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A REVIEW ON REACTIVE OXYGEN AND NITROGEN SPECIES

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ABSTRACT

Generally Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) consist of free radicals and hasty species in these two groups and breakdown yield of lipids proteins, nucleic acids and carbohydrates. Free radicals (FR) contain one or more unpaired electrons and could be positively or negatively charged or neutral in nature. Superoxide anion (O^{-2}), free hydroxyl radical (OH^{-}) and nitric oxide ($NO\cdot$) are important free radicals in human body and produce numerous additional free radicals mostly from unsaturated fatty acids. Physiologically they can be defined as overactive disjointed atoms or molecules which are capable of upsetting and fragmenting other molecules. Free hydroxyl is the mainly reactive neutral free radical with half life of about 10^{-9} second. It is capable of insulting fragmenting and mutating any cellular molecule with forceful passion. Superoxide anion (O^{-2}) in human body arises from metabolic reactions, irradiation and leakage from electron transport chain. Superoxide is often referred as primary ROS as most of other ROS and RNS arise from it and are therefore termed as secondary ROS and RNS. These free radicals are produced in cellular membrane mitochondria, nucleus, lysosomes, peroxisomes, endoplasmic reticulum and cytoplasm. Redox-sensitive proteins with important cellular functions are confined to signalling microdomains in cardiovascular cells and are not readily available for quantification. A popular approach is the measurement of stable by-products modified under conditions of oxidative strain that have entered the circulation. However, these may not accurately reflect redox stress at the cell/tissue height. Many of these modifications are "functionally silent". Functional importance of the oxidative modifications enhances their validity as a proposed biological marker of cardiovascular disease, and is the strength of the redox cysteine modifications such as glutathionylation. We assess selected biomarkers of oxidative stress that show promise in cardiovascular medicine, as well as new methodologies for high-throughput measurement in research and clinical settings. Although associated with disease severity, supplementary studies are necessary to examine the usefulness of the most promise oxidative biomarkers to forecast prognosis or rejoinder to treatment.

KEYWORDS: Reactive oxygen species, Reactive Nitrogen Species, Free radicals, Antioxidants, Oxidative stress.

INTRODUCTION

Although the existence of FR and their use in biochemistry is known for over hundred years, their presence in biological system was suggested by Harmon (1956) about 50 years ago. (1) In next few decades they were implicated in a large number of diseases. Later their role in physiology is also documented. Currently free radicals have found a place in the etiology of many diseases and there is a great deal of enthusiasm regarding the role of free radicals in many previously unexplained disease phenomenons. (2-6)

What are free radicals?

These are chemical species that possess one or more unpaired electrons in the molecule. This is the key factor in the structure of the species and is the reason why they are highly reactive. These species are in reality composed of a group of molecular fragments that are capable of independent existence and can fragment other molecules. (7) The fact that they are highly reactive means that they have low chemical specificity that is why they can react with most molecules in its vicinity. This includes proteins, lipids, carbohydrates and DNA.

Hence, free radicals attack the nearest stable molecule usually "stealing its electron". When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process starts, it can cascade, finally resulting in the disruption of living cell. In 1924, it was established that molecular oxygen has two unpaired electron in its volume orbit, therefore it is biradial. However, because of quantum mechanical restrictions O_2 is not extremely reactive. (8) The two unpaired electrons of oxygen are located in different antibonding orbitals and have the same spin quantum number with parallel spins. This electronic arrangement provides the most stable to the oxygen known as ground state of oxygen. Since most electrons exist in a paired state, free radicals often end up reacting with paired electrons. (9)

What free radicals do?

Free radicals damage our body silently and invisibly.

Everything in our body is at risk, proteins, lipids, hormones, cells, tissues, genetic code etc. Free radicals damage leads to loss of energy, disease pain, aging and eventually death. Free radicals are scientifically proven to cause heart disease, cancer, diabetes and a variety of degeneration diseases. (10-12)

Where do free radicals come from?

Free radicals come from a variety of sources. They come from normal body metabolism, exercise, stress, sunlight, air and H_2O pollution and a variety of other sources. Even food preparation causes free radical formation. Cigarette smoke is filled with free radical.

The Biochemistry of free radical generation

Free radicals can be generated both *in vivo* and *in vitro* by one of the following mechanisms:

1. Homolytic cleavage of a covalent bond, in which a normal molecule fragments in two, each fragment staining one of the paired electrons. Homolytic cleavage occurs less commonly in biological system, as it requires high-energy input from ultraviolet light, heat or ionising radiation.
2. Loss of a single electron from a normal molecule.
3. Addition of an electron to a normal molecule.

