

Chlorine Dioxide (ClO₂) as a Non-Toxic Antimicrobial Agent for Virus, Bacteria and Yeast (Candida Albicans)

Abstract

The use of a family of chemical agents, releasing active species of oxygen, effective against infectious microorganisms and viruses is described with emphasis on Chlorine Dioxide (ClO₂), one of the oxides of chlorine. Also discussed are ozone, hypochlorite, periodate and the known mechanisms of action by which certain white blood cells attach and eradicate infectious microorganisms and primitive bacteria also known as viruses.

Explanation of the biochemical mechanisms of acid of ClO₂ as an anti-microbial agent, is presented. Particular attention is given to Candida albicans, cytomegalovirus, polio virus, Herpes I and II, HTLV-III and Pseudomonas responding to the clinical application of ClO₂. It is implied that these biochemical mechanisms are so fundamental that the development of resistant strains of bacteria and/or yeast would not occur with other anti-infectious agents. Limited lists of health abnormalities that respond to ClO₂ are discussed.

Keywords: Chlorine dioxide; Ozone; Chlorite; Chlorate; Chloride

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Introduction

ClO₂ is an inorganic compound composed of oxygen; chloride; potassium and gold. Compounds of oxygen and chloride are formed from two electronegative elements. This chemical property of ClO₂ makes possible the release of nascent oxygen upon decomposition during its action as an anti-microbial agent. Certain aspects of the cellular immune system (specifically white blood cells) utilize other mechanisms in the generation of highly reactive oxygen derivatives (free radicals) for the purpose of combating the invasion of foreign organisms. Without these protective mechanisms provided by the immune system involving oxygen derivatives; the ability to fight infection is greatly impaired.

The immune system of many persons; particularly the elderly; is deficient in the ability of providing these highly reactive oxygen derivatives (free-radicals) which are so necessary for attacking the great variety of viral; and bacterial invaders become easy targets for the many diseases they produce with the accompanying and sometimes bizarre symptoms.

The use of ClO₂ assists the natural protective mechanisms of the body in counteracting these infectious agents which; if not adequately neutralized; will most certainly lead to disease. There will be described below the biochemical mechanisms by which ClO₂ assists the immune system in destroying pathogenic organisms by making available an active form of oxygen [1].

Clinical Applications of Anti-Microbial Agents

Physical properties and chemical structure

ClO₂ in pure form (anhydrous) is a liquid at 0°C. Having a deep red color. When mixed with water and at high dilution it is colourless [2].

Bohr atomic models indicate a "coordinate covalent" bond between the elements of ClO₂. This type of bond represents the sharing of a pair of electrons between two atoms as in the "covalent" bond; but in this bond both electrons are contributed by one of the atoms (chlorine) and none by the other. In the covalent bond one electron is contributed by each of the atoms forming the bond (Figure 1).

When ClO₂ reacts as an anti-microbial agent the oxygen atom first binds to a single atom (the one being oxidized) and then is dissociated from chlorine. An electron is then given up to chlorine forming the chloride ion. When one realizes that there are 5.3 g. of chloride ion per liter of human plasma it becomes obvious that the small amount of chloride generated through the use of ClO₂ is negligible.

Chemical definition of ClO₂

ClO₂ is an inorganic (containing no carbon) sodium salt of only electronegative elements bound together by electrostatic; covalent and coordinate covalent bonds (Figure 2). The chemical structure

of ClO₂ is pH-dependent; that is; the stability or instability of this substance is determined in large measure by the concentration of hydrogen ions (H⁺ or protons) in the surrounding medium.

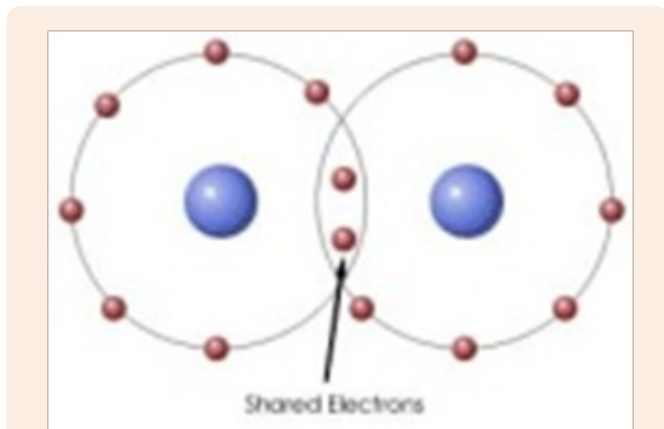


Figure 1: shows the sharing of electrons forming a covalent bond.

