

Neonatal oxygen toxicity and its prevention: D-penicillamine offers benefits without harmful side-effects

G OROSZLÁN, L LAKATOS, L KARMAZSIN

Department of Paediatrics, University Medical School,
Debrecen

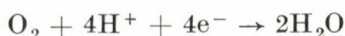
Oxygen toxicity in the newborn and the possibilities of its prevention are discussed. The role of active radicals developing from molecular oxygen, lipid peroxidation and its prevention by antioxidants, oxygen toxicity and its importance in neonatology, and facilities for overcoming hyperoxia of the newborn are considered. The antioxidant effects based on experimental and clinical observations of vitamin E and D-penicillamine are described. Since the introduction of D-penicillamine therapy the incidence of retrolental fibroplasia has steeply decreased. It is emphasized that in the neonatal period the drug has no adverse effects.

Oxygen (O₂) is indispensable for the highly organized functions of life. Oxygen therapy may be life-saving in hypoxia, but hyperoxia may lead to severe damage to cells and tissues.

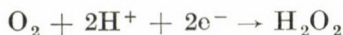
The role of active radicals arising from molecular oxygen

During inspiration, O₂ dissolved in the lipids and the water of lung tissue diffuses through the alveolar membranes into the capillaries where it is bound to the haemoglobin of the erythrocytes; oxygen is then carried by the blood to the various tissues. It is well known that O₂ is indispensable for a large number of biochemical processes working at cellular and tissue levels. Most oxygen undergoes the four-electron reduction carried out by mitochondrial cytochrome oxidase; the end product of this

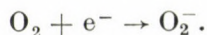
process is water [12]:



Reduction by two electrons performed by other enzymes results in hydrogen peroxide

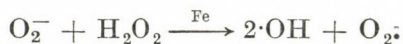


while a reaction using one electron leads to the superoxide radical



In addition, O₂⁻ is produced by auto-oxidation of normal cellular components like thiol derivatives, haemoglobin, or epinephrine [67, 69, 70]. The bactericidal effect of O₂⁻ developing during phagocytosis by polymorphonuclear leucocytes is a characteristic property [3, 85]. H₂O₂ is comparatively well tolerated by most aerobic organisms in spite of its much higher reactivity to biological molec-

ules. The compound may be harmful if it is transformed to hydroxyl radical ($\cdot\text{OH}$) in the presence of certain metal ions [33]. This radical is extremely aggressive. It may also be formed during the reaction of O_2^- and H_2O_2 catalyzed by iron chelates [24, 34]:



The interaction between various oxygen and peroxide radicals may result in a very reactive oxygen derivative toxic to most biological systems, in singlet oxygen ($^1\text{O}_2$). In this radical, one electron of the oxygen is pushed to a high energy orbit [48, 91].

H_2O_2 , O_2^- , $\cdot\text{OH}$ and $^1\text{O}_2$ are the main factors responsible for the tissue damage provoked by hyperoxia; "oxygen radical" is an oversimplified common term for them.

Lipid peroxidation and its biological consequences

According to the most accepted hypothesis, O_2 toxicity is provoked by free radicals arising from O_2 and by other active oxygen metabolites; their effect is mediated by increased lipid peroxidation (LP) of biological membranes, thus leading to specific cell damage [25, 67]. Oxidability of fatty acids depends on the number of double bonds, i.e. only unsaturated fatty acids (linolic acid, linolenic acid, arachidonic acid, etc.) are prone to peroxidation. Cellular membranes,

containing a considerable quantity of unsaturated fatty acids, are especially vulnerable to peroxidative damage [16, 81, 99]. $\cdot\text{OH}$ and probably also O_2^- , react with the lipid components of biological membranes; this results in peroxide ($\text{ROO}\cdot$) and hydroperoxide (ROOH) radicals of lipids and in other compounds like malonyldialdehyde [5, 17, 47]. Malonyldialdehyde forms covalent bonds with the free amino-groups of proteins, lipids and nucleic acids, and on this reaction is based the chemical detection of LP processes [9]. This chain reaction, initiated by the free oxygen radicals is catalysed by heavy metals, especially iron, and copper at several levels [51, 53, 99] leading to a loss of the integrity of membranes of erythrocytes, mitochondria, microsomes and lysosomes, to inactivation of enzymes in these structures and to damage to nucleic acids [50, 65, 71].

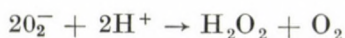
LP may accompany a number of physiological and pathophysiological events. Such normal physiological processes are prostaglandin synthesis, phagocytosis and aging [3, 37, 40, 65, 85]. Certain experimental and clinical observations have indicated the damaging effect of LP in haemolytic anaemia, dysfunctions of reproduction, certain pulmonary disorders, muscular atrophies, liver necrosis, encephalomalacia, atherosclerosis, testicular atrophy, diabetes mellitus, irradiation disease, postresuscitation syndrome, mutagenesis, carcinogenesis [16, 18] and other conditions including certain neonatal disorders to be described in this paper.