There is another aspect of FR in human body which is also very important from both physiological and pathological point of view FR produce many non radical species, which are also highly reactive viz. hydrogen peroxide, peroxy nitrite, perchlorous acid and many other. The FR and RS ions comprised a group of highly reactive biomolecules. In human body they have broadly been divided into "Reactive oxygen species" (ROS) "Reactive nitrogen species" (RNS). These are illustrated in table.

Reactive oxygen species (ROS) is a collective term

Radicals	Non radicals
Reactive oxygen species (ROS)	
Superoxide $O_{2}^{\cdot -}$[a]	Hydrogen peroxide, H_2O_2[a]
Hydroxyl, $OH^{\cdot}$[a]	Hypobromous acid, HOBr
Hydroperoxyl, $HO_2^{\cdot}$[a]	Ozone O_3
Lipid peroxyl, $LO_2^{\cdot}$[a]	Singlet oxygen $(O_2{}^1\Delta g)$[a]
Lipid alkoxyl, $LO^{\cdot}$[a]	Lipid peroxides, LOOH[a]
	Maillard reaction products[a]
Reactive chlorine species (RCS)	
Atomic chlorine, CT	Hypochlorous acid, HOCl[a]
	Nitryl (nitronium) chloride NO_2Cl[b]
	Chloramines
Reactive nitrogen species (RNS)	
Niric oxide, $NO^{\cdot}$[a]	Nitrous acid, HNO_2[a]
Nitrogen dioxide, $NO_2^{\cdot}$[a]	Nitrosyl cation, NO^+
	Nitroxyl anion, NO^-
	Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3
	Peroxynirite, $ONOO^-$
	Peroxynitrous acid, ONOOH
	Nitronium (nitryl) cation, NO_2^+
	Alkyl peroxynitrites, ROONO
	Nitryl (nitronium) chloride, NO_2Cl[b]

Table: The "Reactive Species"

that includes both oxygen radicals and certain nonradicals that are oxidizing agents or are easily converted into radicals (HOCl, O_3, $ONOO^-$, $^1O_2$, H_2O_2). RNS is also a collective term including nitric oxide and nitrogen dioxide radicals, as well as such non radicals as HNO_2 and N_2O_4. $ONOO^-$ is often included in both categories. Reactive is not always an appropriate term: H_2O_2, $NO^{\cdot}$, and $O_2^{\cdot-}$ react quickly with only a few molecules, whereas $OH^{\cdot}$ reacts quickly with almost everything. $RO_2^{\cdot}$, $RO^{\cdot}$, HOCl, $NO_2^{\cdot}$, $ONOO^-$, and O_3 have intermediate reactivities. HOBr could also be considered a "reactive bromine species." [a]Reactive species particularly relevant to foods. [b]NO_2Cl is a chlorinating and nitrating species produced by reaction of HOCl with NO_2. (12-16)

Super oxide radical ($O_2^{\cdot-}$)

The major source of superoxide *in vivo* is the leakage that results from the electron transfer chain of the mitochondria. On its own it isn't particularly damaging. However, the superoxide radical anion appears to play a central role as other reactive intermediates are formed from it. (7)

HYDROXYL RADICALS

The hydroxyl radical is considered potentially the most potent oxidant encountered in biological systems and has extremely short life (microseconds). The hydroxyl radical is an extremely reactive oxidizing radical that will react to most biomolecules at diffusion controlled rates which means that reactions will occur immediately with biomolecules such as those found in organic lipids by removal or addition.

FENTON REACTION

Ferrous ion catalyze the decomposition of H_2O_2 to $OH^{\cdot}$ in Fenton reaction.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

HABER WEISS REACTION

Interaction of superoxide with hydrogen peroxide leads to formation of hydroxyl radical through the Haber Weiss reaction

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + H_2O + OH^{\cdot}$$

Hydroxyl radicals can also be generated when reduced forms of transition metal ions such as copper come into contact with H_2O_2(Yu, 1994).

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^{\cdot} + OH^-$$

PEROXYL RADICALS

They are unrepairable organic molecules formed by fixing of other free radicals by oxygen.

Lipid radical + O_2 → Lipid – $O^{\cdot}$ Peroxyl radical ($LOO^{\cdot}$)

ALKOXYL RADICALS

When reduced iron compounds react with lipid peroxides, alkoxyl radicals are formed due to fission of O-O bonds.