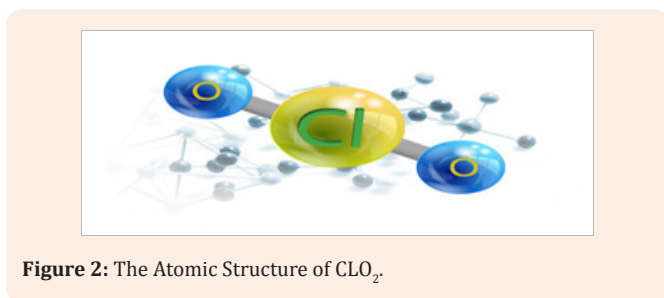


Figure 2: The Atomic Structure of ClO₂.

Under acidic conditions; sodium chlorite becomes unstable and decomposes into a variety of products including but not limited to chloride ions; hyperchlorous ions and nascent (atomic) oxygen. It is for this reason that ClO₂ when bottled and stored for long periods of time; is buffered. Under acidic conditions; ClO₂ results in a neutral molecule consisting of three electronegative atoms held together by covalent and coordinate covalent bonds.

From this cluster; a single atom of highly reactive nascent oxygen is liberated onto the target microorganisms. As you will see below; nascent oxygen is the active agent of ClO₂ and; unless it is liberated; the antiviral; antibacterial and antifungal activity will not occur.

Virus inactivation

A virus typically consists of an outer shell or coating of protein encapsulating a nucleic acid which may be either DNA or RNA (e.g. Retroviruses). The skeletal backbone of nucleic acids includes derivatives of phosphoric acid (H₃PO₄; a very strong acid) in which two of the original three hydroxyl groups (-OH) are substituted; leaving only one active hydroxyl group per phosphate.

Segment of extended dna chain showing alternating deoxyribose- phosphate linkage

Some viruses may have glycoproteins incorporated into their protein coat; that is; proteins to which polysaccharides (sugar

chains) have been attached. The bound polysaccharides may attach to specific sites on the protein coat; effectively converting a protein surface into a poly- sugar surface [3]. This new surface has specificity for certain polysaccharides found on the surfaces of specific cell types; thereby conveying specificity to virus binding as well as a degree of immunological protection.

Once bound to the appropriate cell type; the nucleic acid component of the virus is injected into the cell and in many ways; takes over the protein synthesis processes of the cell. Certain segments of the viral nucleic acid consist of genes that are responsible for the replication of the coat. The nucleic acid component replicates by a process known as "base-pairing"; in which each base of the original strand attracts and binds the corresponding base; forming a pair (A-T and C-G; described more fully below) through hydrogen bonding. The result is the replication of the complete virus until the cell bursts; releasing many additional viral particles into the surrounding medium. In the presence of these acidic nucleic acids; the ClO₂ molecule becomes unstable and releases nascent oxygen into the medium.

Nucleic acids; both RNA and DNA; have many characteristics in common; in fact; they are almost chemically identical. Each consists of a chain of alternating sugar (a modification of ribose; deoxyribose) and phosphate groups; known as the "phosphate-sugar backbone". Attached to a specific carbon of each deoxyribose group is an organic ring compound known as a "base". Altogether; there are only four types of bases found in DNA; namely; guanine (G); cytosine (C); adenine (A) and thymine (T). It is the sequence of these four units along the chain that makes one segment of DNA differs from another. RNA differs chemically from DNA in that the base thymine is replaced by the base uracil (U); representing a very subtle biochemical difference. There is also a subtle difference in the sugar deoxyribose.

The release of nascent oxygen from chlorine dioxide ClO₂ in an acidic environment

The base guanine; found in both RNA and DNA; is very sensitive to oxidation; forming 8-oxoguanine as the oxidation product [4]. The release of ClO₂ results in the oxidation of the guanine residue with the formation of 8-oxoguanine; thereby disallowing the replication of the viral nucleic acid by base pairing. Although the replication of the protein coat may continue; the formation of a complete functional virus has been blocked by ClO₂ oxidation.

Aerobic and anaerobic bacteria

When life began on earth but before the presence of oxygen in the earth's atmosphere; bacteria consisted of what are known today as the anaerobes. These bacteria survive only in the absence of oxygen and include many pathogenic organisms. With the appearance of algae; carbon dioxide; present in large proportion in the primordial atmosphere; was converted by algae to carbohydrates; generating oxygen as a by product. At first; oxygen reacted with iron salts in the primitive oceans to generate extensive deposits of iron oxide (iron ore). When soluble iron in the oceans became exhausted; oxygen concentration in the atmosphere began to rise. At the present time; oxygen comprises approximately 20% of the earth's atmosphere while carbon dioxide levels have dropped to about 2%.

