in adults.[28] Although caeruloplasmin levels were lower in preterm babies, their ferroxidase antioxidant activity was similar to that of term babies. This might be explained by differences in the proportions of apocaeruloplasmin and caeruloplasmin.[29] However differences in plasma vitamin C levels are not a likely explanation because we have shown these to be similar in the two groups of babies.[14]

The low transferrin concentration and the relatively high iron concentration resulted in a low latent iron-binding capacity in the newborn.[30] In fact, a large number of infants had no latent iron-binding capacity at all. This explains the strongly diminished ability of cord blood plasma to protect liposomes against iron-induced lipid peroxidation in assay 3. Many plasma samples from babies, whose latent iron-binding capacity was absent or very low (see Figure 3), actually stimulated peroxidation. In the babies' plasma iron-binding antioxidant activity correlated positively with the latent iron-binding capacity and negatively with the level of bleomycin-detectable iron. Furthermore peroxidation could be inhibited completely by addition of ovotransferrin (conalbumin), apotransferrin or desferrioxamine. We suggest that non-protein-bound iron similar to that described in haemochromatosis[21] and other conditions of iron-overload is present in cord blood.

CLINICAL CONSIDERATIONS

Low caeruloplasmin ferroxidase antioxidant activity and high transferrin saturation of newborn babies may predispose them to iron-induced oxygen damage of brain, eyes and lung.[7] The brain and eye have relatively low concentrations of cellular antioxidant enzymes, such as catalase[31] and the protection provided by primary plasma antioxidants may therefore be important.[32] Animal and adult studies show that caeruloplasmin and transferrin also help protect the lung, which has high antioxidant enzyme concentrations, against oxygen toxicity.[33,34] In the newborn the role of caeruloplasmin in the development of acute and chronic lung problems has also been studied. Decreased concentrations of caeruloplasmin and caeruloplasmin-oxidase-activity at birth have been reported in children who developed hyaline membrane disease and bronchopulmonary dysplasia.[35,36] We are not aware, however, of clinical studies to assess the antioxidant role of transferrin and caeruloplasmin in protecting the newborn against oxidative damage in relation to the presence of low molecular mass iron in the plasma.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK

The potential risk of damage by reactive oxygen species, because of deficiencies in caeruloplasmin and transferrin, would be greatly amplified by iron-overload and further exacerbated by iron supplementation of preterm feeding formulas, by blood transfusions[37] or by vitamin C therapy.[15] On the other hand, raising the concentration of primary antioxidants, by stimulating their endogenous production by corticosteriod therapy[38,39] or providing exogenous sources via plasma transfusions,[40] may inhibit iron-induced formation of reactive oxygen species, and provide rational therapeutic approaches for the future.

Acknowledgements

The financial support of the foundation "De Drie Lichten" (grant JHNL) and Milupa International (EH) is gratefully acknowledged. Professor John Gutteridge's advice and Ralf Moison's assistance were invaluable. Without the help of the obstetric nursing staff the immediate collection and preparation of the babies' samples would not have been possible.

References

1. D.L. Phelps (1982) Neonatal oxygen toxicity – Is it preventable? *Pediatric Clinics of North America*, **29,** 1233–1240.
2. J.R. Wispe and R.J. Roberts (1987) Molecular basis of pulmonary oxygen toxicity. *Clinics in Perinatology*, **14,** 651–666.
3. W.B. Karp and A.F. Robertson (1986) Vitamin E in neonatology. *Advances in Pediatrics*, **33,** 127–148.
4. J.M.C. Gutteridge and B. Halliwell (1989) Iron toxicity and oxygen radicals. *Ballière's Clinical Haematology*, **2,** 195–256.
5. T.F. Slater, K.H. Cheeseman, M.J. Davies, K. Proudfoot and W. Xin (1987) Free radical mechanisms in relation to tissue injury. *Proceedings of the Nutrition Society*, **46,** 1–12.
6. B. Halliwell and J.M.C. Gutteridge (1990) The antioxidants of human extracellular fluids. *Archives of Biochemistry and Biophysics*, **280,** 1–8.
7. J.L. Sullivan (1988) Iron, plasma antioxidants, and the 'oxygen radical disease of prematurity'. *American Journal of Diseases of Children*, **142,** 1341–1344.
8. J.M.C. Gutteridge (1986) Antioxidant properties of the proteins caeruloplasmin, albumin and transferrin. A study of their activity in serum and synovial fluid from patients with rheumatoid arthritis. *Biochimica et Biophysica Acta*, **869,** 119–127.
9. L.M. Cranfield, J.L. Gollan, A.G. White and T.L. Dormandy (1979) Serum antioxidant activity in normal and abnormal subjects. *Annals of Clinical Chemistry*, **16,** 299–306.
10. G.J. Kloosterman (1970) On intrauterine growth. *International Journal of Gynaecology & Obstetrics*, **8,** 895–912.
11. E.A. Catlin, M.W. Carpenter, B.S. Brann, S.R. Mayfield, P.W. Shaul, M. Goldstein and W. Oh (1986) The Apgar score revisited: influence of gestational age. *Journal of Pediatrics*, **109,** 865–868.
12. S.N. Chatterjee and S. Agarwal (1988) Liposomes as membrane model for study of lipid peroxidation. *Free Radicals Biology and Medicine*, **4,** 51–72.
13. M. Kates (1972) Techniques of lipidology: isolation, analysis and identification of lipids. In *Laboratory Techniques in Biochemistry and Molecular Biology* (eds. T.S. Work and E. Work), North Holland, Amsterdam, pp. 347–353 and 393–395.
14. J.H.N. Lindeman, D. Van Zoeren-Grobben, J. Schrijver, A.J. Speek, B.J.H.M. Poorthuis and H.M. Berger (1989) The total free radical trapping ability of cord blood plasma in preterm and term infants. *Pediatric Research*, **26,** 20–24.
15. J.M.C. Gutteridge (1991) Plasma ascorbate levels and inhibition of the antioxidant activity of caeruloplasmin. *Clinical Science*, **81,** 413–417.
16. J.M.C. Gutteridge, D.A. Rowley and B. Halliwell (1981) Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. *Biochemical Journal*, **199,** 263–265.
17. J.M.C. Gutteridge and B. Halliwell (1985) Bleomycin assay for catalytic iron salts in body fluids. In *CRC Handbook of Methods for Oxygen Radical Research* (ed. R.A. Greenwald), CRC Press, Boca Raton, pp. 391–394.
18. L.M. Silverman, R.H. Christensen and G.H. Grant (1986) C-reactive protein. In *Textbook of Clinical Chemistry* (ed. N.W. Tietz), Saunders WB, Philadelphia, pp. 598–599.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK

19. C. Hershko (1987) Non transferrin plasma iron. *British Journal of Haematology*, **66,** 149–151.
20. B. Halliwell and J.M.C. Gutteridge (1989) *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford.
21. J.M.C. Gutteridge, D.A. Rowly, E. Griffiths and B. Halliwell (1985) Low molecular-weight iron complexes and oxygen radical reactions in idiopathic haemochromatosis. *Clinical Science*, **68,** 463–467.
22. C. Hershko, G. Graham, G.W. Bates and E.A. Rachmilewitz (1978) Non-specific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. *British Journal of Haematology*, **40,** 255–263.
23. A.M.M. van der Kraaij, L.J. Mostert, H.G. van Eijk and J.F. Koster (1988) Iron-load increases the susceptibility of rat hearts to oxygen reperfusion damage. *Circulation*, **78,** 442–449.
24. P. Merry, P.G. Winyard, C.J. Morris, M. Grootveld and D.R. Blake (1989) Oxygen free radicals, inflammation, and synovitis: the current status. *Annals of the Rheumatic Diseases*, **48,** 864–870.
25. B. Halliwell, O.I. Aruoma, G. Mufti and A. Bomford (1988) Bleomycin-detectable iron in the serum from leukaemic patients before and after chemotherapy. *FEBS Letters*, **241,** 202–204.
26. H.M. Berger, J.H.N. Lindeman, D. van Zoeren-Grobben, E. Houdkamp, J. Schrijver and H.H. Kanhai (1990) Iron overload, free radical damage, and rhesus haemolytic disease. *Lancet*, **335**, 933–936.
27. J.L. Sullivan and R.B. Newton (1988) Serum antioxidant activity in neonates. *Archives of Disease in Childhood*, **63**, 748–750.
28. P. Haga and S. Kran (1981) Ceruloplasmin levels and erythrocyte superoxide dismutase activity in small preterm infants during the early anemia of prematurity. *Acta Paediatrica Scandinavica*, **70,** 861–864.
29. K.E. Mason (1979) A conspectus of research on copper metabolism and requirement of man. *The Journal of Nutrition*, **109,** 1979–2066.
30. P.H. Scott, H.M. Berger, C. Kenward, P. Scott and B.A. Wharton (1975) Effect of gestational age and intrauterine nutrition on plasma transferrin and iron in the newborn. *Archives of Disease in Childhood*, **50,** 796–798.
31. T. Yusa, J.S. Beckman, J.D. Crapo and B.A. Freeman (1987) Hyperoxia increases H_2O_2 production by brain *in vivo*. *Journal of Applied Physiology*, **63,** 353–358.
32. E.D. Hall and J.M. Braughler (1989) Central nervous system trauma and stroke. II. Physiological evidence for involvement of oxygen radicals and lipid peroxidation. *Free Radical Biology and Medicine*, **6,** 303–313.
33. S.A. Moak and R.A. Greenwald (1984) Enhancement of rat serum ceruloplasmin levels by exposure to hyperoxia. *Proceedings of the Society for Experimental Biology and Medicine*, **177,** 97–103.
34. E.R. Pacht and W.B. Davis (1988) Role of transferrin and ceruloplasmin in antioxidant activity of lung epithelial lining fluid. *Journal of Applied Physiology*, **64,** 2092–2099.
35. J.A. Omene, A.C. Longe, J.C. Ihongbe, R.H. Glew and I.R. Holzman (1981) Decreased umibilical cord serum ceruloplasmin concentrations in infants with hyaline membrane disease. *Journal of Pediatrics*, **99,** 136–138.
36. K. McCarthy, M. Bhogal, M. Nardi and D. Hart (1984) Pathogenic factors in bronchopulmonary dysplasia. *Pediatric Research*, **18,** 483–487.
37. J.C.L. Shaw (1982) Iron absorption by the premature infant. *Acta Paediatrica Scandinavica (suppl.)*, **299**, 83–89.
38. U.M. Chockalingam, E. Murphy, J.C. Ophoven and M.K. Georgieff (1987) The influence of gestational age, size for dates, and prenatal steroids on cord blood transferrin levels in newborn infants. *Journal of Pediatric Gastroenterology and Nutrition*, **6,** 276–280.
39. J.J. Cummings, D.B. D'Eugenio and S.J. Gross (1989) A controlled trial of dexamethasone in preterm infants at high risk for bronchopulmonary dysplasia. *New England Journal of Medicine*, **320**, 1505–1510.
40. J.H.N. Lindman, E.G.W.M. Lenthes, E. Houdkamp, D. van Zoeren-Grobben, J. Schrijver and H.M. Berger (1992) Effect of an exchange transfusion on plasma antioxidants in the newborn. *Pediatrics*, in press.

Accepted by J.M.C. Gutteridge

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/10/11
For personal use only.

RIGHTSLINK