L – O – H + Fe^{++} complex → Fe^{+++} + OH^- + $LO^{\cdot}$ – Alkoxyl radical

NON RADICAL MOLECULES

Although H_2O_2 is not by definition considered an oxygen free radical, it remains the most extensively studied metabolic. A major source of H_2O_2 in the body is the dismutation of $O_2^{\cdot-}$ by superoxide dismutase (SOD)

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

Generally, H_2O_2 itself is not reactive enough to oxidize. Many organic molecules in an aqueous environment. Nevertheless, it is a biologically important oxidant. It has the availability to generate highly reactive hydroxyl free radicals though its interaction with redox-active transitional metals.

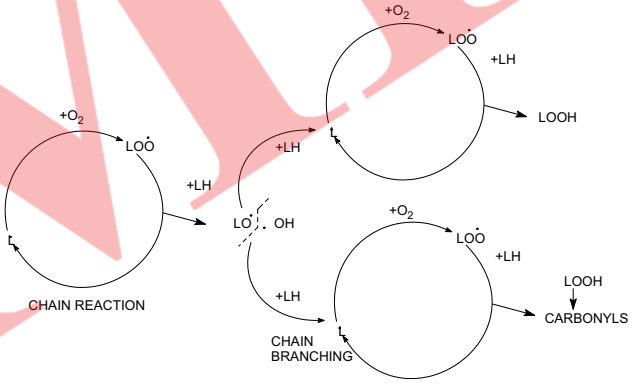

Fig: 1. Mechanisms of free radical chain reactions leading to formation of unsaturated fatty and hydroperoxides homolytic decomposition of hydroperoxide leads to chain branching reactions or autocatalysis.

[LH = Fatty acids; LOOH = Lipid hydroperoxides; $L^{\cdot}$ = Lipid alkyl radical; $LOO^{\cdot}$ = Lipid peroxy radicals]. (17-31)

SINGLET OXYGEN (O_2^1 G)[A]

It is a non radial (does not have an impaired electron) reactive oxygen species often associated with oxygen free radicals that has strong oxidixing activity. Singlet oxygen (O_2) is an electronically excited and mutagenic form of oxygen. It is generated by input of energy, example radiation, but can also be generated enzymatically by the action of peroxidases or lipooxidases or by the reaction of H_2O_2 with hypochloride or peroxy nitrite thermodecomposition of dioxetanes, or during the respiratory burst of phagocytes they are also generated in biological systems in a number of pigment reactions including

chlorophylls, retinal and flavins when they are illuminated in the presence of oxygen. It is also formed by the following mechanisms.

1. Haber – Weiss reaction
2. Light induced conversion of molecular oxygen to singlet oxygen in the presence of photosenstizer.
3. Spontaneous dismutation of superoxide radical (O_2^-).

OZONE (O_3)

The damage caused by O_3 is mediated by free radical production and is associated with lipid peroxidation in membranes. Lung is the major site of O_3 toxicity.

OXIDES OF NITROGEN

Nitric oxide (NO) and nitrogen dioxide (NO_2) contain unpaired electrons and are therefore free radicals; where as the "laughing gas" nitrous oxide (N_2O) is not. Generation of free radicals in body leads to the development of oxidative stress. (32-37) The constant exposure to lipid peroxides makes the endothelium very susceptible to oxidative free radical mediated damage. (38) It has been reported that lipid peroxide levels may provoke the disturbances in endothelial or intimal cells of the blood vessels which in turn causes vasospasm (thus rise in B.P.) and the general increase in the sensitivity to the vasopressors. (39)