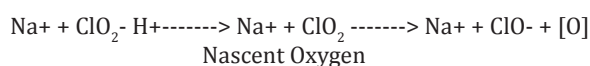
Some bacteria were able to survive the increased levels of oxygen in the atmosphere by any of three paths. The presence of oxygen in the atmosphere resulting from algae and other plants containing the green pigment chlorophyll; allowed the development of the wide range of life forms both in the ocean and on land known today as animals. Some anaerobic bacteria took up residence in oxygen-free environments within the bodies of animals; notably; the intestines. Other anaerobes solved the problem of oxygen toxicity by evolving metabolic ways to cope with its universal presence. These include the development of the enzyme superoxide dismutase (SOD); which in its most primitive form; contained iron [5]. Later refinements included the incorporation of manganese (in mitochondria) and finally; a combination of copper and zinc.

It is widely believed that still another solution to oxygen toxicity lay in the development of organisms that have the ability to utilize oxygen in their metabolism. These organisms are known today as the aerobes or aerobic bacteria. One form of these organisms may have been the precursor of an inclusion organism found in many animal and human cells known as the mitochondria; having its own circular DNA.

It is well known that cultures of many bacteria become acidic; typically generating lactic; acetic and other simple carboxylic (organic) acids. The acidic medium surrounding many bacteria triggers the decomposition of ClO₂ and the subsequent liberation of nascent oxygen. Nascent oxygen is a particularly potent oxidizing agent for anaerobic organisms because it is essentially a free radical seeking not one; but two electrons. Anaerobic organisms have not developed adequate defences against the onslaught of oxygen; particularly nascent oxygen; and quickly succumb to its lethal action.

One possible mechanism for the liberation of nascent oxygen from the chlorite ion (ClO₂⁻) in an acidic environment involves the association or binding of H⁺ (proton; normally bound to the oxygen of water as H₃O⁺) to any of the three pairs of unused electrons in the outer shell of chlorine. This association is equivalent to the formation of hydrogen – chlorine (H – Cl) covalent bond. Because of the single electron deficiency of the chlorine atom; only one covalent bond is permitted at any one time. Since the H – Cl bond is stronger (the H – O bond of water is almost the strongest bond known) than the existing O – Cl covalent bond; the O – Cl bond is disrupted by the ejection of nascent oxygen. This leads to the dissociation of the acidic proton from chlorine and the formation of the hypochlorous ion (ClO⁻) and sodium hypochlorite (NaOCl) and the release of nascent oxygen from chlorine dioxide in an acidic environment.

The decomposition of sodium chlorite to nascent oxygen and sodium hypochlorite in an acidic environment



Sodium Ion + Chlorine Dioxide + Sodium Hypochlorous Ion + Ion Sodium Hypochlorite -----> Nascent Oxygen

(Figure 3) The formula for decomposition of sodium chlorite to nascent oxygen and sodium hypochlorite in an acidic environment]. During World War I; this instability of chlorite ion

was exploited by Alexis Carrel (1873–1944; Nobel laureate 1912); best known for his prolonged culture of chicken heart cells at Rockefeller University; New York. Carrel successfully administered a crude solution topically to war casualties having embedded shell fragments [6]. The anaerobic organisms responsible for gas gangrene in these wounds were quickly quenched; saving many soldiers that would have otherwise perished [7].

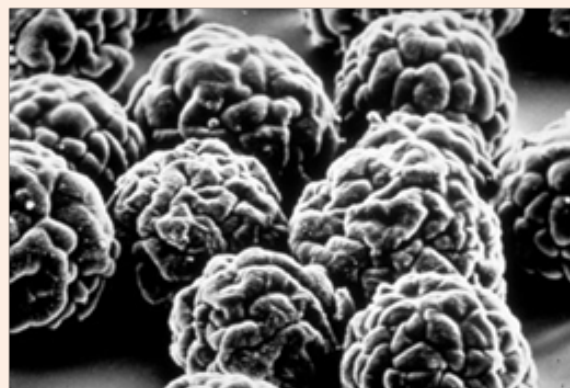


Figure 3: An electron micrograph showing the intact polio virus before exposure to ozone (O₃).

Absence of ClO₂ activation

There are only a small number of basic biological substances found in living organisms. These include proteins; lipids; carbohydrates and nucleic acids. The ability of nucleic acids (in the form of viruses) to activate chlorine dioxide and then nascent oxygen has been discussed previously. Lipids and carbohydrates are both neutral substances; carrying neither an electric charge nor acidic groups. The possibility of proteins activating ClO₂ is discussed below.

Many proteins; particularly those that are soluble and found free in both the blood and in the medium surrounding tissues; contain on their surfaces organic groups; both acidic and basic (alkaline) in nature. In most soluble (globular) proteins either one or the other of these two types of groups predominate. Proteins in which the acidic groups outweigh the basic groups are acidic proteins and those in which the basic groups predominate are known as basic proteins.

