On the etiopathogenesis and therapy of Down syndrome

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ABSTRACT: The etiopathogenesis of Down syndrome is reviewed concentrating on the possible consequences of over-expression of cytoplasmic superoxide dismutase gene located in chromosome 21. Increased superoxide dismutase activity may generate free radical stress through overproduction of hydrogen peroxide. The significance of inadequate adaptive responses, i.e. increase of the selenoenzyme glutathione peroxidase activity in the central nervous system and in the thyroid gland is discussed.

Suggestions are made for prevention of the progress of Down syndrome and intervention studies with antioxidant supplementation are proposed.

KEY WORDS: *Down syndrome, free radicals, antioxidants, etiopathogenesis, therapy*

Introduction

In spite of the intensified antenatal diagnostics and termination of affected pregnancies, the prevalence of Down syndrome (DS), first described in 1866, is still so high that it constitutes an important health care problem.

DS phenotype is readily recognized at birth. Karyotype analysis confirms the diagnosis of DS associated with trisomy of chromosome 21. Since the further development of the child is predestined to be hampered by a multitude of clinical symptoms including mental retardation, premature aging, and immunological disorders such as hypothyroid states, attempts have been made to increase the understanding of the etiopathogenesis of the syndrome and to influence its progress.

The genetic imbalance due to an extra set of normal genes located in chromosome 21 means that the expression of these, or actually of only some genes in the q arm, leads to the disturbed development. Cumulative effects of increased amounts of primary gene products may be deleterious if no compensatory mechanisms exist. Genes of the q arm in chromosome 21, which may contribute to the DS pathology, include alpha/beta interferon receptor, phosphoribosylglycinamide synthetase (cf. McCoy and Sneddon, 1983; Epstein *et al.*, 1985), cystathionine beta synthase (Chadefaux *et al.*, 1985), and cytoplasmic Cu/Zn-superoxide dismutase (SOD-I, Sinet, 1982).

DS, described in a karyotypically normal 18-month-old boy, has been explained by a microduplication of a chromosome 21 fragment (not exceeding 2000-3000 kilobase pairs) containing the SOD-1 gene (Huret *et al.*, 1987). Further evidence of the involvement of additional SOD-1 gene in the neuropathological symptoms of DS has been derived from studies performed with transgenic cell lines and from mice carrying the human SOD gene (Avraham *et al.*, 1988; Elroy-Stein and Groner, 1988).

Consistent with the gene dosage effect, SOD-1 activity is increased in the cerebral cortex of DS fetuses as well as in erythrocytes, blood platelets, leukocytes and fibroblasts of DS patients (cf. Kedziora and Bartosz, 1988). According to Sinet (1982), elevated SOD-1 activity may constitute an oxygen free radical 'stress'. Normally SOD-1 protects cells from the harmful effects of oxygen radicals by catalyzing formation of H_2O_2 from O_{-2}. Enzymes that remove excessive peroxides are catalase and glutathione peroxidase (GSHPx). NADPH is needed for the regeneration by glutathione reductase of glutathione (GSH), the utilization of which is increased via GSHPx as a defence against peroxide formation. Thus several enzymes with structural loci other than those of chromosome 21, including glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase, show elevated activity in erythrocytes of Down syndrome patients (cf. Francke, 1981).

Balazs and Brooksbank (1985) noticed that the adaptive response to elevated SOD-1 activity, i.e. increased GSHPx activity found in other tissues, is not detected in fetal DS brain. The level of GSHPx activity in neural tissues seems to be as such too low to provide protection from peroxide-induced lesions (DeMarchena *et al.*, 1974). Furthermore, if H_2O_2 production is high, catalase might be a superior scavenger, because restoration of glutathione becomes a limiting factor for the activity of GSHPx (Brawn and Fridovich, 1980). However, catalase level is normal in DS erythrocytes (Mattei *et al.*, 1982; cf. McCoy and Sneddon, 1983), and its activity is practically absent in brain tissue (Sailer, 1969). Consequently brain cells may be extremely susceptible to oxygen-free radicals.

To unravel the potential pathological sequellae of free radical stress in Down syndrome, we concentrate here on the etiopathogenesis of brain and thyroid disorders (see Table 1).

Free radicals in the brain

Unlike many other tissues, brain is a highly aerobic and totally oxygen-dependent tissue. Oxygen reduction produces reactive radical intermediates, such as superoxide and hydroxyl radical which are thought to be major agents of oxygen toxicity. Hydrogen peroxide is formed through dismutation of superoxide anion catalyzed by Cu-Zn and Mn forms of SOD, both found in the central nervous system. In addition to Cu-Zn SOD (SOD-1), the activity of which is increased in DS, hydrogen peroxide is generated in association with D- and L- amino acid oxidase, monoamine oxidase, α-hydroxyacid oxidase, xanthine oxidase, and cytochrome P-450 system.

Unlike charged oxygen radicals, H_2O_2, being rather unreactive and stable, rapidly crosses cell membranes. Cellular damage is accomplished when H_2O_2 decomposes to the highly reactive hydroxyl radical in reactions catalyzed by iron (II) or copper (I). If the scavenging of H_2O_2 and the contemporaneous prevention of hydroxyl radical formation does not take place, the hydroxyl radical may attack e.g. fatty acid side chains and start a chain reaction of lipid peroxidation. Lipid peroxidation causes gradual

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loss of membrane fluidity and membrane potential and increases membrane permeability to ions (cf. Gutteridge *et al.*, 1985; Halliwell and Gutteridge, 1985b). Abnormalities in the fatty acid composition found in fetal DS brain phospholipids suggest that from the early stage of ontogenesis lipoperoxidation may have pathological significance (Brooksbank and Balazs, 1984; Balazs and Brooksbank, 1985). Oxidative degradation and polymerization of lipids lead to the accumulation of lipofuscin, the age pigment. Current evidence permits the interpretation that a high proportion of DS subjects later develop the neuropathological changes associated with Alzheimer's disease (Mann, 1988).