Antioxidant prevention

The antioxidant defence mechanisms are indispensable in maintaining life since the potentially destructive free radicals are continuously produced in the organism and they keep combining with each other [28]. The first line of defence consists of various physiological barriers hindering the oxygen to react with the tissues while entering from the alveolar space into the pulmonary capillaries and carried by haemoglobin to the cells [36]. The second type of defence is bound to specific enzymes: superoxide dismutase, catalase, peroxidases, glutathione and its enzyme system, and the NADPH producing system closely coupled to the hexose-monophosphate shunt [87, 106]. Since these enzymes exhibit activity induction during adaptation to hyperoxia, their function will be described in some detail.

Superoxide dismutase (SOD)

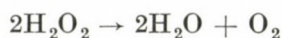
This metalloprotein enzyme has been found in three main forms. In eukaryotic cells the metal is copper and zinc in the cytoplasm, and manganese in the mitochondria. A third type of SOD contains iron and occurs in a number of bacteria. Their main task is to enhance the dismutative inactivation of O_2^- radicals produced during auto-oxidation of various biological components or arising during enzymatic processes. The dismutation can be described by the reaction,



As a result of this reaction, O_2^- is rapidly removed before it could induce direct cellular damage or lead to production of aggressive $\cdot OH$ radicals by combination with H_2O_2 [25, 66, 100].

Catalase and other peroxidases

These are enzymes of haem-protein nature. Their main biological role consists in the elimination of H_2O_2 :



Although H_2O_2 itself participates in LP at a low degree and slow reaction rate, its elimination is imperative because it reacts with O_2^- in the presence of metal ions, leading thus to $\cdot OH$, the most dangerous radical from the aspect of oxygen toxicity [27, 33].

Glutathione and NADPH producing systems

Glutathione is a thio-tripeptide. A high concentration of its reduced form (GSH) and a low concentration of its oxidized form (GSSG) are essential in the maintenance of animal life. The antioxidant effect of GSH is exerted by two routes: it prevents the oxidation of sulfhydryl groups of the proteins by serving as a substrate for the oxidants; in addition, GSH is a substrate of the enzyme glutathione peroxidase which inactivates both H_2O_2 and lipid peroxides, stopping thus the chain reaction initiated by the oxygen radicals [35, 68]. The optimum GSH-GSSG ratio is maintained by the enzymes glutathione reductase and glucose-6-phosphate

dehydrogenase. The latter forms an essential step of the hexose-monophosphate shunt. Its production is greatly enhanced by the NADPH (reduced nicotinic amide adenine dinucleotide phosphate) system, supporting in this way the important biosynthetic reactions necessary for the reparation of tissues damaged by lipid peroxidation [11].

The third line of antioxidant defence comprises endogenous antioxidants, the adequate tissue level of which offers satisfactory protection against oxygen toxicity. These compounds are vitamin E, selenium, sulfur containing amino acids, vitamin C [27, 97], and transferrin and caeruloplasmin, proteins performing the task of extracellular transport of iron or copper [19].

The special situation of neonates and low-birth-weight newborns

As shown above, oxygen toxicity is the resultant of two biological processes acting in two opposite directions. On the one hand, production of free radicals and the chain reaction initiated by them would tend to lead to disintegration of the cell and the cellular organelles. On the other hand, there exists a sophisticated defence system acting at several levels that prevents or even repairs the tissue damage. Under physiological conditions there is an equilibrium between the two systems; the identical rate of production and elimination secures only the "normal" function of lipid peroxidation. Pathological newborns and preterm infants

are, however, in a peculiar situation. In these infants and to some extent in normal term babies as well, a disequilibrium resulting in increased vulnerability to oxygen toxicity may develop during adaptation to extrauterine life. In addition, defence mechanisms against the oxygen radicals are also immature in them. There are two main reasons for this increased vulnerability, (i) an increased exposure to situations of prolonged oxidative stress; (ii) deficient defence at all levels.

Ad (i)

1. Before and during birth the newborn may be exposed to maternal and fetal injuries resulting in an increased rate of LP: EHP-gestosis, intrauterine infections, various maternal diseases, placenta praevia, premature rupture of the membranes, exposure of the mother to drugs and radiation, especially tocolytics, procedures during labour like oxygen inhalation, anaesthesia, analgesia, oxytocin etc. [89].

2. The greatest risk is oxygen therapy some time after birth, frequently applied as a life-saving measure necessary for the prevention of sequelae of hypoxia [42]. The most vulnerable individuals in this respect are babies of very low birth weight and newborns in whom the partial tension of arterial oxygen continuously exceeds the tolerable level. For this reason, in newborn units all over the world attempts at measuring arterial oxygen tension are made to avoid prolonged hyperoxaemic

periods [49, 96]. Unfortunately, only partial success has been achieved as even in intensive care units not all the grave sequelae of oxygen toxicity can be avoided [83].

