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EDITORIAL REVIEW

Oxidative Stress in Childhood Malnutrition and Diarrhoeal Diseases

Oxidative stress as induced by oxygen free radicals has been implicated in the pathogenesis of many human diseases in recent years (1). Oxygen free radicals are highly unstable atoms or molecules or chemical species. In addition to oxygen free radicals there are varieties of radicals of carbon, nitrogen and sulphur that can cause oxidative stress. All these radicals possess one or more unpaired electron(s) which make them unstable and highly reactive. While oxygen free radicals are produced from the metabolism of oxygen derived exogenously, nitrite and thiyl radicals are formed endogenously. Although atmospheric oxygen is not toxic in the sense of its oxidative action with biologic molecules, Priestley more than 200 years ago (in 1771) stated that "pure oxygen has adverse effects on the healthy state of the body". So it is not surprising that our own metabolism produces free radicals even during healthy conditions. It is however surprising to find that these toxic species are also very useful in immunological defense mechanisms. Many diseases including cancer, diabetes, AIDS and even the process of ageing are all found to be intimately associated with varying grades of oxidative stress in the human body. Considerable literature dealing with the studies of oxidative stress in human has already appeared (2-5). Although malnutrition is an underlying cause of many infectious diseases, very little effort has been made to evaluate the role of oxidative stress in the infectious diseases of malnourished children, particularly the diarrhoea diseases. In this review an attempt has therefore been made to put together some evidences to implicate the role of oxidative stress in the pathogenesis of diarrhoea diseases of malnourished children.

OXIDATIVE STRESS

Every aerobic organism requires oxygen to sustain life. While most of the inhaled oxygen undergoes reduction to water during the normal course of metabolism, some of them are also converted to reactive oxygen species (ROS). ROS are capable of causing damage to cell membranes and altering subcellular organelle structure and their functional integrity (6,7). The electronic composition of these ROS is shown in Table I.

From their electronic orbital properties shown in Table I it can be seen that the superoxide, $\cdot O_2^-$ and hydroxyl ion, $\cdot OH$, are the most reactive species because of their lone pair electron in the outer orbit (as indicated by half arrows in Table I). These ROS may be produced by many exogenous factors, including ionizing radiation, heat, sunlight, drugs etc., and by many endogenous factors such as mitochondrial respiration, oxidant enzymes, autooxidation and most importantly by the phagocytic and bactericidal activity of polymorphonuclear leukocytes (PMN) and the macrophages (8-10).

Table I. Electronic configuration of oxygen radicals

ROS	Electronic orbital		
	π		σ
Singlet O_2	$1\downarrow$		
Superoxide, $\cdot O_2^-$	$1\downarrow$	1	
Hydrogen peroxide, H_2O_2	$1\downarrow$	$1\downarrow$	
Hydroxyl ion, $\cdot OH$	$1\downarrow$	$1\downarrow$	1

The following reactions illustrate the biochemical pathways of formation of ROS from the inhaled molecular oxygen.

$$O_2 \xrightarrow{+e} \cdot O_2^-$$

$$\cdot O_2^- \xrightarrow{2H+e} H_2O_2$$

$$\cdot O_2^- + H_2O_2 \xrightarrow{Fe^{2+}} O_2 + \cdot OH + {}^-OH \text{ (Heber-Weiss or Fenton reaction)}$$

From these reactions it can be seen that the molecular oxygen (O_2) is reduced to the reactive superoxide ($\cdot O_2^-$) during the normal mitochondrial respiration. This superoxide in combination with two protons and one electron is transformed into hydrogen peroxide (H_2O_2). Later both $\cdot O_2^-$ and H_2O_2 reacted under normal physiologic condition and catalyzed by a transition metal ion like ferrous ion (Fe^{2+}) to produce highly reactive hydroxyl radical ($\cdot OH$). This is known as Heber-Weiss or Fenton reaction (11).

Although these ROS, i.e. $\cdot O_2^-$, H_2O_2 and $\cdot OH$, are difficult to detect and monitor *in vivo*, they can react extremely rapidly with the living cells initiating the process of chemical injury to the cell membranes (12). Three most common and dominant metabolic damages include: (i) oxidation of DNA bases (13), (ii) oxidative cross-linking of protein and inactivation of enzymes (14) and (iii) lipid

peroxidation (15). Lipid peroxidation has been proposed as the primary mechanism for cellular dysfunction and tissue injury (16-18). The ROS-induced lipid peroxidation proceeds in three steps:

(a) Initiation

In this step a polyunsaturated fatty acid (PUFA) is transformed into a radical by reacting with ROS that abstracts a hydrogen atom from PUFA as follow:

$$PUFA - H \xrightarrow{ROS} PUFA^{\cdot}$$

(b) Propagation

In the second step the $PUFA^{\cdot}$ reacts with a molecular oxygen to form a lipid peroxy radical i.e. $PUFA\text{-}OO^{\cdot}$ which in turn reacts with another PUFA to produce more $PUFA^{\cdot}$ and thus a chain of reactions takes place as presented below (19):

$$PUFA\text{--}OO^{\cdot} + PUFA\text{-}H \longrightarrow PUFA\text{-}OOH + PUFA^{\cdot}$$

The lipid hydroperoxide, PUFA-OOH, may be catalyzed by transitional metal ions, e.g. Fe and Cu, to give lipid alkoxy radicals ($PUFA\text{-}O^{\cdot}$) and lipid peroxy radicals ($PUFA\text{-}OO^{\cdot}$). Both of these radicals being formed very rapidly are capable of reacting with proteins, nucleic acids or another PUFA thus setting up a vicious cycle of lipid peroxidation (20).