A technique has been developed to determine; for a given protein; in which category it may lie. Either acidic or basic proteins migrate under the influence of an applied electric field. This laboratory technique; known as electrophoresis; has been used to determine the acidity or basicity of proteins. As the pH of the surrounding medium into which a protein has been solubilized is changed; the protein will migrate either in one direction or the other. When a pH is found that will result in no migration; the protein is said to be at its isoelectric point. The pH value at which this occurs for a given protein is known as the isoelectric point of that protein. The isoelectric points of most proteins are not far from 7 or neutrality. Such proteins are not capable of activating ClO₂ to decomposition; partly accounting for low toxicity.

ClO₂ as an antifungal agent

Fungi are considered as plants without chlorophyll and lack the ability to generate carbohydrates from sunlight and carbon dioxide. They were probably derived in an oxygen-free atmosphere but some have developed the ability to tolerate low levels of oxygen. Most fungi prefer an oxygen-poor environment and live best under these conditions. They obtain their energy requirements from the decomposition (through enzyme activity) of existing organic matter and; in this light; may be considered as parasitic.

Because of their low tolerance for nascent oxygen and the acidic medium in which they thrive (from the liberation of organic acids); fungi in the mycelial form are sensitive to the destructive action of ClO₂. One example of a human pathogenic fungus is *Candida albicans*; invading finger and toenails. A second example is the fungus responsible for "athlete's foot"; thriving between toes in a moist environment while still another example is "ringworm"; propagating on the dead cells found on skin (as well as generating many mycotoxins affecting homeostasis).

From all of the above considerations; it is apparent that ClO₂ is essentially activated only by viruses; acidic bacteria and fungi. Other Cytotoxic Oxidizing Agents Used Clinically ClO₂ is not the only anti-microbial agent in clinical use. Another agent also providing active oxygen is hydrogen peroxide; which has been used in the treatment of arthritis; cancer and other metabolic diseases. Hydrogen peroxide is commercially available in low concentrations for the treatment of topical microbial infections.

Found to be more effective as a sporicide than as a bactericide; hydrogen peroxide is bacteriostatic at concentrations greater than 0.15 millimolar [8]. Peracetic acid; CH₃COOOH; is simply acetic acid; CH₃COOH; with one additional oxygen atom inserted between the carbon and OH group. This bonding is unstable; decomposing to acetic acid and one atom of oxygen is being made available as bactericidal; fungicidal or virucidal. Peracetic acid exhibits rapid activity against spores and yeasts [8].

An anti-microbial agent having chemical similarity to ClO₂ is sodium hypochlorite (NaOCl) in which only one atom of oxygen is bound to halogen rather than two. Solutions containing 125ppm were found effective within 30minutes in decontaminating objects which had been massively contaminated by both gram-positive and gram-negative bacteria and fungi. Drinking water heavily contaminated by a polymicrobial suspension became sterile in 30 minutes by a solution containing 10 ppm of sodium hypochlorite [9].

Still another oxidizing agent having increased application is ozone. Ozone is an unstable gas and is not commercially available but must be generated as required. It has been shown that ozone selectively inhibits the growth of human cancer cells [10]. Under specified conditions cancer cell growth was inhibited more than 90 percent while control cells were inhibited less than 50 percent [11].

These results indicate that cancerous cells; because of their altered oxygen metabolism; are less able to handle the oxidative stress presented by ozone than normal cells. Ozone has also been shown to inactivate polio virus with an effect noticeable after

only 0.5 seconds of contact and is very strong after 2.5seconds. These results parallel those caused by ClO₂ in relation to polio virus (see below, anti-viral activity of ClO₂). Ozone is being used both in Europe and the U.S. to treat various diseases including a cancerous condition; blood coagulation disorders and liver diseases; among others [12-14] (Figure 4).

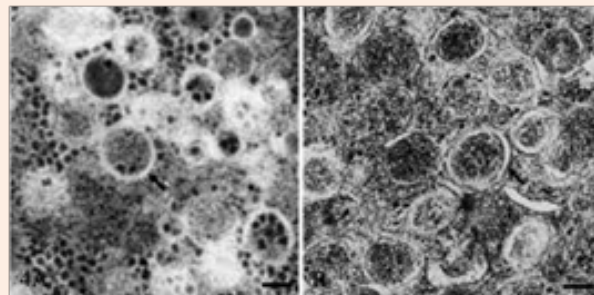


Figure 4: shows a Phase contrast micrograph of the polio virus after exposure to ozone (O₃) [11].