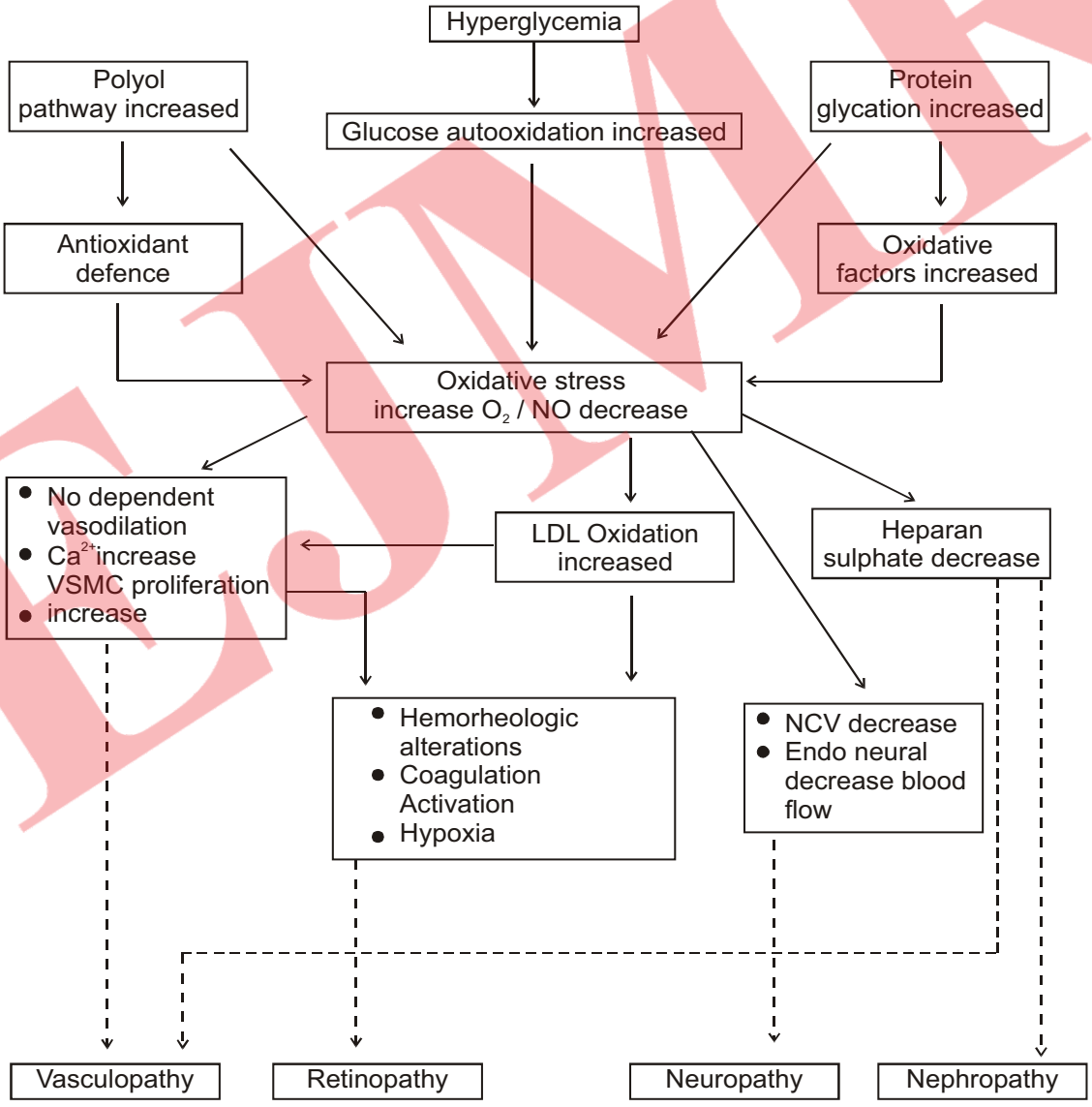

Fig.2:Possible Links Between Hyperglycemia Induced Oxidative Stress And Diabetic Complications.

NCV - Nerve conduction velocity,

VSMC - Vascular smooth muscle cell. (40-42)

LIPID PEROXIDATION

Indicator of lipid peroxidation is used to evaluate oxidative stress. The lipids within the membrane of cells from higher organism contain large number of polyunsaturated fatty acids side chains. Such fatty acids are prone to undergo a process known as **"Lipid peroxidation"**, which involves the generation of carbon radicals followed by production of peroxide radicals. Lipid peroxidation is oxidative deterioration of polyunsaturated fatty acids (PUFAS) i.e. those which contain two or more double bonds. Lipid peroxidation has been identified as a basic deteriorative reaction in the cellular mechanism of aging process, in cells damaged by environmental pollutants an in variety of pathological conditions. (43-44)