Radical attack may also destroy membrane-bound enzymes and receptors, e.g. the binding of serotonin is decreased. However, the brain tends to need radical reactions for the generation of physiological responses. Cellular redox adjustments generally regulate functional sulfhydryl groups of proteins.

There is evidence supporting the suggestion that free radical intermediates are involved e.g. in the coupling between depolarization of the plasma membrane Ca^{2+} fluxes and neurotransmitter release (Zoccarato *et al.*, 1987). It has also been hypothesized that the elimination of neurofilaments at nerve terminals is regulated by oxygen radicals, and that a contact of a growing neurite with a neighboring neuron induces the production of oxygen radicals. Rapid elimination of oxygen radicals during synaptogenesis would result in a reduced number of synaptic connections (Elomaa and Virtanen, 1985). Accordingly, transgenic mice bearing the human SOD gene develop abnormalities in neuro-muscular junctions of the tongue which closely resemble those of DS patients (Avraham *et al.*, 1988). However, these abnormalities may be explained by a decreased level of superoxide anion as well as by increased formation of highly active and toxic hydroxyl radicals and singlet oxygen.

Phorbol esters, strong superoxide producers through NADPH oxidase activation (DeChatelet *et al.*, 1976; Suzuki and Lehrer, 1980), induce biosynthesis of polyamines (O'Brien *et al.*, 1975). The role of polyamines is also associated with the architectural modeling of brain regions and generation of synaptic connections (Slotkin and Bartolome, 1986). Thus effective scavenging of superoxide by excess SOD-1 may contribute even to the polyamine biosynthesis.

In addition to the complex enzyme systems, biochemical defenses include low molecular weight free radical scavengers, antioxidants. Lipid soluble vitamin E lowers the steady state concentrations of many free radical species (Nishikimi *et al.*, 1980). Ubiquinone may exert, similarly to vitamin E, a protective effect against lipid peroxidation (Ernster, 1984). The concentration of vitamin E in fetal DS brain does not significantly differ from that of controls (Metcalfe *et al.*, 1984). We have found the serum vitamin E concentrations of DS patients to be normal ($1.01+0.35$ $n=7$ vs. $1.13+0.39$ mg/100ml $n=13$, Antila *et al.*, 1988b). In addition to conventional antioxidant systems, brain has been found to contain specific or high endogenous levels of free radical scavengers such as dopamine, norepinephrine (Misra and Fridovich, 1972), catechol estrogens (Nakano *et al.*, 1987), carnosine (Kohen *et al.*, 1988) and taurine (Oja and Kontro, 1983; Pasantes-Morales *et al.*, 1985). The transport of taurine to platelets is impaired in DS (Boullin *et al.*, 1975). Taurine and hypotaurine, found in high concentrations in the brain (Perry *et al.*, 1971), could act as intracellular superoxide scavengers which would not only inhibit lipid peroxidation but also inactivation of SOD by both superoxide and H_2O_2 (Sinet and Garber, 1981; Alvarez and Storey, 1983). The presence of 1-10mM taurine protects cultured lymphoblastoid cells from the injurious effect of iron-ascorbate (Pasantes-Morales *et al.*, 1985).

The role of dietary betacarotene in the central nervous system is not elucidated. Betacarotene acts as an antioxidant at low oxygen pressures (Burton and Ingold, 1984). Being converted to retinol or retinoic acid it causes a marked decrease in superoxide generation. Through a still unknown mechanism retinoic acid induces human neuroblastoma cell differentiation *in vitro* (Sidell *et al.*, 1986), and it also contributes to neural differentiation of embryonal carcinoma cells *in vitro* (Jetten *et al.*, 1986).

Serum betacarotene and vitamin A levels are generally normal in DS, although vitamin A levels lower than in normal subjects have also been reported. Relatively high carotene/vitamin A ratio suggests decreased efficiency in converting carotene to vitamin A. In addition the utilization of vitamin A in DS may be impaired at its site of action (Barden, 1977).

Thyroid hormones improve the cleaving of betacarotene to vitamin A. This conversion to retinol is decreased in hypothyroidism. Thus, in hypothyroidism the vitamin A concentration decreases even when the dietary betacarotene remains the same or rises (carotinemia, Mandal and Dastidar, 1985).

Integration of the thyroid gland into the etiopathogenesis of DS

Thyroid hormone has a regulatory function on mitosis and differentiation of neural cells, and it is hence intimately involved in normal brain maturation (Bass *et al.*, 1977). Experimentally trijodothyronine enhances neural outgrowth *in vitro* (Romijn *et al.*, 1982). Even subclinical hypothyreosis during the postnatal period may contribute to the delayed and incomplete maturation of the cerebral and cerebellar cortices.