3. A frequent damaging factor is hyperbilirubinaemia. Apart of its unfavourable effect on the central nervous system [63], bilirubin has been shown to be a photodynamic agent i.e. it produces 1O_2 in the presence of oxygen and light, thus enhancing the peroxidation of unsaturated fatty acids [15, 29, 32].

4. Certain therapeutic measures destined to surmount neonatal hyperbilirubinaemia like phototherapy and exchange transfusion may also be potentially harmful in this respect. Phototherapy enhances the isomerisation of bilirubin into rapidly eliminated and less toxic compounds. This process is accompanied by an increased production of 1O_2 . This radical has a minor role in bilirubin oxidation but it may be responsible for the potential hazards of phototherapy: damage to DNA, mutagenesis, carcinogenesis, sexual dysfunction, etc. [14, 93]. Similarly, exchange transfusions and transfusion of adult blood may increase the oxygen toxicity by the lower oxygen affinity of adult haemoglobin compared to that of fetal haemoglobin [13].

5. Septic and hypotensive conditions, including the idiopathic respiratory distress syndrome (IRDS), resuscitation and reparation after resuscitation increase the risk of oxygen damage. In chronic and acute inflammations the polymorphonuclear

leucocytes, macrophages and monocytes produce a considerable amount of O_2^- ; this radical has a direct bactericidal effect and promotes the formation of the superoxide dependent chemotactic factor and the production of $^{\cdot}OH$ radicals. Both compounds have a favourable effect in the killing process directed against the bacteria and other invasive agents [3, 85]. In the immature organism, however, with its deficient antioxidant defence, the aggressive radicals may damage the cellular integrity.

6. Certain drugs may increase the proneness to oxygen toxicity. Some have a direct sensitizing effect in favour of oxygen toxicity, e.g. riboflavin, methylene blue, salicylates, etc. [92]. Others enhance the albumin-bilirubin dissociation or weaken the activity of glucuronyl transferase thus promoting bilirubin and oxygen toxicity at the same time [63]; sulfonamides and chloramphenicol are in this group. Finally, artificial ventilation and X-ray exposure, not infrequent in modern intensive care, directly lead to overproduction of harmful oxygen radicals [4].

Ad (ii)

1. In newborn babies the physiological barriers lining the internal route of oxygen transport are immature. Immaturity and gestational age show a direct correlation. Size, weight, alveolarity, collagen content, concentration of the pulmonary surfactant, the proportion of interstitial elements, capillary microcirculation, the number of alveolar epithelial

cells, respiratory capacity and compliance of the lung all differ from the situation prevailing in the baby born at term [41].

2. The activity of specific antioxidant enzymes gradually increases during gestation and in the early postnatal period. The most potent inductor of this activity is the oxygen itself, and hyperoxia speeds up the enzyme adaptation process [1, 6, 107]. This may explain the finding that under prolonged oxygen exposure newborn animals survive much longer than adult animals; this does not imply that they do not suffer permanent tissue damage. Similarly, pretreatment with antioxidants abolishes the usual biochemical reaction to hyperoxia [103]. Our own experiments with SOD on the effects of D-penicillamine resulted in similar findings [46].

The neonatal insufficiency of the enzymatic defence is markedly promoted by heavy metals, especially by iron and copper ions. These latter accumulate in a considerable degree in the fetal tissues during the last weeks of gestation [88]. After birth an additional unfavourable factor is the increased haemolysis of fetal erythrocytes. The metal ions directly influence the oxidative processes and, in addition, they inhibit the activity of delta-aminolaevulinic acid synthetase, an enzyme governing the production of haeme-containing proteins like catalase, peroxidase and cytochrome P-450. They also stimulate haemoxygenase, an enzyme initiating haeme catabolism [62].

3. Newborn babies, especially very low birth weight preterm babies, are insufficiently supplied by endogenous antioxidants like vitamin E, selenium, transferrin and coeruloplasmin [23, 38].

Neonatal disorders related to oxygen toxicity

These disorders are specific to the newborn period. They have a multi-causal aetiology, their common feature being the chain reaction leading to tissue damage, initiated by free oxygen radicals.

Haemolytic diseases

Auto-oxidation of the newborn's erythrocytes proceeds at a rate three to five times higher than in adults [94]. An especially unfavourable situation is encountered in babies affected by congenital deficiency of glucose-6-phosphate dehydrogenase, pyruvate kinase or glutathione reductase [95].

Retrorenal fibroplasia (RLF)

Terry [98] gave the first description of RLF of premature babies in 1942. The pathology of the condition was clarified 10 years later [26] when it was also recognized that the principal cause was an uncontrolled high oxygen supply [10, 80]. Although this view has repeatedly been confirmed, no efficacious prophylactic or therapeutic measure has been available and RLF has become the heaviest burden of modern intensive care treatment [90]. The increasing

survival rate of very low-birth weight infants has led to an increase in the number of blind people [42, 89, 96].

Bronchopulmonary dysplasia (BPD)

A better understanding of the pathomechanism of IRDS has led to the introduction of continuous positive airway pressure (CPAP) ventilation, bringing about a dramatic improvement in the prognosis of the disease [31].