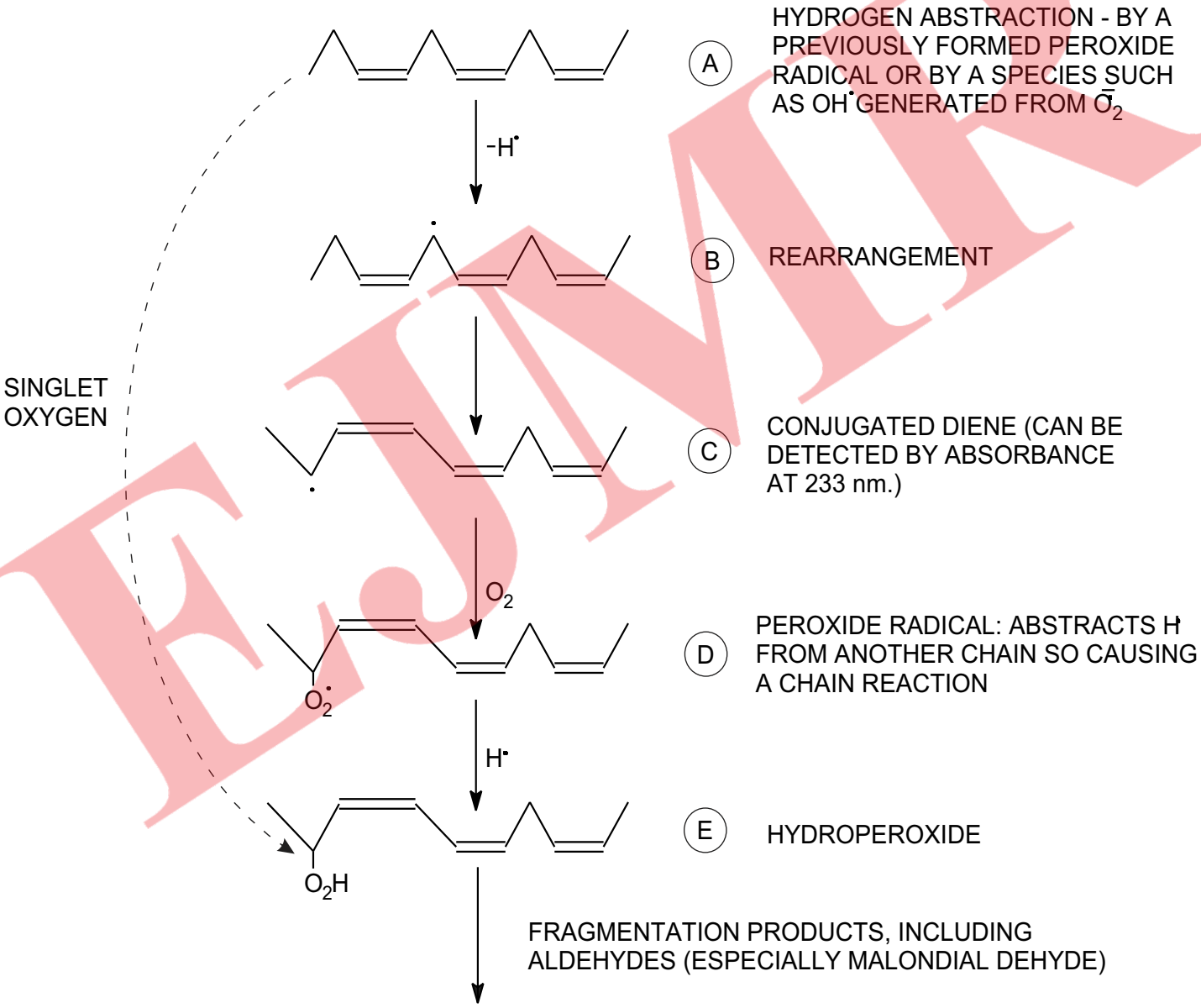

Fig.3:Biochemical Pathway Of Peroxidation Of Polyunsaturated Fatty Acids

CONCLUSION

Persuade of ROS, RNS and antioxidants had been under scanner. Evidence is mounting to suggest: a) ROS and RNS are significant regulators of metabolism in blood and tissues and this deregulation may trigger T-2DM and may set supplementary complications in due course of time. b) The FR could be causative factors is the source of IR. c) Redox perturbations in mitochondria resulting to altered ATP creation in electron transport chain results in improved outflow of electrons to form more superoxide anion. d) Metabolic change in cytosol resulting to changes in enzyme activities of NADPH oxidase, xanthine oxidase uncoupled nitric oxide synthase and many others increase the generation of reactive species. Both of these activities may participate in the pathogenesis of T-2DM. e) a lot of environmental factors awesomely tilt the redox homeostasis toward proxy-dizing conditions which affect the insulin signaling cascade and genetic disposition to diabetes and lastly. f) The reactive species may promote risk of any metabolic disorder by provoking genetic factors. Antioxidants are undoubtedly essential spokes of human life but their excess intake is undesirable. Further our studies on several series of diabetic patients and those of others indicate that raised OS is not an inevitable phenomenon in diseases.

Therefore, although all the loaded evidence for the involvement of reactive species in the diabetes, the argue continues on three points: 1) is it discriminating in patients or present in all patients but not measurable by available methods, 2) is it facultative, that is, it is competent of causing disease but does not essentially do so in all patients and 3) is it compulsory, that is, it universally participate in the genesis of all disease. Thus we can say that free radicals are root cause of all diseases.

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