Increased prevalence of thyroid dysfunction is associated with DS in infants as well as in older children and adults (cf. Fort *et al.*, 1984; Pueschel and Pezzullo, 1985). Hypothyroidism is reported in 17 to 50% of the DS patients studied depending on the age and sex distribution of the population (cf. Pueschel and Pezzullo, 1985). The relatively high incidence of autoimmune thyroiditis suggests that impaired immune surveillance is the primary mechanism in hypothyroidism. Pueschel and Pezullo (1985) conducted a study which showed that approximately 28% of their 151 DS patients had elevated antimicrosomal antibodies. They found a highly significant correlation of antimicrosomal antibodies to T_4 (-.324) and to TSH (.388). Recent studies indicate that antimicrosomal antibodies are mainly, if not exclusively, against thyroid peroxidase (Ruf *et al.*, 1987).

Thyroid peroxidase is a membrane-bound enzyme associated with the endoplasmic reticulum and the apical membrane of the thyroid cell. Immunoelectron microscopical observations of the thyroid microsomal antigen in the apical plasma membrane are compatible with the notion that microsomal antigen is identical with thyroid peroxidase (Nilsson *et al.*, 1987).

SOD-1 system, which generates H_2O_2, is essential in the iodination and coupling reactions catalyzed by thyroid peroxidase (cf. Nuñez and Pommier, 1982).

However, an excess of H_2O_2 may inactivate the peroxidase complex (Keilin and Hartree, 1951; cf. Nuñez and Pommier, 1982). The irreversible loss of catalytic activity, caused by H_2O_2 or by the reactive oxygen species generated, may result from oxidation of functionally important amino acid residues to car-

TABLE 1

FREE RADICAL-MEDIATED PATHOLOGY DUE TO OVEREXPRESSION OF CYTOPLASMIC Cu/Zn SUPEROXIDE DISMUTASE

TISSUE AND ORGANISMIC LEVEL	SUBCELLULAR LEVEL	BIOCHEMICAL LEVEL
impaired synapto-genesis	elimination of neurofilaments	decrease of superoxide anion
neuromuscular junction abnormality, terminal axon atrophy	degenerative structures	decrease of poly-amine synthesis?
	impaired uptake of biogenic amines	overproduction of H_2O_2
loss of coordination	loss of membrane fluidity and membrane potential	hydroxyl radical formation catalyzed by Fe^{2+} and Cu^{+1}
accelerated aging	lipofuscin accumulation	lipoperoxidation
autoimmune thyroiditis leading to hypo-thyroidism	expression of HLA-DR antigen on thyrocytes anti-gen presentation	inactivation and proteolysis of thyroid peroxidase?

See the text for details and references. Hypothetical consequences indicated by a question mark. Consequences considered to be adaptive are not included.

bonyl derivatives. This may also render the protein susceptible to proteolytic attack and to detachment of its cellular compartment. If so, the maintenance of the thyroid peroxidase function and its integrity would require correct steady state production of H_2O_2 and strict control of its level. This requirement is mainly met with by GSHPx and catalase.

Antigenity of the modified and disintegrated thyroid peroxidase should be recognized by helper lymphocytes only in the context of MHC Class II products coded by genes in the HLA-D region. This region, normally expressed only by a restricted variety of cell types, is found in autoimmune disorders in target cells. Thus an aberrant expression of HLA-DR antigen found on thyrocytes in Graves' and Hashimoto's disease indicates its potential importance in antigen presentation of thyroid autoimmune disorders.

The presence and intensity of DR expression in Graves' thyroids correlate positively with the titer of microsomal antibodies. Curiously, in cultured thyroid cells, plant lectins are able to induce the expression of HLA-DR by a mechanism unrelated to the known mitogenic effects. On the other hand, the inducing effect of gamma-interferon (IFN-γ) could have physiological significance (IFN-α and IFN-β did not induce Class II expression in thyrocytes, Pujol-Borrell and Todd, 1987).

Release of IFNs and lymphokines, best candidates for inducers, must be triggered by other factors.

On the basis of the gene dosage effect we suggest possible mechanisms for the sensitization of DS thyroids to autoimmunization:

— viral challenge including enhanced production of oxygen radicals due to macrophage and neutrophil activity may exert additional requirement for GSHPx and selenium. If these needs are not met with, overproduction of oxygen radicals and constitutive excess of both LFA-1 and its beta-chain may hinder normal immune response (Taylor, 1987).

— excess H_2O_2 causes fragmentation of thyroid peroxidase, which detaches from the cell membrane and turns into an autoantigen.

— increased expression of IFN-α / IFN-β receptors, gene of which is found in chromosome 21, may sensitize thyrocytes to the induction of HLA-DR antigen by γ-IFN.

Development of antioxidant therapy

In the past ten years, through intervention efforts, patients with DS have received medical treatment and stimulation of sensory, motor and cognitive areas (Pueschel, 1981). In 1981 Harrell *et al.* reported that the administration of a megavitamin-mineral supplement to a heterogenous group of 16 mentally retarded children, four of which had DS, had led to encouraging results. However, controlled double blind studies on somewhat larger groups using similar megadoses of vitamins and minerals but no thyroid supplementation did not result in any beneficial effects (Bennet *et al.*, 1983; Smith *et al.*, 1984). However, these studies were devoid of a detailed theory of the mechanism of action, and the age distribution of the patients was disadvantageous in relation to possible targets in the developing brains.

Our primary survey and theory of antioxidant therapy in DS (Antila *et al.* , 1988, 1989) rests on the present concepts of the etiopathogenesis of DS (Balazs and Brooksbank, 1985; Kedziora and Bartosz, 1988). Earlier, selected antioxidants have been used e.g. in the therapy of neuronal ceroid lipofuscinosis. The role of selenium as antioxidant in neurological disorders will be reviewed elsewhere (Westermarck and Antila, 1989).