Cytotoxicity of ClO₂

Proof that ClO₂ is cytotoxic to bacteria; fungus and virus clinically is shown by data indicating its effectiveness as a disinfectant (outside the body). ClO₂ has been found to inactivate the organism causing Legionnaire's Disease (*Legionella pneumophila*) [15]. The chemically related compound sodium periodate (NaIO₄) inhibited the virulence; decreased the respiration of and increased the sensitivity to phagocytosis of the common pathogen *Listeria monocytogenes* [16].

A germicidal solution has been developed containing ClO₂ at an acid PH (lactic acid). The solution gave complete kill of *Staphylococcus aureus*; *Pseudomonas* and *Candida albicans* spores within 10 minutes. If used in an ultrasound cleaning device complete killing occurred in less than five minutes [17]. The bacterial virus ϕ 2 was rapidly inactivated with ClO₂ at pH 5.9 only GMP (guanosine monophosphate) reacted while the amino acids cystine; tryptophan and tyrosine reacted rapidly [18].

ClO₂ applied to polio virus separated the RNA from the protein coat (capsid). ClO₂ reacted with the capsid protein and prevented the adsorption; penetration and normal un-coating of the virus. It also reacted with the viral RNA and impaired the ability of the nucleic acid to act as a template for replication [19].

Fate of ClO₂ following ingestion

The half-life for the elimination of ClO₂ from experimental animals (rat) was 43.9hours. When rats drinking 100mg of ClO₂ per liter for 15days were then given 3ml of 300mg/liter orally the half-life was 31hours.

The distribution of ClO₂ in tissues following administration of ClO₂ labelled material is as follows

Plasma; 2.74; Kidney; 2.45; Lung; 2.25; Stomach; 2.15; Liver; 1.16; Spleen; 0.76; Thymus; 0.68 [20]. The metabolites of ClO₂ are chlorite (ClO₂⁻); chlorate (ClO₃⁻); chloride (Cl⁻) and oxygen (O₂) [21,22] (Figure 5).

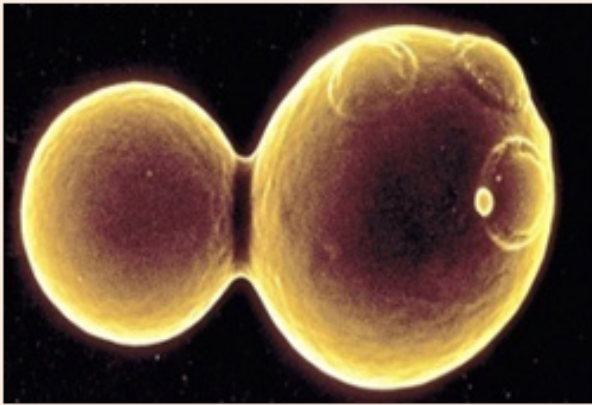


Figure 5: Is a scanning electron micrograph of Candida albicans showing the bud scars.

Toxicity of ClO₂

It has been shown by toxicity studies in man that daily ingestion of 500ml ClO₂ having a concentration of 5ppm is safely tolerated [23]. In other studies with rats the LD of NaClO₂ was established as 140mg/kg [2]. In studies where ClO₂; Na ClO₂ or ClO₃ was included in the drinking water for up to several months where no significant increases in methemoglobin concentrations resulted with doses as high as 1000mg/liter (1000ppm) in the rat; mouse or chicken [20].

ClO₂ in wound healing

It has been demonstrated clinically that ClO₂ is highly effective in accelerating wound healing with particular applications to burns. There may at first be no apparent explanation for such a relationship but careful biochemical analysis has revealed a mechanism of action relating ClO₂ to wound healing. Nucleic acids (RNA and DNA) are formed of only four basic units linked together into a chain. One of the basic units is designated; guanosine monophosphate; or GMP. If the phosphate group is brought close to one of the hydroxyl groups of the sugar component ribose; water is eliminated and the product is known as cyclic GMP (cGMP).

It has been shown that cGMP stimulates cell division and is activated during the regeneration process. It is also known that the enzyme synthesizing cGMP; guanylate cyclase; is stimulated by one of the highly reactive oxygen derivatives; hydroxyl radical; symbolized by OH⁻. An oxidizing agent chemically similar to ClO₂ is periodic acid; HIO₄; in which four oxygen atoms are bound to a single halogen atom; iodine. In ClO₂; two oxygen atoms are bound to the halogen chlorine. It has been determined that hydroxyl radical is present in solutions of periodic acid [24] and; by inference; may also be present in solutions of ClO₂. The presence of hydroxyl radicals in ClO₂ activates the enzyme guanylate cyclase; which; in turn; synthesizes cGMP leading to cell proliferation and wound healing [1].