However, BPD, a new clinical entity has appeared on the scene [75]. The condition is a progressive pulmonary fibrosis leading to severe respiratory failure; it is caused, in addition to oxygen therapy, by prolonged mechanical ventilation and patent ductus arteriosus [104].

Hyaline membrane disease or IRDS

This condition is specific to preterm babies and pathological newborns and leads to alveolar hyaline membrane production. The process is related to oxygen toxicity in several respects. Increased catabolism due to the severe condition of the newborn may increase the production of free radicals, and the therapeutic efforts may directly enhance LP. Recently it has been shown that the peroxidative chain reaction triggered by oxygen radicals may play a primary role, since the hyaline membrane is basically a LP product [8]; it can be induced experimentally by ionizing radiation and type II pneumocytes producing the surfactant are damaged by oxygen radicals [2].

Possible methods for surmounting the adverse effects of hyperoxia

Theoretically, there are two possibilities to increase the defence against oxygen toxicity. Antioxidant agents are substances directly neutralizing the free radicals. Other compounds enhance the activity of enzymes counteracting the effect of oxygen radicals and repair the damage caused by lipid peroxidation. Vitamin E is a good example of antioxidants while D-penicillamine plays an active role in both respects.

Vitamin E

Vitamin E has been known for more than forty years [22] but the exact mechanism of its antioxidant effect has not been clarified [82]. Vitamin E deficiency was observed to lead in certain animals to disorders probably due to increased auto-oxidation, i.e. infertility, encephalomalacia, muscle dystrophy or demyelination. Human hypovitaminosis E occurs only in preterm infants or in chronic fat malabsorption [30, 105]. Several authors attribute a primary aetiological role in neonatal oxygen toxicity to the low vitamin E concentration in the preterm babies' tissues [78, 79]. Vitamin E has been applied for its antioxidant effect by several authors; some reported on success in the prevention of BPD [20] or of RLF [45, 39], others have not been able to observe any convincing effect [42, 44, 84, 89, 102]. We feel that Dormandy's remark still holds [18]: "Forty years after its discovery,

vitamin E remains a biochemical Don Basilio, not to be trusted or believed but impossible to dismiss”.

D-penicillamine (DPA)

In previous studies we have shown that intravenous DPA is a potent drug in the treatment and prevention of neonatal hyperbilirubinaemia [56, 58]; it was especially effective in haemolytic disease due to incompatibility in the Rh and ABO systems [57]. Its use combined with phototherapy allowed to avoid exchange transfusions in preterm babies affected by hyperbilirubinaemia [7]. A new impetus to our research was the observation of a reduced incidence of RLF among very low-birth-weight infants treated with DPA during the newborn period. This retrospective observation was later confirmed by a comparative study of preterm babies weighing less than 1500 g at birth, 109 of whom were treated with DPA and 86 without the drug [54, 55]. The two groups were shown to be comparable in

a number of perinatal factors including those known to increase oxygen toxicity. The incidence of cicatricial RLF was 0.9% in the treated group while it was as high as 11.6% among the babies not treated with DPA. Since our first observation in 1979, all preterm babies weighing less than 1500 g at birth and undergoing oxygen therapy have been given DPA treatment. Table I shows our recent results. It is seen that while intensive perinatal care has brought about a marked increase in the survival rate of very low-birth-weight infants, this was accompanied by a sharp increase in the incidence of loss of sight due to RLF. On the other hand, ever since the introduction of DPA treatment, only one case of RLF has been observed and none during the last two years. Mortality, a sensitive indicator of adequate oxygen therapy, has been unchanged during the same period.

I. DPA directly neutralizes free oxygen radicals. DPA is dimethylcysteine and like all compounds contain-

TABLE I

Number, mortality rate and incidence of retrolental fibroplasia in premature infants weighing less than 1500 g at birth, 1977-1981

Year	Intensive neonatal care				
	Without DPA		With DPA		
	1977	1978	1979	1980	1981
All cases	90	142	140	138	134
Deaths, No.	36	56	64	54	51
per cent	40.0	39.4	45.7	39.1	38.1
No. of survivors	54	86	76	84	83
RLF, No.	6	4	1	0	0

DPA was administered to all preterm babies from May, 1979.

ing SH-groups it is a hydrogen and electron donor [21, 60, 74]. It has been shown that the red-violet complex of copper and DPA exerts SOD activity [61]; other authors, however, assumed that not the complex itself but other copper complexes of low molecular weight liberated by gradual decomposition of the whole complex are responsible for the dismutation of O_2^- [86].

It is highly probable that the antioxidant property of DPA is largely due to its chelating capacity, binding heavy metal ions known to catalyze LP [43].

As every thiol-derivative, DPA forms mixed disulfide bonds with serum proteins containing sulfhydryl groups; during this process it undergoes oxidation and in this way prevents the development of free radicals [73].