(c) Termination

In the final phase, $PUFA^{\cdot}$ radicals formed can be decreased either by bond rearrangement to form dien conjugates, degradation products being malondialdehyde (MDA) and lipofuscin or catalyzed by metal in beta-scission reaction with PUFA-OOH to generate volatile hydrocarbons, such as ethane and pentane (21,22). These end products constitute the basis for the assessment of lipid peroxidation (23). The chain reactions can be terminated by many endogeneous antioxidant enzymes and exogeneous antioxidant nutrients (24-26).

Among the endogeneous antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) are notable. SOD, in the first place, dismutases the superoxide radicals as shown below (27):

$$\cdot O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The hydrogen peroxide (H_2O_2) thus formed is further reduced to water by the CAT (28). Glutathione (GSH), although a low molecular weight tripeptide, has a dual functions in its antioxidative activity as shown diagramatically in Figure 1 (29).

In addition to converting H_2O_2 into H_2O and O_2, the remarkable property of GSH lies in its ability to destroy the radical nature of lipid peroxide by transforming it into the hydroxy fatty acids (PUFA-OH). As shown in Figure 1 the selenium dependent glutathione peroxidase (GPX) first converts GSH to GSSG at the expense of H_2O_2. The oxidized GSH (GSSG) is reduced back to GSH by glutathione reductase (GRX) and at the same time metabolizes $PUFA\text{-}OO^{\cdot}$ into PUFA-OH (30), thus breaking the chain reaction.

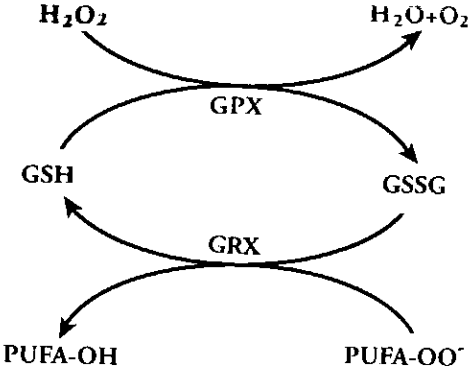

Fig. 1. Glutathione (GSH) antioxidative pathways

If initiation (step a) and propagation (step b) predominate over the chain-breaking process as in the step (c) described above, or in other word, if the oxidative process overwhelms the antioxidative protection, an oxidative stress is thereby created in a living organism. Such oxidative stress may very well be prevailing in malnourished children as exemplified below.

MALNOURISHED CHILDREN

During the normal course of metabolism body tissues oxidise nutrients to maintain growth, functions and most importantly cellular structure (31,32). Nutrients are divided into macro- and micronutrients. Among the macronutrients fat, protein and carbohydrates provide the body's fuels that generate energy for every aerobic life to live and function normally (33). Each of these macronutrients however has particular metabolic pathways, e.g. towards carbohydrate in cerebral tissues, fatty acids in cardiac muscle and amino acids in muscular tissue (34-36). An insufficient and/or disproportionate supply of these energy sources is liable to produce nutritional aberrations in the human body. For example, protein energy malnutrition (PEM), which is highly prevalent in the preschool children of the developing countries, is caused by the insufficient supply of energy from the protein sources (38-40).

The PEM children are classified either as marasmic or kwashiorkor or marasmic-kwashiorkor types (41). The state of undernutrition is invariably caused by the protein-energy deficiency with variable supply of other energy sources. For example, children with kwashiorkor are also known as

"sugar babies" since most of their energy supply comes from the carbohydrate sources (42,43). It has been proposed that if a weaning diet containing very low protein energy and very high carbohydrate energy is given to a malnourished child for a prolonged period, the child is likely to develop the signs of kwashiorkor (44). Pathophysiologically, a marasmic child can be differentiated from a kwashiorkor child by the presence of oedema or retention of body water in kwashiorkor (45). In marasmus, tissue content of visceral protein is preserved for longer time at the expense of skeletal muscle protein catabolism (46). Protein deficit diet leads to hypoalbuminemia which is the underlying cause for the manifestation of kwashiorkor along with oedema (47).

Albumin is present in human plasma which maintains osmotic pressure and transports a wide range of bioactive substances (48). Most importantly, albumin has a specific site for copper ion. By virtue of this property albumin is able to inhibit copper-stimulated peroxidation and hemolysis of erthrocyte membranes (49). Albumin-bound copper ions may still be accessible to $\cdot O_2^-$, H_2O_2 and ascorbate which are likely to produce $\cdot OH$ radicals. However, because of high concentration of plasma albumin these radicals are not released into free solution and thus albumin acts as an active extracellular antioxidant (49). In conjunction with other enzymatic antioxidants, such as SOD, CAT and GSH, albumin may therefore form an effective defense mechanism against the ROS. The synthesis of these essential antioxidative components requires adequate protein energy supply. The hypoalbuminemia in children with PEM could therefore be an activating factor in creating oxidative stress. In Table II the concentrations of these endogeneous antioxidants are listed as obtained from some previous publications on malnourished children (50-52).