Prevention of free radical damage should be executed by agents focused on the tissues, cellular compartments and processes where the generation of free radicals is critical.

In DS, elevated cytoplasmic SOD-1 activity causes free radical stress through H_2O_2. An excess of H_2O_2 may be activated in iron- or copper-catalyzed reactions to generate highly reactive hydroxyl radical (·OH) or singlet oxygen. The extent and nature of the damage depends on the precise site of the ·OH production, which in turn depends on the intra- or extra-cellular location of the critical metal ions (Halliwell and Gutteridge, 1984; 1985a).

However, GSHPx, which gives protection against elevated H_2O_2, is low in brains with intensive oxygen metabolism. Histochemical studies by Slivka and co-workers (1987) are indicative of the relative absence of GSH in neuronal somata and locate GSH to non-neuronal elements and fibrous and terminal regions of neurons. Furthermore, in normal newborn infants the activity of GSHPx is physiologically low (Miller, 1972). Just a reflection of this condition could be the low blood selenium level found in neonates (Westermarck *et al.*, 1977). Controversial results have been published on the level of selenium in plasma and erythrocytes of DS patients (cf. Kedziora and Bartosz, 1988).

Peripherally decreased GSHPx activity may be corrected by selenium supplementation (Westermarck and Sandholm, 1977; Thompson *et al.*, 1982). Unlike catalase, benefit of optimal GSHPx activity is gained through its capability to reduce both H_2O_2 and organic hydroperoxides, including lipid peroxides.

The mean plasma selenium concentration in DS patients has

been shown not to differ significantly from that of the control subjects although the GSHPx activity is 130% of the normal (cf. Anneren, 1984). Néve and co-workers (1983) reported normal erythrocyte but significantly decreased plasma selenium levels in 29 DS patients. These discrepancies may be explained by the differences in the population groups studied and by the distribution of the blood selenium pool. Only 10 to 15% of the erythrocyte selenium in man is reported to be incorporated into the GSHPx, whereas the corresponding value in rat is 75 to 85% (Beilstein *et al.*, 1984).

We noticed in DS patients of different ages that the mean compensatory increase of erythrocyte GSHPx was lower than expected, 33.4% (n=29) vs 50%. The finding that the erythrocyte SOD/GSHPx ratio was higher than in healthy controls confirmed our belief of insufficient compensation (Antila *et al.*, 1988, 1989).

In DS the whole body retention of 5-8 kBq ^{75}Se-sodium selenite, with 0. 4 ug Se as carrier/kg b.w., is estimated to be 53.3±21.1% (n=10). Stable selenium supplementation increased ^{75}Se elimination indicating a saturated selenium pool in the body (Westermarck *et al.*, 1985). Selenium supplementation 0.025 mg Se/kg/d in the form of sodium selenite increased E-GSHPx activity by 28% (59.9% above normal). This was also demonstrated by the 23.9% decrease of SOD/GSHPx ratio ($P < 0.01$, n=7) (Antila *et al.*, 1988, 1989).

The consequences of selenium supplementation on the brain antioxidant balance, and thus its therapeutic value, are difficult to monitor. However, certain clinical, experimental and *in vitro* observations may be indicative.

A highly positive correlation has been reported between erythrocyte GSHPx-values and IQ in DS (Sinet *et al.*, 1979). The plasma selenium concentration and erythrocyte GSHPx activity was found to be higher in DS girls than in DS boys, which is consistent with the finding of significantly higher IQ scores for female than for male DS patients. The positive correlation of E-GSHPx activity between DS subjects and their siblings suggests the influence of environmental and/or additional genetic factors (Anneren, 1984).

Estimates of the amount of selenium in the rat brain indicate that the GSHPx may account for only 1/5 of the total selenium in the brain (Prohaska and Ganther, 1976).The finding that selenoproteins other than GSHPx are distributed mainly in the brain and endocrine organs raises a question of their physiological role. After the administration of very small amounts of selenium, severely depleted rats retained in the brain and in the reproductive and several endocrine organs (including thyroid) a dose which was 20-50 times the dose found in adequately-fed control animals. This indicates the existence of regulatory mechanisms which ensure a sufficient level of selenium in critical organs, above all in the brain and the thyroid even during depletion (Behne *et al.*, 1988).

In concentrations of 6×10^{-7} to $\times 10^{-6}$M, selenite induces a 30-fold increase of GSHPx activity in neuroblast cells *in vitro* (Germain and Arneson, 1977). Recent studies on the rat liver suggest that selenium regulates the level of GSHPx-mRNA as well as GSHPx protein concentration and GSHPx activity (Saedi *et al.*, 1988). Concentrations of 10^{-5}M exert obvious toxic effects on nerve fibers *in vitro* (Lindner and Grosse, 1985).

Trace elements, as «doping impurities» in organic material, could be key variables that regulate conductivity in biological semiconduction structures (Parantainen and Atroshi, 1988). We suggest that the abnormally high levels of copper and iron and the low level of zinc ion in erythrocytes and blood mononuclear cells are reflections of disturbed oxygen radical metabolism in DS. However, increased concentration of copper can be explained as gene dosage evident by the increased activity of SOD-1 (Johansson *et al.*, 1989). Except for the decreased iron content in erythrocytes, the results were in accordance with an earlier study (Anneren *et al.*, 1985). Ferrous-ion (Fe II) and copper-ion (Cu I) react with H_2O_2 producing ·OH radicals. Titanium in erythrocytes may indicate insufficient protection by GSHPx. If titanium is present as Ti (IV), stable compounds may be formed with hydrogenperoxides and probably with superoxide anions to give 1:1 adducts (Schwarzenbach, 1970; cf. Johansson *et al.*, 1989). The low plasma zinc concentration in DS children has been recognized in earlier studies as well (Halsted *et al.*, 1970; Milunsky *et al.*, 1970). The homeostasy of zinc is regulated by the intestine. The absorption of zinc seems to be decreased in DS, mean retention of zinc being 30% (n=9) compared with 58% in healthy adults (n=4). Stable zinc supplementation in one DS patient did not increase the ^{65}Zn elimination indicating an unsaturated zinc pool (Westermarck *et al.*, 1985).