The significance of candida albicans in therapy with ClO₂

Candida albicans is a normal resident of the human digestive

system; and vaginal tract and; unlike many other yeasts; is found in two forms (dimorphic). Baker's or brewers yeast is a familiar example of a yeast which occurs in only one form; that of single ovoid-shaped cells which reproduce by the process of "budding." An older; large cell constricts near one end and eventually closes off thereby forming a daughter cell which breaks free and continues the reproductive cycle.

Candida albicans is said to be "dimorphic" because the ovoid single-cell may; under certain conditions; continue to elongate into an almost perfect cylinder (mycelium); occasionally laying down a wall or partition separating individual cells. This process is also accompanied by branching in which a new tube may start to grow laterally from a specific point. By changing the culture conditions the form of Candida albicans may be changed from one form to the other and vice versa. This phenomena is known as pleomorphism (Figure 6).

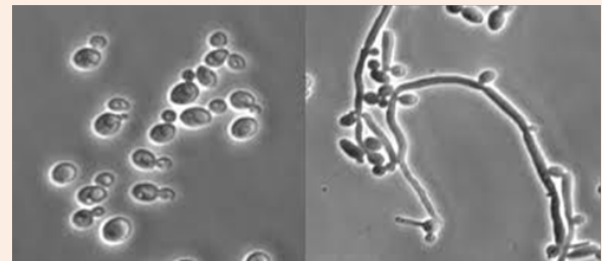


Figure 6: Y-form or round Candida albicans can biologically transform into a M-form or mycelial Candida albicans.

It is believed that only the mycelial form is pathogenic in man; who will be discussed in greater detail below. The incidence of both superficial and invasive candidosis has been increased markedly over the last few decades; probably resulting from the widespread usage of immunosuppressive treatment; antibiotics; etc. The organism can reasonably be described as the most common and most serious fungal pathogen of man [25].

Candida albicans has a worldwide distribution and is commonly found in normal individuals. The incidence of this fungus in various groups undergoing medical treatment is in general higher. Candida vaginitis is at present the second most common form of vaginal infection in the United States. Of the women who develop vaginal candidosis; a significant proportion suffer frequent recurrences or show chronic intractable symptoms. Candida albicans can infect virtually every tissue in the human body. Clearly there is a requirement for new and more effective antifungal drugs [26].

For Candida albicans to successfully colonize and infect mucosal surfaces it has to adhere to epithelial surfaces. In germ free animals the mucosa is colonized in higher numbers than in that of control animals (normal). These results suggest that the indigenous bacterial flora suppresses Candida albicans colonization. This; in turn; implies that at least one factor contributing to Candida albicans infection may be the balance between the various components of the intestinal flora [26].

Lysis of candida by ClO₂

The lysis of Candida (and other infectious yeasts) by ClO₂ may be accounted for by a simple mechanism related to the oxidizing ability of this substance. There follows a description and rationale for the mechanism as related to chitin; one of the constituents of the fungal cell wall. Chitin is a polymeric substance (formed by linking together a basic subunit) forming the bulk of the hard parts of insects (shell; legs; head; etc.). It is a water-insoluble substance formed by linking together the glucose derivative; N-acetylglucosamine (NAG) (contains nitrogen while glucose does not). Chitin is biochemically synthesized by the enzyme chitin synthetase; and provides rigidity to the fungal cell wall [27].

Yeast cells reproduce asexually by budding in which a daughter cell is formed by a constriction near one end of a mature cell. The construction continues until the walls almost meet followed by a thickening of the wall at this point. The space remaining is filled with a plug of chitin (ki-tin). When the daughter cell breaks free what would otherwise be an open hole in the cell becomes a bud scar of chitin plugging the hole and preventing cell disruption through lysis [28] see Figure 3. Thus; any agent which inhibits the synthesis of chitin should; in the light of these considerations; act as a lytic agent for yeast.

In an effort to answer the question as to whether the cellular immune system (white blood cells) has an anti-fungal action against *Coccidioides immitis*; polymorphonuclear leukocytes (PMN cells) were incubated with the fungus under culture conditions. The results of contacting PMNs with the fungus indicated that the incorporation of NAG into chitin was inhibited from 54 to 85 percent [28].

PMNs from patients with chronic granulomatous disease (CGD) are apparently equivalent in every way to similar cells from normal individuals but lack the ability to generate active oxygen species (members of the Reactive Oxygen Toxic Species or ROTS family) used by these cells to fight infection (including superoxide; hydrogen peroxide; hydroxyl radical and singlet oxygen). One measure of the production of reactive oxygen toxic species or ROTS by these cells is the chemiluminescence (chemical generation of light) resulting from the presence of foreign particles. Tests made with PMNs isolated from patients with CGD are remarkably low in chemiluminescent response to stimulation.