It has been shown [59] that the radioprotective property of DPA is more expressed in suckling mice than in adult animals.

The fact that the drug markedly decreases the serum bilirubin level, seems to point to its potent antioxidant activity [13].

DPA treatment leads to an increased intracellular concentration of free glutathione [72].

II. DPA increases the activity of enzymes participating in the antioxidant defence of newborn babies. This may explain the age dependent differences in the action of DPA. It was shown earlier [64] that in newborn animals DPA enhances the activity of hepatic peroxidase and catalase

while such an effect could not be observed in adult animals. Similar results were obtained concerning lipid peroxidation in erythrocyte membranes, induced by hydrogen peroxide or phenylhydrazine [76].

The age dependence of the DPA effect was shown in rat experiments by measuring hexobarbital sleeping time. A marked reduction of the parameter by DPA treatment could be achieved in newborn animals. In addition, DPA induced an increase in microsomal cytochrome P-450 and led to multiplication of mitochondria in the liver cells only in newborns. In newborn animals, DPA inhibits haemoxygenase, the enzyme initiating haeme catabolism and so it not only inhibited bilirubin production but also ensured the persistence of high levels of the antioxidant enzymes of haeme-protein structure [77].

Recently, direct evidence has been produced of the sight protective effect of DPA, demonstrating that the drug significantly inhibited the proliferative processes in the corpus vitreum [101].

DPA treatment in the newborn may deeply affect some complex biochemical events and as a result it may shorten the postnatal adaptation period and reduce in that way the incidence and severity of some pathological phenomena appearing characteristically in the course of adaptation. In spite of this, many paediatricians are reluctant to apply DPA in newborn babies. This may be attributed to a fear of the unpleasant and dangerous side-effects of pro-

longed DPA therapy observed in some patients affected by rheumatoid arthritis [52]. It must, however, be emphasized that preterm infants are not small adults with rheumatoid arthritis. Even a high dose of DPA administered for some days has no acute side-effect in babies nor could any chronic side-effect be detected by prolonged observations. The drug had no effect on the albumin-bilirubin complex [7], and none of the outward effects described in adults have ever been encountered in our material. To date a total of 2500 newborn babies has been treated with DPA during the last 9 years, without any complication whatever.

REFERENCES

1. Autor PA, Frank L, Roberts RJ: Developmental characteristics of pulmonary superoxide dismutase: Relationship to idiopathic respiratory distress syndrome. *Pediatr Res* 10:154, 1976
2. Avery ME: *The Lung and Its Disorders in the Newborn Infant*. 2nd ed. Saunders, Philadelphia 1968, p. 149
3. Babior BM, Kipnes RS, Curnutte JT: Biological defense mechanisms. *J Clin Invest* 52: 741, 1973
4. Bacq ZM: *Chemical Protection against Ionizing Radiation*. Charles C, Thomas, Springfield Illinois, 1965, p. 189
5. Bidlack WR, Tappel AL: Damage to microsomal membrane by lipid peroxidation. *Lipids* 8:177, 1973
6. Bonta BW, Gawron ER, Warshaw JB: Neonatal red cell superoxide dismutase enzyme levels: Possible role as a cellular defense mechanism against pulmonary oxygen toxicity. *Pediatr Res* 11: 754, 1977
7. Brodersen R, Lakatos L, Karmazsin L: D-Penicillamine, a non-bilirubin-displacing drug in neonatal jaundice. *Acta Paediatr Scand* 69: 31, 1980
8. Brown RE, Craver R, Drake RM: Lipid peroxidation and pulmonary hyaline membranes of the newborn. *Ann. Clin Lab Sci* 11: 25, 1981
9. Buege JA, Aust SD: *Microsomal Lipid Peroxidation* In: *Methods in Enzymology*, eds Fleischer S, Packer L, Academic Press, New York 1978
10. Campbell K: Intensive oxygen therapy as a possible cause of retrolental fibroplasia: A clinical approach. *Med J Aust* 2: 48, 1951
11. Carson PE, Brewer GJ, Ickes C: Decreased glutathione reductase with susceptibility to hemolysis. *J Lab Clin Med* 58: 804, 1961
12. Chance B: Spectra and reaction kinetics on respiratory pigments of homogenized and intact cells. *Nature* 169: 215, 1952.
13. Clark C, Gibbs JAH, Maniello R, Outerbridge EW, Aranda JV: Blood transfusion: A possible risk factor in retrolental fibroplasia. *Acta Paediatr Scand* 70: 535, 1981
14. Cohen AN, Ostrow JD: New concepts in phototherapy: Photoisomerization of bilirubin IX α and potential toxic effects of light. *Pediatrics* 65: 740, 1980
15. Cukier JO, Maglalang AC, Odell GB: Increased osmotic fragility of erythrocytes in chronically jaundiced rats after phototherapy. *Acta Paediatr Scand* 68: 903, 1979
16. Del Maestro RF: An approach to free radicals in medicine and biology. *Acta Physiol Scand (Suppl)* 492:153, 1980
17. Deneke SM, Fanburg BL: Normobaric oxygen toxicity of the lung. *N Engl J Med* 303: 76, 1980
18. Dormandy TL: Vitamin E and antioxidant activity. *Proc R Soc Med* 70: 91, 1977
19. Dormandy TL: Free-radical oxidation and antioxidants. *Lancet* 1: 647, 1978
20. Ehrenkranz RA, Bonta BW, Ablow RC, Warshaw JB: Amelioration of bronchopulmonary dysplasia after vitamin E administration. *N Engl J Med* 299: 564, 1978
21. Emerit L, Emerit J, Levy A, Keck M: Chromosomal breakage in Crohn's disease: Anticlastogenic effect of D-Penicillamine and L-cysteine. *Hum Genet* 50: 51, 1979
22. Evans HM, Bishop KS: The production of sterility with nutritional regimens adequate for growth and its cure with other foodstuffs. *J Metab Res* 3: 233, 1923
23. Farrel PM: Vitamin E deficiency in premature infants. *J Pediatr* 95: 869, 1979