Interestingly, the primarily high blood mononuclear cell levels of copper decreased, whereas the concentration of iron and zinc was not affected during selenium supplementation. No significant alterations were observed in the erythrocyte concentrations of magnesium, calcium, iron, copper, zinc, sulphur, titanium and manganese. Aluminium was not found in erythrocytes nor in neutrophils of DS patients (Johansson *et al.*, 1989).

Future prospects

DS may be explained etiopathogenetically by constitutive overexpression of SOD-1. Permanent distortion of oxygen radical production, ultimately involved in normal cellular metabolism, exposes the cells to free radical stress. Due to their availability, strong reactivity and accuracy, i.e. short half-life and short effective distance, free radicals/reactive oxygen metabolites are especially useful in the mediation of functionally important modifications in proteins. Therefore free radical damage comprises not only structural correlates but important mediators in signal transmission in differentiation, neurotransmission, and immunological processes.

Constitutive dismutation of superoxide anion leads to overproduction of hydrogenperoxide and hence to loss of balance needed for cellular regulation.

H_2O_2 reaches extra and intracellular compartments more easily than ordinary radicals. Therefore the damage caused by decomposition of H_2O_2 to hydroxyl radical in iron (Fe II) or copper (Cu I) ion catalyzed reactions expands. Peroxisomal catalase and cytoplasmic GSHPx can cope with the normal production of H_2O_2. The functional balance of SOD-1, catalase, and GSHPx is concomitantly influenced by a variety of factors, especially by H_2O_2. However, superabundant H_2O_2 may interact with infrequent elements such as titanium (Ti IV) to produce stable complexes or with free/low molecular weight chelates of iron or copper ions to generate hydroxyl radicals. Adaptive changes may maintain the integrity of cells for a limited time. Many of the pathological alterations in DS are in accordance with the oxygenic theory of aging and are associated with Alzheimer's disease as well. In contrast to Alzheimer's disease, the early onset of distortions in DS affects embryonic and postnatal neurogenesis and differentiation.

Recent developments in basic research have confirmed the relationship between etiopathogenesis and supplementation therapy with vitamins and trace elements. We have described the theoretical basis for antioxidant therapy in DS.

Supplementation therapy studies should be conducted, in addition to those with selenium and vitamin E, with zinc, vitamin A, B_2, B_6, betacarotene and ubiquinone. We need more clinical experience and a longer follow-up period with our DS patients receiving antioxidant therapy to reach more final conclusions concerning efficacy in the control of optimal development.

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References

ALVAREZ, J.G. and STOREY, B.T. (1983). Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. *Biol. Reprod. 29:* 548-555.

ANNEREN, G. (1984). Down's syndrome. A metabolic and endocrinological study. Acta Univ. Upps., 483., Uppsala.

ANNEREN, G., JOHANSSON, E. and LINDH, U. (1985). Trace elemental profiles in individual blood cells from patients with Down's syndrome. *Acta Paediatr. Scand. 74:* 259-263.

ANTILA, E., NORDBERG, U-R. and WESTERMARCK, T. (1988a). Antioxidant therapy in Down syndrome (DS). A theory and primary survey. In *Selenium in Biology and Medicine. Fourth International Symposium. July 18-21, 1988,* University of Tübingen, W-Germany. (Abstr.).

ANTILA, E., NORDBERG, U-R., SYVAOSA, E-L. and WESTERMARCK, T. (1989). Selenium therapy in Down syndrome (DS). A theory and a clinical trial. In *Antioxidants in Therapy and Preventive Medicine* (Eds. I. Emerit, L. Packer and C. Auclair). Plenum Publ. Co., New York. *In press.*

AVRAHAM, K.B., SCHICKLER, M., SAPOZNIKOV, D., YAROM, R. and GRONER, Y. (1988). Down's syndrome: abnormal neuromuscular junction in tongue of transgenic mice with elevated levels of human Cu/Zn-superoxide dismutase. *Cell 54:* 823-829.

BALAZS, R. and BROOKSBANK, B.W.L. (1985). Neurochemical approaches to the pathogenesis of Down's syndrome. *J. Ment. Defic. Res. 29:* 1-14.

BARDEN, H.S. (1977). Vitamin A and carotene values of institutionalised mentally retarded subjects with and without Down's syndrome. *J. Ment. Defic. Res. 21:* 63-74.

BASS, N.H., PELTON, E.W. and YOUNG, E. (1977). Defective maturation of cerebral cortex: an inevitable consequence of dysthyroid states during early postnatal life. In *Thyroid Hormones and Brain Development* (Ed. G.D. Grave). Raven Press, New York, pp. 199-214.

BEHNE, D., HILMERT, H., SCHEID, S., GESSNER, H. and ELGER, W. (1988). Evidence for specific selenium target tissues and new biologically important selenoproteins. *Biochim. Biophys. Acta 966:* 12-21.