Table II. Endogeneous antioxidant enzymes in malnourished children

Parameter	Kwashiorkor	Marasmus	Control
Albumin (g/l)	20.0	35.0	39.0
SOD (IU/g Hb)	3662	3382	3406
CAT (IU/g Hb)	13.2	13.5	12.9
GSH (mmol/l)	0.9	1.8	2.0
GPX (IU/g Hb)	20.0	30.0	30.0
TBARS* (mmol/l)	0.99	0.83	0.26

*TBARS is an index of lipid peroxidation

Based on the usual concentrations of plasma albumin in human which is about 40 g /l, the marasmic children are marginally hypoalbominic compared to the kwashiorkor group. While SOD and CAT are almost similar in all three groups (Table II), GSH and GPX are markedly lower in children with kwashiorkor. Determinations of blood thiobarbituric acid reacting substances (TBARS) which are marker for lipid peroxidation indicated that both marasmic and kwashiorkor children are suffering from oxidative stress, albeit higher in kwashiorkor. It is not yet clear why there is a marked decline of GSH in kwashiorkor while other enzymatic antioxidants i.e. SOD and CAT, are maintained in an almost similar level in kwashiorkor, marasmic and normal children.

Two recent publications may provide some probable explanations for the above mentioned observations, particularly the depletion of GSH. Ramdath and Golden (53) reported that there is a considerable elevation of glutathione S-transferase (GST) in malnourished children. GST is required in *in vivo* productions of leukotrienes (LT) from oxidative products of arachidonic acid via the 5-lipoxygenase system. This is depicted schematically in Figure 2.

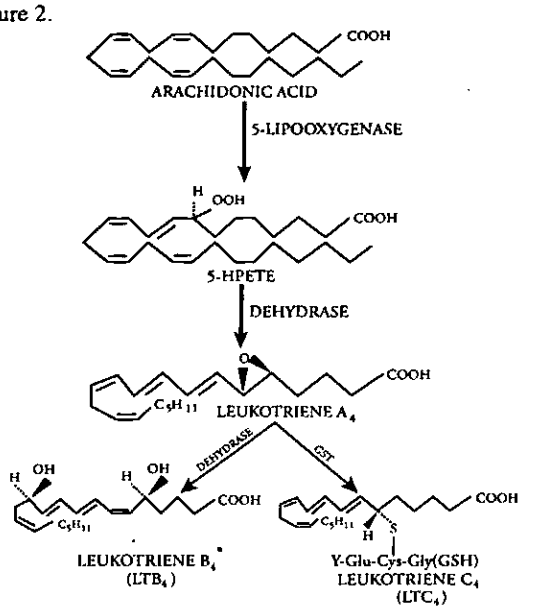

Fig. 2. Arachidonic acid pathways to produce Leukotrienes

One of the basic properties of cysteinyl LTs, i.e. LTC_4 and its metabolites, is to produce oedema in living tissues/cells (54). LTB_4 is an immunoregulator in that it causes adhesion and chemoattraction of phagocytes in an inflammatory response (55). Mayatepek *et al.* (52) recently found that LTC_4 and its metabolite LTE_4 excreted in the urine of kwashiorkor children are significantly higher than in the urine of marasmic or normal children. LTB_4 excretion, on the other hand, was found to be less in kwashiorkor than in marasmic or in normal children.

Oxidative stress may play a crucial role in the above mentioned situations by producing lipid peroxide 5-HPETE via 5-lipoxygenase (Fig. 2). The GSH, while forming cysteinyl-LTs, may create its own depletion and thus promote further lipid hydroperoxide production as shown in Figure 1. In malnutrition therefore particularly in kwashiorkor, limited availability of protein energy may be responsible for this vicious cycle of lipid peroxidation rendering a malnourished child prone to various opportunistic and inflammatory diseases. This contention is

also supported by a recent animal study (56) where it has been shown that a diet with insufficient protein and high polyunsaturated fat is highly capable of producing oxidative stress.

DIARRHOEA

In developing countries the most common childhood diseases are diarrhoea and respiratory tract infections which are often associated with malnutrition and result in death (32). The childhood diarrhoea, according to Roediger (33), could be classified as "starvational (malnutritional?) diarrhoea" and "infective diarrhoea". Before going into further discussions regarding these classes of diarrhoea it is important to elaborate here the possible mechanisms of involvement of oxidative stress in the gastrointestinal tract.

As mentioned earlier, the ROS can reversibly or irreversibly damage various kinds of biochemical substances thus influencing membrane fluidity and function, cellular metabolism and gene expression (19). For example, lipid peroxidation may disable membranes to maintain ionic gradients resulting an aberration in ion transport, particularly affecting potassium efflux and sodium/calcium influx. An increased intestinal permeability allows antigen to penetrate epithelium which in turn initiates a vigorous immune response. In this cellular immunologic defense mechanism, the noxions ROS are utilized by the phagocytic activity to destroy ingested microorganisms (57). While the ROS released by the phagocytes are bactericidal, they also damage normal cells. Most important in this phenomenon is the production of polymorphonuclear neutrophils (PMN) and the activation of arachidonic acid pathways. These immunologic activities can generate chemotactic factors that enhance inflammatory reactions leading to epithelial cell injury, cytolysis, haemolysis, haemorrhage and the activation of intravascular coagulation (57). Recently, it has also been reported that the cytokines as released by mononuclear phagocytes are associated with the granulocyte activation and aggregation in numerous inflammatory disorders (58). These will be dealt with in detail later.