24. Fridovich I: Superoxide dismutases. *Annu Rev Biochem* 44:147, 1975
25. Fridovich I: The biology of oxygen radicals. *Science* 201: 875, 1978
26. Friedenwald JS, Owens WC, Owens EU: Retrolental fibroplasia in premature infants: III. The pathology of the disease. *Trans Am Ophthalmol Soc* 49: 207, 1951
27. Gilbert DL: The role of pro-oxidants and antioxidants in oxygen toxicity. *Radiat Res (Suppl)* 3: 44, 1963
28. Gilbert DL: Introduction: Oxygen and life. *Anesthesiology* 37:100, 1972
29. Girotti AW: Bilirubin-sensitized photo-inactivation of enzymes in the isolated membrane of the human erythrocyte. *Photochem Photobiol* 24: 525, 1976
30. Gordon HH, Nitowsky HM, Tildon JT, Levin S: Studies of tocopherol deficiency in infants and children. An Interim Summary. *Pediatrics* 21: 673, 1958
31. Gregory GA, Kitterman JA, Phibbs RH, Tooley WH, Hamilton WK: Treatment of the idiopathic respiratory distress syndrome with continuous positive airway pressure. *N Engl J Med* 284:1333, 1971
32. Hackney DD: Photodynamic action of bilirubin on the inner mitochondrial membrane. Implications for the organization of the mitochondrial ATP-ase. *Biochem Biophys Res Commun* 94: 875, 1980
33. Halliwell B: Biochemical mechanisms accounting for the toxic action of oxygen on living organisms: the key role of superoxide dismutase. *Cell Biol Int Rep* 2:113, 1978
34. Halliwell B: Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates: is it a mechanism for hydroxyl radical production in biochemical systems? *FEBS Lett* 92: 321, 1978
35. Haugaard N: Cellular mechanisms of oxygen toxicity. *Physiol Rev* 43: 311, 1968
36. Hedley-Whyte J, Winter TM: Oxygen therapy. *Clin Pharmacol Ther* 8: 696, 1967
37. Hemler ME, Lands WEM: Evidence for a peroxide-initiated free radical mechanism of prostaglandin biosynthesis. *J Biol Chem* 255: 6253, 1980
38. Henkin RI, Schulman JD, Schulman CB, Bronzert DA: Changes in total, nondiffusible and diffusible plasma zinc and copper during infancy. *J Pediatr* 82: 831, 1973
39. Hittner HM, Godio LB, Rudolph AJ, Adams JM, Garcia-Prats JA, Friedman Z, Kautz JA, Monaco WA: Retrolental fibroplasia: Efficacy of vitamin E in a double-blind clinical study of preterm infants. *Engl J Med* 305:1365, 1981
40. Hochstein P, Jain SK: Association of lipid peroxidation and polymerization of membrane proteins with erythrocyte aging. *Fed Proc* 40:183, 1981
41. Inselman LS, Mellins RB: Growth and development of the lung. *J Pediatr* 98: 1, 1981
42. James LS, Lanman JT: History of oxygen therapy and retrolental fibroplasia. *Pediatrics (Suppl)* 57: 591, 1976
43. Jocelin PC: Biochemistry of the SH-group. Academic Press, London, New York 1972
44. Johnson L: Retrolental fibroplasia: A new look at an unsolved problem. *Hosp Pract* 16:109, 1981.
45. Johnson L, Schaffer D, Boggs TR Jr: The premature infant, vitamin E deficiency and retrolental fibroplasia. *Am J Clin Nutr* 27:1158, 1974
46. Karmazsin L, Lakatos L, Balla Gy, Makay A, Hatvani I: Experimental data on the prevention of retrolental fibroplasia by D-penicillamine. *Acta Paediatr Acad Sci Hung* 21: 131, 1980
47. Kellog EW, Fridovich I: Liposome oxidation and erythrocyte lysis by enzymically generated superoxide and hydrogen peroxide. *J Biol Chem* 252: 6721, 1977
48. Kahn AU: Activated oxygen: singlet molecular oxygen and superoxide anion. *Photochem Photobiol* 28: 429, 1978
49. Kinsey VE: Retrolental fibroplasia. Cooperative study of retrolental fibroplasia and the use of oxygen. *Arch Ophthalmol* 56: 481, 1956
50. Kong S, Davison AJ: The relative effectiveness of OH, H₂O₂, O₂⁻ and reducing free radicals in causing damage to biomembranes. *Biochim Biophys Acta* 640: 313, 1980
51. Kornbrust DJ, Mavis RA: Microsomal lipid peroxidation. I. Characterization of the role of iron and NADPH. *Mol Pharmacol* 17: 400, 1980
52. Kreysel HW: D-Penicillamin. Chemie, Pharmakologie, therapeutische Anwendung und unerwünschte Wirkungen. Schattauer Verlag, Stuttgart 1977
53. Kunitomo M, Inoue K, Nojima S: Effect of ferrous ion and ascorbate-induced lipid peroxidation on liposomal membranes. *Biochim Biophys Acta* 646:169, 1981
54. Lakatos L, Hatvani I, Oroszlán Gy, Karmazsin L: Prevention of retrolental fibroplasia in very low birth weight