BEILSTEIN, M.A., BUTLER, I.A. and WHANGER, P.D. (1984). Metabolism of ^{75}Se-selenite by rhesus monkeys. In *Handbook on the Toxicology of Metals* (Eds. L. Friberg, G. Nordberg,G.F. Folke and V. Vouk). Elsevier Science Publ. B. V., pp. 482-520.

BENNET, F.C., MCCLELLAND, S. KRIEGSMANN, E.A., ANDRUS, L.B. and SELLS, C.J. (1983). Vitamin and mineral supplementation in Down's syndrome. *Pediatrics 72:* 707-713.

BOULLIN, D.J., AIRAKSINEN, E.M. and PAASONEN, M. (1975). Platelet taurine in Down's syndrome. *Med. Biol. 53:* 184-186.

BRAWN, K. and FRIDOVICH, I. (1980). Superoxide radical and superoxide dismutases; threat and defence. *Acta Physiol. Scand. 492 (Supp):* 9-18.

BROOKSBANK, B.W.L. and BALAZS, R (1984). Superoxide dismutase and lipoperoxidation Down's syndrome fetal brain. *Dev. Brain. Res. 16:* 37-44.

BURTON, G.W. and INGOLD, K.U. (1984). β-carotene: an unusual type of lipid antioxidant. *Science 224:* 569-573.

CHADEFAUX, B., RETHORE, M.O., RAOUL, O., CEBALLOS, I., POISSONNIER, M., GILGENKRANZ, S. and ALLARD, D. (1985). Cystathionine beta synthase: gene dosage effect in trisomy 21. *Biochem. Bioph. Res. Co. 128:* 40-44.

DECHATELET, L.R., SHIRLEY, P.S., and JOHNSTON, R.B. (1976). Effect of phorbol myristate acetate on the oxidative metabolism of human polymorphonuclear leukocytes. *Blood. 47:* 545-5543.

DEMARCHENA, O., GUARNIERI, M., and MCKHANN, G. (1974). Glutathione peroxidase levels in brain. *J. Neurochem. 22:* 773-776.

ELOMAA, E. and VIRTANEN, I. (1985). Is the mental deterioration in Down's syndrome linked to impaired terminal degradation of neurofilaments? *Med. Hypotheses 16:* 171-172.

ELROY-STEIN, O. and GRONER, Y. (1988). Impaired neurotransmitter uptake in PC12 cells overexpressing human Cu/Zn-superoxide dismutase -- implication for gene dosage effects in Down syndrome. *Cell 52:* 259-267.

EPSTEIN, C.J., COX, D.R. and EPSTEIN, L.B. (1985). Mouse trisomy 16: an animal model of human trisomy 21 (Down syndrome). *Ann. N Y Acad. Sci. 450:* 157-168.

ERNSTER, L. (1984). Ubiquinone: redox coenzyme, hydrogen carrier, antioxidant. In *Biomedical and Clinical Aspects of Coenzyme Q, Vol. 4* (Eds. K. Folkers and Y. Yamamura). Elsevier Science Publ. B.V., pp. 3-13.

FORT, P., LIFSHITZ, F., BELLISARO, R., DAVIS, J., LANES, R., PUGLIESE, M., RICHMAN, R., POST, E.M. and DAVID, R. (1984). Abnormalities of thyroid function in infants with Down syndrome. *J. Pediatr. 104:* 545-549.

FRANCKE, U. (1981). Gene dosage studies in Down syndrome. A review. In *Trisomy 21 (Down syndrome)* (Eds. F.F. de la Cruz and P.S. Gerald). University Park Press, Baltimore, pp. 237-251.

GERMAIN, G.S. and ARNESON, R.M. (1977). Selenium induced glutathione peroxidase activity in mouse neuroblastoma cells. *Biochem. Biophys. Res. Commun. 79:* 119-123.

GUTTERIDGE, M.C., WESTERMARCK, T. and HALLIWELL, B. (1985). Oxygen radical damage in biological systems. In *Free Radicals, Aging and Degenerative Diseases* (Eds. J.E. Johnson, Jr., R. Walford, D. Harman and J. Miquel). Alan R. Liss, New York, pp. 99-139.

HALLIWELL, B. and GUTTERIDGE, M.C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J. 219:* 1-14.

HALLIWELL, B. and GUTTERIDGE, M.C. (1985a). The role of transition metals in superoxide mediated toxicity. In *Superoxide Dismutase. Vol. III* (Ed. L.W. Oberley). CRC Press Inc., Boca Raton, Florida, pp. 46-82.

HALLIWELL, B. and GUTTERIDGE, M.C. (1985b). Oxygen radicals and the nervous system. *TINS 8:* 22-26.

HALSTED, J.A. and SMITH, J.C., Jr. (1970). Plasma-zinc in health and disease. *Lancet 1:* 322-324.

HARRELL, R.F., CAPP, R.H., DAVIS, D.R., PEERLESS, J. and RAVITZ, L.R. (1981). Can nutritional supplements help mentally retarded children? An exploratory study. *Proc. Natl. Acad. Sci. 78:* 574-578.

HURET, J.L., DELABAR, J.M., MARLHENS, F., AURIAS, A., NICOLE, A., BERTHIER, M., TANZER, J. and SINET, P.M. (1987). Down syndrome with duplication of a region of chromosome 21 containing the CuZn superoxide dismutase gene without detectable karyotypic abnormality. *Hum. Genet. 75:* 251-257.

JETTEN, A.M. (1986). Induction of differentiation of embryonal carcinoma cells by retinoids. In *Retinoids and Cell Differentiation* (Ed. M.I. Sherman). CRC Press Inc., Boca Raton, Florida, pp. 105-136.