Another important factor that can induce gut injury is the ischemia/reperfusion (57). During gastric and intestinal ischemia, metabolism occurs in the following pathways: ATP $\rightarrow$ AMP $\rightarrow$ adenosine $\rightarrow$ inosine $\rightarrow$ hypoxanthine. After restoration of circulation (reperfusion), hypoxanthine produces the following metabolites:

$$\text{Hypoxanthine} \xrightarrow{\text{xanthine oxidase}} \text{Xanthine} + \cdot O_2^- + H_2O_2$$

As mentioned earlier, $\cdot O_2^-$ and H_2O_2 can react together to produce the obnoxious $\cdot OH$ ion. Involvement of the above oxidative pathways in the "malnutritional" and "infective" diarrhoeal diseases will now be illustrated.

Malnutritional Diarrhoea

All the organ-specific nutrients are processed through the gastrointestinal tract. In the same process the intestinal nutritional demand is also met by the following way. In the intestinal mucosa the energy supply comes from luminal and vascular substrates (32). Most of the protein energy however comes from the luminal substrates while energy for the jejunum and ileum is almost equally supplied by the luminal and vascular substrates. The energy for the colon comes mostly from the luminal nutrients which provides short chain fatty acids (SCFA) as its preferred fuel (33). In malnourished children the cell growth of mucosa is altered more in the colon than in the small intestine. Because of this intestinal atrophy diarrhoea occurs on refeeding when the intestine cannot salvage ions and other nutrients like fat, protein and carbohydrates (33). This situation may occur in severe malnutrition, such as in marasmus and kwashiorkor, thus contributing to the development of malnutritonal diarrhoea. Animal studies reflecting the above situations have recently shown that oxidative stress is a causal factor in such intestinal dysfunction (59-61).

Basal electrical e.g. short-circuit current (Isc) properties are usually considered to indicate total electrogenic ion movement across the jejunum. These animal studies using rats have reported such Isc values in the context of malnutrition and diarrhoea. The Isc values from these published reports are presented in Table III.

Table III. Basal electrical (short-circuit current [Isc]) characteristics of jejunum of malnourished rats

Animal conditions	Isc($\mu A/cm^2$)	Ref
Control	93	(59)
Starved	101	
Control	73	(60)
Vit. E-Deficient	91	
Control	45.3	(61)
Protein-Deficient	69.5	

Young and Levin (59) starved the rats continuously for three days and observed the electrical properties (Isc) of jejunum and electrogenic ion movement. They found that as the starvation prolonged the Isc increased with the concomitant increase in concentration of chloride ion in the luminal fluid. It was concluded that progressive starvation in the rats induces secretory hypersensitivity which is thus responsible for "starvational" or "malnutrional" diarrhoea.

Lindley *et al.* (60) however put control and experiment rats on similar diet except that the control group received alpha-tocopheryl acetate (vitamin E) in an amount of 40 mg/kg of feed while the other group did not receive any vitamin E. The feeding started when the animals were 21 days and continued for one year. The concentration of vitamin E declined rapidly in the vitamin E-deficient group while it rose to a stable plateau in the control group. Vitamin E was also abundant in the mucosa of the control group, but undetectable in the experimental group (vitamin E-deficient group). The basal Isc as listed in Table III shows that it is significantly higher in the vitamin E-deficient group. These authors also measured the TBARS as an index of lipid peroxidation (oxidative stress) as mentioned earlier. The TBARS value in the vitamin E deficient group was greater, 0.83 mmol/l versus 0.67 mmol/l (for the vitamin E-supplemented group). In addition, this study related the increased Isc with secretagogue-induced electrogenic anion secretion in the vitamin E-deficient animals. This study therefore strongly supported the notion that oxidative damage, as produced by the deficiency of a chain-breaking exogenous antioxidant vitamin E within the small intestinal mucosa, brought about a change in enterocyte apical membrane which in turn enhanced basal and secretagogue-induced secretion leading to diarrhoea.

The last report by Darmon *et al.* (61) in this series gave a detailed description of the association of oxidative stress with the intestinal dysfunction of experiment rats as induced by a low-protein diet. The control rats were fed a standard diet containing 22 g/100 g of casein while the experiment rats were fed low-protein diet containing 6 g/100 g of casein. Both diets however were made isoenergetic. In addition to the jejunal Isc values as listed in Table III, other important related parameters were also observed. For example, they reported that the body weights, including the jejunal weight of the low-protein diet group were significantly lower than of the control group. From the absorptional secretion and macromolecular transport studies they indicated dysfunctions of intestinal mucosa. Most important contributions that can be derived from this study are oxidative and antioxidative activities in the gastrointestinal tract. While the concentrations of SOD, CAT and GPX did not differ significantly between the control and the protein-deficient animals, there were significant differences in GSH and TBARS values between the groups of animals. The GSH values were 18.6±1.7 and 16.0±1.7 mmol/mg of protein for the control and protein-deficient rats respectively. Similarly, the TBARS values were 101±5 and 125±6 mmol/mg of protein for the control and protein-deficient rats respectively. This situation reminiscences the oxidative status in malnourished children as described earlier.