- infants by D-penicillamine. *Eur J Pediatr* 138: 199, 1982
55. Lakatos L, Hatvani I, Oroszlán Gy, Karmazsin L, Matkovics B: D-penicillamine in the prevention of retrolental fibroplasia. *Acta Paediatr Acad Sci Hung* 23: 327, 1982
 56. Lakatos L, Kövér B, Péter F: D-penicillamine therapy of neonatal hyperbilirubinaemia. *Acta Paediatr Acad Sci Hung* 15: 77, 1974
 57. Lakatos L, Kövér B, Oroszlán Gy, Vekerdy Zs: D-penicillamine therapy in ABO hemolytic disease of the newborn infant. *Eur J Pediatr* 123:133, 1976
 58. Lakatos L, Kövér B, Vekerdy Zs, Dvoráček É: D-penicillamine therapy of neonatal jaundice: Comparison with phototherapy. *Acta Paediatr Acad Sci Hung* 17: 93, 1976
 59. Lakatos L Oroszlán Gy Dézsi Z Hatvani I Karmazsin L.: Age related difference in radioprotective effect of D-Penicillamine. *Dev Pharmacol Ther* (in press)
 60. Lal M Lin W S, Gaucher GM Armtong DA: The repair, protection and sensitisation of papain with respect to inactivation by H_2O_2 and OH: Effects of dithiothreitol, penicillamine, cystine and penicillamine disulphide. *Int J Radiat Biol* 28: 549, 1975
 61. Lengfelder E, Elstner EF: Determination of the superoxide dismutating activity of D-penicillamine copper. *Hoppe-Seylers Z Physiol Chem* 359: 751, 1978
 62. Maines MD, Kappas A: Metals as regulators of heme metabolism. *Science* 198:1215, 1977
 63. Maisels MJ: Bilirubin. On understanding and influencing its metabolism in the newborn infant. *Pediatr Clin North Am* 19: 447, 1972
 64. Matkovics B, Lakatos L, Szabó L, Karmazsin L: Effects of D-penicillamine on some oxidative enzymes of rat organs in vivo. *Experientia* 37: 79, 1981
 65. McCay PB: Physiological significance of lipid peroxidation. Introduction. *Fed Proc* 40: 173, 1981
 66. McCord JM, Fridovich I: Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *J Biol Chem* 244: 6049, 1969
 67. McCord JM, Fridovich I: The biology and pathology of oxygen radicals. *Ann Intern Med* 89:122, 1978
 68. Mills GC: Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J Biol Chem* 229: 189, 1957
 69. Misra HP: Generation of superoxide free radical during the autoxidation of thiols. *J Biol Chem* 249: 2151, 1974
 70. Misra HP, Fridovich I: The generation of superoxide radical during the autoxidation of hemoglobin. *J Biol Chem* 247: 6960, 1972
 71. Mukai FH, Goldstein BD: Mutagenicity of malonaldehyde, a decomposition product of peroxidized polyunsaturated fatty acids. *Science* 191: 868, 1976
 72. Munthe E, Guldal G, Jellum E: Increased intracellular glutathione during penicillamine treatment for rheumatoid arthritis. *Lancet* 2:1126, 1979
 73. Munthe E, Jellum E, Aaseth J: Some aspect of the mechanism of action of penicillamine in rheumatoid arthritis. *Scand J Rheumatol* 28: 6, 1979
 74. Nagata Ch, Yamaguchi T: Electronic structure of sulfur compounds and their protecting action against ionizing radiation. *Radiat Res* 73: 430, 1978
 75. Nothway WH Jr, Rosan RC, Porter DY: Pulmonary disease following respirator therapy of hyaline-membrane disease: Bronchopulmonary dysplasia. *N Engl J Med* 276: 357, 1976
 76. Oroszlán Gy, Lakatos L, Balázs M: D-penicillamine reduced lipid peroxidation of erythrocyte membranes. *J Pharmacol Exp Ther* (in press)
 77. Oroszlán Gy, Matkovics B, Lakatos L, Szabó L, Karmazsin L: unpublished data
 78. Oski FA, Barnes LA: Vitamin E deficiency: A previously unrecognized cause of hemolytic anemia in the premature infant. *J Pediatr* 70: 211, 1967
 79. Owens WC, Owens EU: Retrolental fibroplasia in premature infants. *Am J Ophthalmol* 32:1, 1949
 80. Patz A, Eastham A, Higginbotham DH, Kleh T: Studies on the effect of high oxygen administration in retrolental fibroplasia. I. Nursery observations. *Am J Ophthalmol* 35:1248, 1952
 81. Pederson TC, Aust SD: NADPH-dependent lipid peroxidation catalyzed by purified NADPH-cytochrome C reductase from rat liver microsomes. *Biochem Biophys Res Commun* 48: 789 1972
 82. Phelps DL: Vitamin E: Where do we stand? *Pediatrics* 63: 933, 1979
 83. Phelps DL: Retinopathy of prematurity: an estimate of vision loss in the United States. *Pediatrics* 67: 924, 1981
 84. Reese AB, King M, Owens WC: Classification of retrolental fibroplasia. *Am J Ophthalmol* 36:1333, 1953