JOHANSSON, E., LINDH, U., WESTERMARCH, T., ANTILA, E., NORDBERG, U.R. and NIKKINEN, P. (1989). Deviation of elements and enzymes in blood cells in Down's syndrome (trisomy 21). *Biol. Trace Elem. Res. In press.*

KEDZIORA, J. and BARTOSZ, G. (1988). Down's syndrome: A pathology involving the lack of balance of reactive oxygen species. *Free Rad. Biol. Med. 4:* 317-330.

KOHEN, R., YAMAMOTO, Y., CUNDY, K.C. and AMES, B.N. (1988). Antioxidant activity of carnosine, homocarnosine and anserine

present in muscle and brain. *Proc. Natl. Acad. Sci. 85:* 3175-3179.
LINDNER, G. and GROSSE, G. (1985). Über die Wirkung einer Selenverbindung (Na_2SeO_3) auf das Nervengewebe *in vitro*. *Z. Mikrosk. Anat. Forsch. 99:* 627-638.
MANDAL, S.K. and DASTIDAR, A.G. (1985). Carotene and retinol levels in the diagnosis of hypothyroidism. *JAPI 33:* 654-655.
MANN, D.M.A. (1988). Alzheimer's disease and Down's syndrome. *Histopathology 13:* 125-137.
MATTEI, J.F., BAETEMAN, M.A., BARET, A., ARDISSONE, J.P., REBUFFEL, P. and GIRAUD, F. (1982). Erythrocyte superoxide dismutase and redox enzymes in trisomy 21. *Acta Paediatr. Scand. 71:* 589-591.
McCOY, E.E. and SNEDDON, J.M. (1983). Cell biological aspects of Down's syndrome. In *Advances in Cellular Neurobiology, Vol. 4* (Eds. S. Fedoroff and L. Hertz). Academic Press, New York, pp. 249-266.
METCALFE, T., MULLER, D.P.R. and BROOKSBANK, B.W.L. (1984). Vitamin E concentration in brains from foetuses with Down's syndrome. *IRCS Med. Sci-Biochem. 12:* 121.
MILLER, D.S. (1972). Hereditary hemolytic anemias. *Pediatr. Clin. North Am. 19:* 865-887.
MILUNSKY, A., HACKLEY, M. and HALSTED, J.A. (1970). Plasma, erythrocyte and leucocyte zinc levels in Down's syndrome. *J. Ment. Defic. Res. 14:* 99-105.
MISRA, H.P. and FRIDOVICH, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem. 247:* 3170-3175.
NAKANO, M., SUGIOKA, K., NAITO, I., TAKEKOSHI, S. and NIKI, E. (1987). Novel and potent antioxidants on membrane phospholipid peroxidation: 2-hydroxyestrone and 2-hydroxyestradiol. *Biochem. Biophys. Res. Commun. 142:* 919-924.
NÈVE, J., SINET, P.M., MOLLE, L. and NICOLE, A. (1983). Selenium, zinc and copper in Down's syndrome (trisomy 21): blood levels and relations with glutathione peroxidase and superoxide dismutase. *Clin. Chim. Acta 133:* 209-214.
NÈVE, J., VERTONGEN, F., CAUCHIE, P., GNAT, D. and MOLLE, L. (1984). Selenium and glutathione peroxidase in plasma and erythrocytes of Down's syndrome (trisomy 21) patients. *J. Ment. Defic. Res. 28:* 261-268.
NILSSON, M., MÖLNE, J., KARLSSON, F.A. and ERICSON, L.E. (1987). Immunoelectron microscopic studies on the cell surface location of the thyroid microsomal antigen. *Mol. Cell. Endocrinol. 53:* 177-186.
NISHIKIMI, M., YAMADA, H. and YAGI, K. (1980). Oxidation by superoxide of tocopherol dispersed in aqueous media with deoxycholate. *Biochim. Biophys. Acta 627:* 101-108.
NUÑEZ, J. and POMMIER, J. (1982). Formation of thyroid hormones. In *Vitamins and Hormones. Advances in Research and Applications* (Eds. P.L. Munson and E. Diczfalusy). J. Glover & R.E. Olson, Academic Press, pp. 175-229.
O'BRIEN, T.G., SIMSIMAN, R.C. and BOUTWELL, R.K. (1975). Induction of the polyamine-biosynthetic enzymes in mouse epidermis by tumor-promoting agents. *Cancer Res. 35:* 1662.
OJA, S.S. and KONTRO, P. (1983). Taurine. In *Handbook of Neurochemistry* (Ed. A. Lajtha). Plenum Press 3, New York, pp. 501-533.
PARANTAINEN, J. and ATROSHI, F. (1988).Trace elements as possible keys to biological semiconduction. *Int. Conf. on Medical, Biochemical and Chemical Aspects of Free Radicals.* (Abstr.).
PASANTES-MORALES, H., WRIGHT, C.E. and GAULL, G.E. (1985). Taurine protection of lymphoblastoid cells from iron-ascorbate induced damage. *Biochem.Pharmacol. 34:* 2205-2207.
PERRY, T.L., HANSEN, S., BERRY, K., MOK, C. and LESK, D. (1971). Free amino acids and related compounds in biopsies of human brain. *J. Neurochem. 18:* 521-528.
PROHASKA, J.R. and GANTHER, H.E. (1976). Selenium and glutathione peroxidase in developing rat brain. *J. Neurochem 27:* 1379-1387.
PUESCHEL, S.M. (1981). Therapeutic approaches in Down syndrome. In *Trisomy 21 (Down syndrome)* (Eds. F.F. Delacruz and P.S. Gerald). University Park Press, Baltimore, pp. 217-224.