The results from these three studies described above support the fact that malnutrition, induced either by the food shortage or by antioxidant micronutrient deficiency or by protein deficiency, makes the gatrointestinal tract vulnerable to oxidative stress. The ROS that are responsible for setting the stage for oxidative stress are also responsible for ion secretions. The lipid peroxidation, resulting from oxidative stress in gastrointestinal tract, is likely to produce arachidonic acid metabolites which are associated with intestinal dysfunctions including diarrhoea as discussed earlier.

Infective Diarrhoea

Gastrointestinal tract of a malnourished child is prone to infection by parasites, toxigenic bacteria or viruses which may alter immunocompetence of the local mucosa. It has already been reported by Sullivan *et al.* (62) that in chronic malnutritional diarrhoea the villous epithelium is infiltrated by the lymphocytes. Even in healthy human subjects the intestine is always inflamed and contains an extensive lymphoid cell populations. As the enteric pathogens cross epithelium, the lamina propria macrophages underlying the colonic epithelium in particular, are phenotypically activated. The phagocytic activity of macrophages plays an important role in destroying the invading pathogens as well as essential constituents of normal flora. The phagocytic cells amplify local immune response by enhancing PMN production in the mucosa (63). Additionally, the macrophages are capable of producing cytokines, such as cachectin or tumor necrosis factor (TNFα), interleukins (IL) and interferons which have various systemic effects (64). For example, TNFα causes mucosal ischemia by altering the intestinal vasculature. Ischemia/reperfusion, as mentioned earlier, produces ROS that are known to create oxidative stress. Furthermore, TNFα and interferonϒ synergistically can cause epithelial cell death. Interferonϒ can also increase epithelial permeability by loosening tight junctions. Convincing evidence exists to show that these cytokines can also be produced by the epithelial cells (65) thereby contributing to mucosal inflammation. All these phagocytic activities of macrophages in the intestinal tract are therefore capable of enhancing inflammatory mediators, destructive enzymes and most of the noxious ROS. The results of two recent studies described briefly below lend support to such phenomena occurring in infective diarrhoea.

In one study Rabbani *et al.* developed a rabbit model for shigellosis (personal communication). In this procedure the animals were infected by direct inoculation of *Shigella flexneri* 2a into the colon through trans-abdominal tube. The animals were found to develop mucoid diarrhoea within 24 to 48 hours after the inoculation. The excreted stool contained inoculated *Shigellae*, and colon of the animals showed histologic signs of acute inflammation. Histopathologic images of normal and infected colonic mucosa of the animals are elucidated in Figure 3. Figure 3A shows normal mucosa while 3B gives the details of inflammatory lesions of the infected colonic mucosa. The mucosa of the control rabbit colon shows normal appearance without any evidence of inflammation. The mucosa of the infected colon however is highly inflamed as characterized by the

superficial ulcerations, crypt abscess formation, and most importantly, by the heavy infiltration of PMN cells in the lamina propria (Figure 3B). It was mentioned earlier that the phagocytic PMN cells activity utilizes noxious oxygen radicals to inhibit any pathogenic infiltrations in the mucosa. Such immunologic action also takes its toll on the normal cellular integrity thereby increasing permeability of the epithelium that contributes to diarrhoea.

Another study by Kabir *et al.* (66) reported a rapid catch-up growth of children when fed a high-protein diet during convalescence from shigellosis. Since protein energy supplementation is likely to increase the concentration of endogeneous antioxidative enzymes that may reduce the lipid peroxidation, a preliminary study was initiated to monitor the level of TBARS (Khaled MA; unpublished data). The TBARS measured in these children before and after the protein-energy (15%) supplementation are presented in Figure 4 to compare with the control subjects who received isocaloric diet containing protein-energy only about 6%. The protein-energy supplemented group showed significant improvement in their protein nutriture i.e. the plasma level of albumin, prealbumin, etc. increased considerably (66). It can also be seen in Figure 4 that the TBARS level diminished considerably on protein-energy supplementation. However, the TBARS levels remained almost the same for the control group (Figure 4) in concordance with the protein nutriture of these subjects which did not change appreciably between the pre- and post-supplement measurments. The reduction in TBARS implies a reduction in lipid peroxidation thus relieving oxidative stress in the protein-supplemented group. These observations concur well with the results of previously reported animal studies (56,61) where protein insufficiency was found to aggravate the lipid peroxidation, a root cause of oxidative stress.

The above discussion was limited only to the invasive type of diarrhoeal diseases. In the case of secretory i.e. noninvasive diarrhoea, the literature is scanty on oxidative stress-induced disease proliferation. However, Deitch *et al.* (67) observed a few years ago an increased level of xanthine oxidase in the intestinal mucosa, infected with endotoxin. Since xanthine oxidase necessarily produces ROS from hypoxanthine (vide-supra), it is tempting to postulate that any enterotoxin-induced mucosal injuries in secretory diarrhoea may be mediated via oxidative stress.