When PMN's from a patient with CGD were incubated with *Coccidioides*; the incorporation of NAG into chitin was inhibited from only 0 to 22 percent under various conditions as compared to 54-85 percent with normal PMNs [28]. These results indicate that the agents responsible for the inhibition were the highly reactive oxygen toxic species (ROTS) mentioned above. Similar oxidative species arise from the breakdown of certain oxidizing agents (including ClO₂) when in contact with the target substances (bacterial proteins; polysaccharides; viruses; etc.). It has been shown that periodic acid (HIO₄); a substance in which four oxygen atoms are bound to iodine (corresponding to chlorine in ClO₂); is capable of forming one of the ROTS family; hydroxyl radical [24].

Another reactive oxygen derivative; superoxide; may result from the breakdown of ClO₂ (during an oxidative reaction) in

which the two oxygen atoms released acquire an electron. It is known to be produced by *Candida albicans* as a metabolic by-product [29]. Thus; three members of the ROTS family of oxygen derivatives; namely; hydroxyl radical; superoxide and singlet oxygen; are produced by both PMNs during the oxidative burst (response to foreign particulate matter) and ClO₂ during an oxidative process. These considerations indicate that ClO₂ as well as PMN cells are capable of inhibiting the biosynthesis of chitin in *Candida* resulting in lysis from the absence of the chitin plug at the bud scar.

Antiviral activity of ClO₂

Viruses; in general; consist of two parts; an inner nucleic acid core consisting of either; DNA or RNA; and an outer protein coat. The protein coat not only serves to protect the vital nucleic acid within but is also instrumental in providing specificity in binding to the surface of particular cells (polio virus binds to nerve cells; influenza virus binds to the nasal mucosa; etc.). It has also been shown that without certain specific polysaccharides attached to the protein coat (glycoproteins); binding to the cell surface will not occur [30].

This aspect will be discussed in greater detail below in relation to the action of ClO₂ on viruses.

Some virus; including the AIDS virus HTLV-III; herpes and cytomegalovirus; are surrounded by a bilayer lipid membrane which is a fragment of the plasma membrane of the cell in which the virus replicated. As the virus ruptures and kills the cell it takes with it a fragment of the plasma membrane of that cell and becomes encapsulated. This membrane is critical for infectivity and without it the virus cannot enter the proper cell type for replication. Anti-viral agents which damage or deteriorate the encapsulating bilayer lipid membrane also inhibit or prevent infectivity. We shall return to this aspect of the anti-viral activity of ClO₂ in a subsequent section.

Biochemical activity of ClO₂

The action of ClO₂ as a cytotoxic agent may include as many as four separate biochemical mechanisms described below.

Oxidation of protein

As mentioned above; the protein coat is highly significant in viral infectivity and provides specificity for binding to the proper cell type. Two amino acids that would undergo oxidation and modification to a significant enough degree to permit inhibition of virus to cell binding are those containing sulfur; namely; methionine and cysteine.

The residue of methionine; -CH⁺CH⁻CR₂; contains the C-S-C thioether linkage subject to oxidation in either one or two stages. In the first stage the binding of one atom of oxygen results in the formation of the sulfoxide -CH₂CH₂SCO₂CH₂; having the ability to bind several molecules of water (hydrophilic). The parent compound; methionine; is hydrophobic (in relation to the residue). A subsequent stage of oxidation leads to the formation of the sulfone; -CH₂CH₂S(O₂)CH₂; which is even more hydrophilic than the sulfoxide. Both methionine sulfoxide and sulfone are well known modifications of this amino acid [29].

The modification of the water binding ability of a protein constituent (methionine) is highly significant in relation to the antiviral activity of ClO₂. In transmission electron micrographs of virus which has been exposed to the action of ClO₂ it appears that the outer coat has swollen much like a sponge. The increased penetration of water into the viral protein coat allows additional ClO₂ (water soluble) to penetrate to still deeper layers of the coat; thus initiating a vicious cycle (Figure 3).

Oxidation of nucleic acid (RNA)

As mentioned above; ClO₂ is capable of generating a variety of extremely reactive oxygen derivatives when acting as an oxidizing agent. Another example of an oxidizing agent releasing atomic or nascent oxygen is ozone; O₃ this molecule consists of a chain of three oxygen atoms held together by covalent bonds. The release of one atom of oxygen results in stable; molecular oxygen; O. The oxidizing activity of ClO₂ is similar to that of ozone; liberating highly active forms of oxygen. Very detailed research has been described related to the oxidation of polio virus by ozone. Exposure of the virus RNA strand to ozone resulted in fragmentation into several short chains. The effect of ozone on the nucleic acid was measured spectrophotometrically indicating that inactivation was complete after only sixty seconds [30].