85. Reiss M, Roos D: Differences in oxygen metabolism of phagocytosing monocytes and neutrophils. *J Clin Invest* 61: 480, 1978
86. Robertson PH, Fridovich I: Does copper-D-penicillamine catalyze the dismutation of O_2^- ? *Arch Biochem Biophys* 203: 830, 1980
87. Saltzman HA, Fridovich I: Oxygen toxicity: Introduction to a protective enzyme: superoxide dismutase. *Circulation* 48: 921, 1973
88. Shaw JCL: Trace elements in the fetus and young infant. *Am J Dis Child* 134: 74, 1980
89. Silverman WA: *Retrolental Fibroplasia: A Modern Parable*. Grune and Stratton, New York 1980
90. Sinclair JC, Torrance GW, Boyle MH, Horwood SP, Saigal S, Sackett, DL: Evaluation of neonatal-intensive-care programs. *N Engl J Med* 305: 489, 1981
91. Singh A: Introduction: interconversion of singlet oxygen and related species. *Photochem Photobiol* 28: 429, 1978
92. Speck WT, Chen CC, Rosenkranz H: In vitro studies of effects of light and riboflavin on DNA and HeLa cells. *Pediatr Res* 9:150, 1975
93. Speck WT, Rosenkranz H: The bilirubin-induced photodegradation of deoxyribonucleic acid. *Pediatr Res* 9: 703, 1975
94. Stocks J Offerman EL, Modell CB, Dormandy TL: The susceptibility to autoxidation of human red cell lipids in health and disease. *Br J Haematol* 23: 713, 1972
95. Sullivan DW, Glader BE: Erythrocyte enzyme disorders in children. *Pediatr Clin North Am* 27: 449, 1980
96. Symposium on retrolental fibroplasia. *Ophthalmology (Rochester)* 86: 1685, 1979
97. Tapeel AL: Lipid peroxidation damage to cell components. *Fed Proc* 32: 1870, 1973
98. Terry TL: Extreme prematurity and fibroplastic overgrowth of persistent vascular sheath behind each crystalline lens. I. Preliminary report. *Am J Ophthalmol* 25: 203, 1942
99. Tien M, Svingen BA, Aust SD: Superoxide dependent lipid peroxidation. *Fed Proc* 40:179, 1981
100. Weisiger RA, Fridovich I: Superoxide dismutase: organelle specificity. *J Biol Chem* 248: 3582, 1973
101. Weiss JF, Belkin M: The effect of penicillamine on posttraumatic vitreous proliferation. *Am J Ophthalmol* 92: 625, 1981
102. Weiter JF: Retrolental fibroplasia: an unsolved problem. *N Engl J. Med* 305:1404, 1981
103. Wender DF, Thullin GE, Warshaw JB: Vitamin E affects lung biochemical response to hyperoxia. *Pediatr Res* 13: 375, 1979
104. Workshop on BPD. *J Pediatr* 95: 815, 1979
105. Wright SW, Filer LJ Jr Mason KE: Vitamin E blood levels in premature and full term infants. *Pediatrics* 7: 386, 1951
106. Yam J, Frank L, Roberts RJ: Age-related development of pulmonary antioxidant enzymes in the rat. *Pediatr Res* 12:115, 1978
107. Yoshioka T, Shimada T, Sekiba K: Lipid peroxidation and antioxidants in the rat lung during development. *Biol Neonate* 38: 161, 1980

Received March 11, 1982

G OROSZLÁN MD

Pf 32

H-4012 Debrecen, Hungary