CONCLUDING REMARKS

In the foregoing discussion an effort has been made to define the term 'oxidative stress' and its involvement in childhood malnutrition and diarrhoeal diseases. Because of nutritional paucity, intestinal cell growth is suppressed as well as the absorptive capacity of the colonic mucosa is diminished. In severe malnutrition, such as in kwashiorkor, childhood diarrhoea could be attributed to mucosal malnutrition. Childhood diarrhoea in such cases could therefore be termed as malnutritional diarrhoea. The gastrointestinal tract in such situations becomes vulnerable to various enteropathic antigens. Through dietary intake many such pathogens enter the intestinal tract and cross intestinal epithelium that could activate local phagocytic action. Oxidative stress resulting from such activity may render the mucosae of small bowel and colon highly depressed. Arachidonic acid metabolites (leukotrienes), neutrophils (PMN) and cytokines (TNFα and Υ-interferon) are responsible as well for creating such a state of the gastrointestinal tract. Infective diarrhoeal diseases are therefore liable to turn a healthy subject to be malnourished unless the compromised nutritional status is replenished.

Fig. 3A. Normal rabbit colonic mucosa

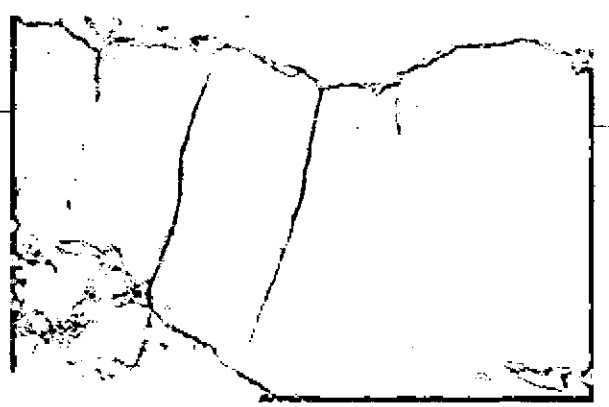

Fig. 3B. Colonic mucosa of rabbit showing a necrotizing mucosal inflammation with congestion, haemorrhage in the lamina propria of the mucosa with crypt absess and marked transmural inflammation with serosites (courtesy: Rabbani, *et al.*)

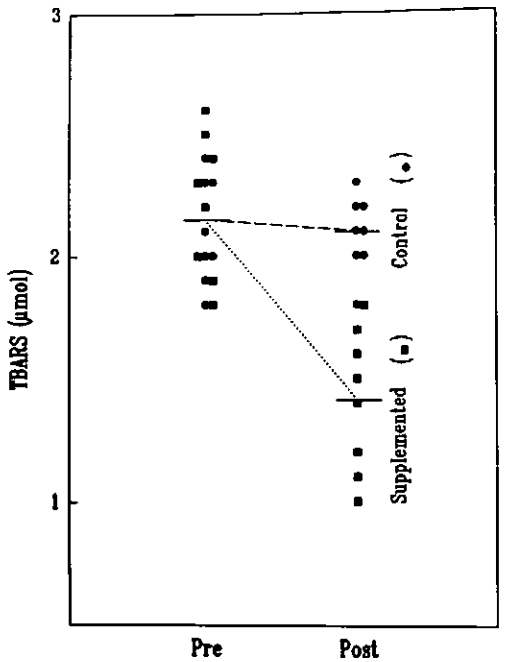

Fig. 4. A plot of TBARS (µmol/l) of the control (•) and protein energy-supplemented (■) groups

Vascular supply of amino acids has been shown to restore the nutritional status of intestinal mucosa (33-36). Protein-energy supplementation was found to enhance nutritional status of children recovering from shigellosis (66) thereby facilitating the absorption of many other micronutrients, particularly vitamin A (68). A large oral dose of vitamin A (100,000 to 200,000 IU) to severely PEM children may not therefore produce any desirable benefits (69) until and unless mucosal nutrition is appropriately restored.