Nucleic acid comprising the RNA or DNA of viruses consists of a chain of four building blocks arranged in some specific sequence. It is the sequence of the four nucleotides (designated by the letters A; C; G and U for RNA) that causes one strand to differ from another resulting in the synthesis of specific proteins for each. When a mixture of the four nucleotides is exposed to ozone it is found that only the unit designated; G (guanine); is degraded in the initial stage. In the exposure of transfer RNA (tRNA) to ozone; guanine again was the first nucleotide to be degraded. Exposure of tobacco mosaic virus (TMV) to ozone caused a rapid loss of infectivity within 30 minutes showing a preferential degradation of guanine [2].

Oxidation of lipids

Some viruses are surrounded by a bilayer lipid membrane as described above. Any substance which acts to disrupt this membrane is an anti-viral agent for that virus since the membrane is essential for infectivity. ClO₂ may act to disrupt this membrane by peroxidizing the unsaturated fatty acids found in the bilayer lipid membrane. The specific positioning of these unsaturations along the fatty acid chain is related to the integrity and fluidity of the membrane. The peroxidation of unsaturations results in the development of hydrophilic (water seeking) groups in the central part of the fatty acid chain which causes distortion in the bilayer lipid membrane. This activity of ClO₂ has not been demonstrated biochemically but is very apparent in the electron micrographs (transmission) of viral membrane disruption shown in Figure 10.

Oxidation of polysaccharides

There are found on the surfaces of viruses; bacteria and fungi (including *C. albicans*) chains of sugars (polysaccharides); some attached to protein as glycoprotein. An example of such a polysaccharide is mannan composed principally of sugar mannose and found on the surface of many yeasts. The degradation of sugars

and polysaccharides has been conducted mainly with periodic acid and its salts having the structure HIO₄. The oxidizing part of this substance is the ion IO₄⁻ (periodate); in which four oxygen atoms are bound to a single atom of iodine (halogen). A similar oxidizing agent is pH Miracle's ClO₂ product called Activator having the structured ClO₂ or in its partially reduced form; ClO.

When an oxidizing agent of this nature is applied to the free aldehyde group of a "reducing sugar" (one in which the ring has opened with the formation of an aldehyde) an extensive degradation is initiated as exemplified by the equations [31] (Figure 7).

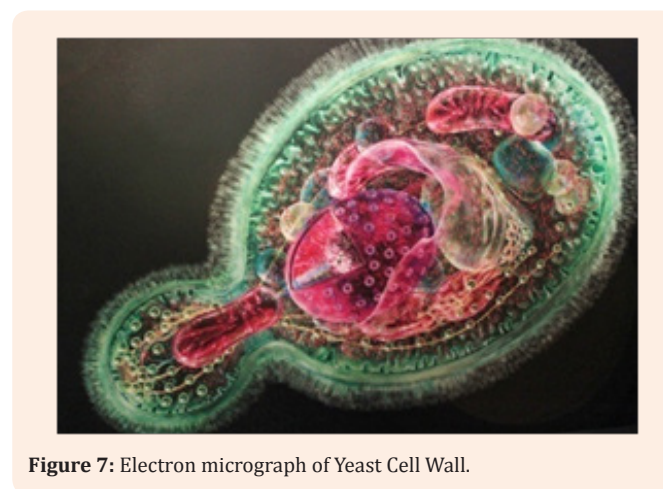


Figure 7: Electron micrograph of Yeast Cell Wall.

H-C-OH illustration

The products formed are low molecular weight substances including formaldehyde; formic acid; carbon dioxide; etc. The surfaces of many organisms and viruses carry polysaccharides that are highly significant in relation to infectivity. It is these polysaccharides showing specificity for the surface of target cells that enables the organism to bind to those particular cells and invade (infectivity) [31]. One of the mechanisms of action of ClO₂ against pathogenic organisms (including *Candida*) is undoubtedly the oxidation and degradation of sugars with a concomitant loss of infectivity and integrity for those organisms.

Significance of the cell wall of candida albicans

The principal cell wall polysaccharides of most yeasts are mannans (formed mainly of the sugar; mannose) and glucans (formed mainly of the sugar; glucose) whose basic structures have been determined. In models of the yeast cell wall recently proposed; a mannan-protein component is found in the outer region while glucan is found mainly in the inner region. Chitin (a polymer of the glucose derivative; N-acetylglucosamine) has been detected not only in the bud scar but also in the glucan network of the cell wall [32].

Treatment of the cells (yeast form) of *C. albicans* with helicase; an enzyme preparation from snail gut; releases mostly mannose from the surface during the first 20 minutes. Holes are formed in the cell wall which allow further penetration into the inner