PUESCHEL, S.M. and PEZZULLO, J.C. (1985). Thyroid dysfunction in Down syndrome. *Am. J. Dis. Child. 139:* 636-639.
PUJOL-BORRELL, R. and TODD, I. (1987). Inappropriate HLA class II in autoimmunity: is it the primary event? In *Baillière Clinical Immunology and Allergy* (Eds. D. Doniach and G. F. Botazzo). Baillière Tindal, London, pp. 1-27.
ROMIJN, H.J., HABETS, A.M.M.C., HUD, M.T. and WOLTERS, P.S. (1982). Nerve outgrowth, synaptogenesis and bioelectric activity in fetal rat cerebral cortex tissue cultured in serum-free, chemically defined medium. *Dev. Brain Res. 2:* 583-589.
RUF, J., CZARNOCKA, B., DEMICCO, C., DUTOIT, C., FERRAND, M. and CARAYON, P. (1987). Thyroid peroxidase is the organ-specific «microsomal» autoantigen involved in the thyroid autoimmunity. *Acta Endocrinol. 281:* 49-56.
SAEDI, M. S., SMITH, C.G., FRAMPTON, J., CHAMBERS, I., HARRISON, P.R. and SUNDE, R.A. (1988). Effect of selenium status on mRNA levels for glutathione peroxidase in rat liver. *Biochem. Biophys. Res. Commun. 153:* 855-661.
SAILER, N. (1969). Enzymes. In *Handbook of Neuro-chemistry, Vol. 1* (Ed. A. Lajtha). Plenum Press, New York, pp. 325-468.
SIDELL, N., SARAFIAN, T., KELLY, M., TSUCHIDA, T. and HAUSLER, M. (1986). Retinoic acid-induced differentiation of human neuroblastoma: a cell variant system showing two distinct responses. *Exp. Cell Biol. 54:* 287-300.
SINET, P.M. (1982). Metabolism of oxygen derivatives in Down's syndrome. *Ann. N Y Acad. Sci. 396:* 83-94.
SINET, P.M. and GARBER, P. (1981). Inactivation of the human CuZn superoxide dismutase during exposure to O_2 and H_2O_2. *Arch. Biochem. Biophys. 212:* 411-416.
SINET, P.M., LEJEUNE, J. and JEROME, H. (1979). Trisomy 21 (Down's syndrome), glutathione peroxidase, hexose monophosphate shunt and IQ. *Life Sci. 24:* 29-34.
SLIVKA, A., MYTILINEOU, C. and COHEN, G. (1987). Histochemichal evaluation of glutathione in brain. *Brain Res. 409:* 275-284.
SLOTKIN, T.A. and BARTOLOME, J. (1986). Role of ornithine decarboxylase and the polyamines in nervous system development: a review. *Brain Res. Bull. 17:* 307-320.
SMITH, G.F., SPIKER, D., PETERSON, C.P., CICCHETTI, D. and JUSTINE, P. (1984). Use of megadoses of vitamins and minerals in Down syndrome. *J. Pediatr. 105:* 228-234.
SUZUKI, Y. and LEHRER, R.I. (1980). NAD(P)H oxidase activity in human neutrophils stimulated by phorbol myristate acetate. *J. Clin. Invest. 66:* 1409-1418.
TAYLOR, G.M. (1987). Altered expression of lymphocyte functional antigen in Down syndrome. *Immunol. Today 8:* 366-369.
THOMPSON, C.D., ROBINSON, M.F., CAMPBELL, D.R. and REA, H.M. (1982). Effect of prolonged supplementation with daily supplements of selenomethionine and sodium selenite on glutathione peroxidase activity in blood of New Zealand residents. *Am. J. Clin. Nutr. 36:* 24-31.
WESTERMARCK, T. and ANTILA, E. (1989). Selenium in CNS diseases. In *Selenium and its Derivatives: from Chemistry to Therapy* (Eds. A. Bast and M.J. Parnham). Marcel Dekker, Inc., New York, N.Y. *In press.*
WESTERMARCK, T., RAHOLA, T., SUOMELA, M., ALANEN, T. and PUHAKAINEN, M. (1985). Biological half-life of ^{75}Se-selenite and ^{65}Zn-chloride in syndroma Down patients. In *Trace Elements in Man and Animals -- Tema 5. Proceedings of the Fifth International Symposium on Trace Elements in Man and Animals* (Eds. C.F. Mills, I. Bremner and J.K. Chesters). Commonwealth Agricultural Bureaux, pp. 792-795.
WESTERMARCK, T., RAUNU, P., KIRJARINTA, M. and LAPPALAINEN, L. (1977). Selenium content of whole blood and serum in adults and children of different ages from different parts of Finland. *Acta Pharmacol. Tox. 40:* 465-475.
WESTERMARCK, T. and SANDHOLM, M. (1977). Decreased erythrocyte glutathione peroxidase activity in neuronal ceroid lipofuscinosis (NCL)-corrected with selenium supplementation. *Acta Pharmacol. Tox. 40:* 70-74.
ZOCCARATO, F., PANDOLFO, M., DEANA, R. and ALEXANDRE, A. (1987). Inhibition by some phenolic antioxidants of Ca^{2+} uptake and neurotransmitter release from brain synaptosomes. *Biochem. Biophys. Res. Commun. 146:* 603-610.