REFERENCES

1. Sies H, editor. Oxidative Stress. New York: Academic Press, 1991.
2. Halliwell B. Current Status Review: free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. *Br J Exp Path* 1989;70:37-757.
3. Cutler RG, Semsei I. Development, cancer and aging: possible common mechanisms of action and regulation. *J Gerontol* 1989;44:25-38.
4. *Annals N.Y. Acad Sci* Vol.663, 1992.
5. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press, 1989.
6. Pryor WA. Formation of free radicals and the consequences of their reactions *in vivo*. *Photochem Photobial* 1978;28:787-95.
7. Willson RL. Organic peroxy free radicals as ultimate agents in oxygen toxicity. Sies H, editor. Oxidative Stress. New York: Academic Press, 1985;41-72.
8. Park MK, Nyers RAM, Marzella L. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents and immunologic responses. *Clin Infect Dis* 1992;14:720-40.
9. Fantone JC, Ward PA. Role of oxygen derived free radicals and metabolites in leukocyte-dependents inflammatory reaction. *Am J Pathol* 1982;107:397-418.
10. Babior BM, Kipnes RS, Curnutte JT. The production by leukocytes of superoxide, a potential bacteriocidal agent. *J Clin Invest* 1973;52:741-4.
11. Willson RL. Vitamin, selenium, zinc and copper interactions in free radical protection against ill-placed iron. *Proc Nutr Soc* 1987;46:27-34.
12. Sohal RS, Allen RG. Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. *Exp Gerontol* 1990;25:499-520.
13. Simic MG. The rate of DNA damage and aging. *In*: Emerit I, Chance B, editors. Free radicals and aging. Birkhauser: Verlag, 1992:20-31.
14. Fridovich I, Handler P. Detection of free radicals generated during enzymetic oxidations by the initiation of sulfite oxidation. *J Biol Chem* 1961;236:1836-44.
15. Logani M, Davis R. Lipid oxidation: biologic effects and antioxidants. *Lipids* 1979;15:485-95.
16. Kappus H. Lipid peroxidation: mechanisms, analysis, enzymology and biochemical relevance. *In*: Sies H, editor. Oxidative Stress. New York: Academic Press, 1985:273-310.
17. Harley JB, Santangelo GM, Rusmussen H, Goldfine H. Dependence of *E.coli* hyperberic oxygen toxicity on the lipid acyl chain composition. *J Bacteriol* 1978;134:808-820.
18. Tappel AL. Lipid peroxidation and flourescent molecular damage to membranes. *In*: Trump BF, Arstila AU, editors. Pathobiology of cell membranes. v. 1. New York: Academic Press, 1975:145-56.
19. Fridovich I. The biology of oxygen radicals. *Science* 1978; 201:875-80.
20. Buege JA, Aust SD. Microsomal lipid peroxidation. *Meth Enzymol* 1978;30:302-10.
21. Baldwin SR, Simon RH, Grum CM, Ketai LH, Boxer LA, Devall LJ. Oxidant activity in expired breath of patients with adult respiratory distress syndrome, *Lancet* 1986;1:11-4
22. Tappal AL. Effects of antioxidants and nutrients on lipid peroxidation flourescent products and aging parameters in the mouse. *J Gerontol* 1973;28:415-21.
23. Satoh K. Serum lipid peroxides in cerebrovuscular disorders detected by a new colorimeter method. *Clin Chim Acta* 1978;90:37-43.
24. Draper HH. Nutritional modulation of oxygen radical pathology. *Adv Nutr Res* 1990;8:119-26.
25. Harman D, Eddy DE, Nuffsinger J. Free radical theory of aging: inhibition of amyloidosis in mice by antioxidants; possible mechanism. *J Am Geriatr Soc* 1976;24:203-7.
26. Baker SS, Baker RD, Campbell C. Short-term malnutrition in neonatal rabbits: effect on function and synthesis of free radical metabolizing enzymes in the gastrointestinal tract. *J Ped Gastroenterol Nutr* 1990;11:247-53.
27. Dalsing MC, Grosfeld JL, Shiffler MA, Vane DW, Hull M, Baehner RL, Weber TR. Superoxide dismatase: a cellular protective enzyme in bowel ischemia. *J Surg Res* 1983;34:589-96.
28. Chow CK. Nutritional influence on cellular antioxidant defense mechanisms. *Am J Clin Nutr* 1979;32:1066-81.
29. Little C, O'Brian PJ. An intracellular GSH - peroxidase with a lipid peroxide substrate. *Biochem Biophys Res Commun* 1968;31:145-50.
30. Barnji MS. Glutathione reductase activity in red blood cells and riboflavin nutritional status in humans. *Clin Chim Acta* 1969;26:263-69.
31. Newsholme EA, Leech AR. Biochemistry for the medical sciences. New York: Wiley, 1983.
32. Roediger WEW. Metabolic basis of starvation diarrhea: implications for treatment. *Lancet* 1986;1:1082-4.
33. Roediger WEW. The starved colon-diminished mucosal nutrition,diminished absorption and colitis. *Dis Colon Rectum* 1990;33:858-62.
34. Davis NC. The influence of diet upon liver injury product by carbon tetrachloride. *J Med Res* 1924;44:601-14.
35. Elwyn DH, Parikh HC, Shoemaker WC. Amino acid movements between gut, liver and periphery in unanesthatized dogs. *Am J Physiol* 1968;215:1260-75.

36. Windemueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism *in vivo* in small intestine of fed rats. *J Biol Chem* 1980;255:107-12.
37. Editorial: Classification of infantile malnutrition. *Lancet* 1970;2:302-3.
38. FAO/WHO/UNO Expert Committee. Energy and Protein Requirements. WHO Tech Rep 1985;Ser 724.
39. Brown KH, Black RE, Robertson AD, Akhter NA, Ahmed MG, Bechker S. Xeropthalmia, protein calorie malnutrition and infections in children. *J Pediat* 1979; 95:651-9.
40. Young VR, Taylor YSM, Rand WM, Scrimshaw NS. Protein requirements of man: efficiency of egg protein utilization at [illegible] in young men. *J Nutr* 1973;164-74.
41. Torun B, Viteri FE. Protein-energy malnutrition. *In*: Shils ME, Young VR, editors. Modern Nutrition in Health and Disease. Philadelphia: Lea and Febiger, 1988:746-73.
42. Torun B, Chen F. Protein-energy malnutrition.ibid 1994;950-76.
43. Brock JF, Autret M. Kwarshiorkor in Africa. FAO Nutrition Studies No. 8. Rome: FAO, 1952.
44. Waterlow JC. Classification and definition of protein-energy malnutrition. *In*: Beaton GH, Bengoa JM, editors. Nutrition in Preventive Medicine. Geneva: WHO, 1976.
45. World Health Organization. Measuring change in nutritional status. Geneva: World Health Organization, 1983.
46. Golden MHN. The consequences of protein deficiency in man and its relationship to the features of kwashiorkor. *In*: Blaxter KL, Waterlow JC, editors. Nutritional adaptation in man. London: John Libby, 1985:169-88.
47. Waterlow JC. Kwashiorkor revisited: the pathogenesis of oedema in kwashiorkor and its significance. *Trans Roy Soc Trop Med Hyg* 1984;78:436-41.
48. Passmore R, Robson JS. A Companion to Medical Studies. Oxford: Blackwell, 1976;1:33-1.
49. Halliwell B. Commentary: Albumin - an important extracellular antioxidant ? *Biochem Pharmacol* 1988;37:569-71.
50. Golden MHN, Ramdath DD. Free radicals in the pathogenesis of kwashiorkor. *Proc Nutr Soc* 1987;46:53-68.
51. Sive AA, Subtzky EF, Malan H, Dempster WS, Heese HDV. Red blood cell and antioxidant enzyme concentrations in kwashiorkor and marasmus. *Ann Trop Paediat* 1993;13:33-28.
52. Mayatepek E, Becker K, Gana L, Haffman GF, Leichsenring M. Leukotrienes in the pathophysiology of kwashiorkor. *Lancet* 1993;342:958-60.
53. Ramdath DD, Golden MHN. Elevated glutathione S-transferase activity in erythrocytes from malnourished children. *Euro J Clin Nutr* 1993;47:658-65.
54. Samuelsson B, Dahlen S-E, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and Lipoxins: structures, biosynthesis and biological effects. *Science* 1987;237:1171-6.
55. Ford-Hutchinson AW. Leukotrienes: their formation and role as inflamatory mediators. *Fed Proc* 1985;44:25-9.
56. Huang CJ, FWU M-L. Protein insufficiency aggravates the enhanced lipid peroxidation and reduced activities of antioxidative enzymes in rats fed diets high in polyunsaturated fat. *J Nutr* 1992;122:1182-9.
57. Otamiri T. Oxygen radicals, lipid peroxidation and neutrophil infiltration after small-intestinal ischemia and reperfusion. *Surgery* 1989;105:593-7.
58. Otamiri T, Sjodahl R. Oxygen radicals: their role in selected gastrointestinal tract. *Digest Dis* 1991; 9:133-41.
59. Young A, Levin RJ. Diarrhoea of famine and malnutrition: investigations using a rat model. I. Jejunal hypersecretion induced by starvation. *Got* 1990;31:43-53.
60. Lindley KJ, Gross-Sampson MA, Muller DPR, Milla PJ. Lipid peroxidation and electrogenic ion transport in the jejunum of the vitamin E deficient rat. *GUT* 1994;35:34-9.
61. Darmon N, Prilissier M-A, Heyman M, Albrecht R, Desjeux J-F Oxidative stress may contribute to the intestinal dysfunction of weanling rats fed a low protein diet. 1993;1068-75.
62. Sullivan PB, Marsh MN, Mirakian R, Hill SM, Milla PJ, Neale G. Chronic diarrhea and malnutrition - histology of the small intestinal lesion. *J Pedia Gastroenterol Nutr* 1991;12:195-203.
63. Allison MC, Cornwall S, Poulter LW, Dhillon AP, Pounder RE. Macrophage heterogeneity in normal colonic mucosa and in inflammatory bowel disease. *GUT* 1988;1531-1538.
64. Nicholls S, Stephens S, Braegger CP, Walker-Smith JA, MacDonald TT. Cytokines in stools of children with inflammatory bowel disease or infective diarrhea. *J Clin Pathol* 1993;46:757-760.
65. Choy MY, Walker-Smith JA, Williams CB, MacDonald TT. Differential expression of CD25 (interleukin-2 receptor) on lamina propria T cells and macrophages in the intestinal lesions in Crohn's disease and ulcerative cells. *GUT* 1990;31:1365-70.
66. Kabir I, Malek MA, Mazumder RN, Rahaman MM, Mahalanabis D. Rapid catch-up growth of children fed a high-protein diet during convalescence from shigellosis. *Am J Clin Nutr* 1993;57:441-5.
67. Deitch EA, Taylor M, Grisham M. Endotoxin induces bacterial translocation and increases xanthine oxidase activity. *J Trauma* 1989;29:1679-83.
68. Khaled MA, Kabir I, Wahed MA, Mahalanabis D. Habte D. Oxidative stress and antioxidant: implications in vitamin A status in malnourished children. Vitamin A workshop, October 31, 1994, Dhaka: International Centre for Diarrhoeal Disease Research, Bangladesh, (In press).
69. Khaled MA, Wahed MA, Alvarez Jo, Mahalanabis D, Habte D. Large-dose vitamin A supplementation in malnourished children. *FASEB J* 1994;8:A892.

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