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# Therapeutic Applications of Squalene - A Review

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## Abstract

The article gives an overview of squalene, a naturally occurring antioxidant and its medical applications for human beings. The sources and properties of squalene and its occurrence in human beings are discussed. Deep sea sharks belonging to the family squalidae are the richest source of squalene. Squalene's cytoprotective activity, antioxidant property, cholesterol lowering property and effect of squalene on testosterone level, obesity and high blood pressure are detailed. Rapid transdermal absorption, creation of moisture barrier, high spreadability, non-greasy texture and antibacterial properties of squalene make it an excellent skin protector and finds application against eczema and in anti-aging and wrinkle protecting cosmetics. Being an adjuvant in vaccines, it stimulates the immune system and increases response to vaccines. Being a safe and naturally occurring antioxidant, its application for human health is very important. Its availability in nature *viz.*, from deep sea shark liver and olive oil cannot meet the growing demand of industry. Synthesis of squalene by organic hemi-synthesis should be explored to meet the demand.

**Key words:** Squalene, deep sea shark, antioxidant, cholesterol, cytoprotective activity

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## Introduction

Squalene ($C_{30}H_{50}$) is a highly unsaturated isoprenoid hydrocarbon widely distributed in nature. Vegetable oils such as olive oil, palm oil, wheat germ oil, amaranth oil and rice bran oil contain varying amounts (0.1 to 0.7%) of squalene. It is also called as spinacene and supraene. Deep sea shark is the richest source of squalene in nature (Gopakumar, 1997). In fact, the compound got the name squalene as it was identified from the oil of the deep sea shark belonging to the genus *Squalus.* The tropical deep sea shark (*Centrophorus artomarginatus*) contains as high as 80% squalene in its liver oil. Basking shark liver oil also contains high amounts of squalene. Squalene is an all trans isoprenoid with six isoprene units. Structurally it is called (all-E) 2, 6, 10, 15, 19, 23-hexamethyl-2, 6, 10, 14, 18, 22-tetrecosahexaene.

The ancient Shoguns of Japan were the first to recognize the beneficial effects of deep sea shark liver oil. They found that it provides strength, vigour, energy, virility and over all good health and called this very rare and precious extract as *"Tokubetsu no Miyage"* meaning "*Special Gift"*. The Japanese fishermen of Suruga Bay in the Izu Peninsula, famous for shark fishing called this elixir as *"Samedawa"* meaning cure-all. The deep sea shark liver also was in use by the coastal people and fishermen of Micronesia and they called it miracle oil. The Spanish mariners called it *"aciete de bacalao"* or the oil of the great fish. The Chinese ancient pharmaceutical book *Honzokomuko* also contains references of the therapeutic uses of deep sea shark liver oil. The credit for discovery of squalene was

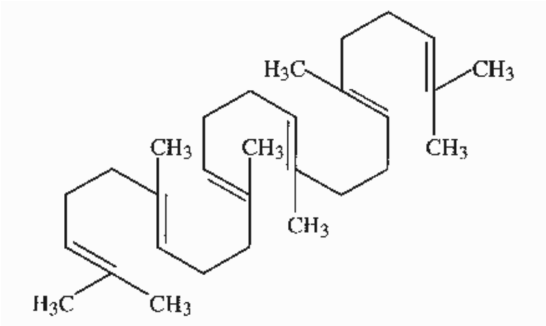


Fig. 1. Structure of squalene

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given to Dr. Mitsumaru Tsujimoto, a chemist on oils and fats at the Tokyo Industrial Testing Station who reported in 1906 that certain shark liver oils contain a highly unsaturated hydrocarbon and is rich in energy. The sharks belong to the family squalidae and the compound was named squalene. But it was after several years, in 1936, that the structural formula of squalene was elucidated by Paul Karrer, a Swiss Chemist working at Zurich University in Switzerland. Given the roles squalene performs, in the human body, it is aptly referred to as 'gift from sea' (Farvin et al., 2009a).

## Sources and properties of squalene

Deep sea sharks live about 900 m under the sea where sunlight and oxygen are almost negligible. Squalene is stored in the bodies of these sharks, which lack a swim bladder and therefore reduce their body density with fats and oils. Squalene, which is stored mainly in the shark's liver, is lighter than water with a specific gravity of 0.855. The ability of this species to withstand high pressure at this depth and to survive is due to squalene. Squalene abstracts oxygen from the water present in the body and releases it to the cells for physiological activities and also to provide strength and stamina.

The richest amount of squalene is in the shark, *Centrophorus moluccensis* (synonym. *Centrophorus scalpratus*) abundantly occurring in the Indian Ocean, particularly in the seas of Andaman and Nicobar Islands. Gopakumar (1997) has reported that the liver oil of this species contained 70 per cent of squalene by weight. Extra virgin olive oil contains about 200-450 mg $g^{-1}$ of squalene (Kelly, 1999). Extensive methodology for purification, estimation and industrial applications of squalene extracted from *C. scalpratus* has been reported (Gopakumar & Thankappan, 1986; Thankappan & Gopakumar, 1991). Characteristics of squalene are given in Table 1.

Table 1. Chemical properties of squalene

| Properties | Value |
|---|---|
| Molecular weight | 410.7 |
| Melting point | -75°C |
| Viscosity at 25°C | 12 centipoises |
| Specific gravity | 0.8 to 0.86 |
| Boiling point at 25°C | 285°C |
| Calorific value | 19 400 BTU $Pound^{-1}$ |
| Flash point | 110°C |

Squalene is present upto 85% by weight of liver in deep sea sharks. Among the plant sources, squalene is present in Amaranthus seed oil (6-8%), olive oil (up to 0.7% by weight), palm oil (0.1 to 2%, depending on species and method of extraction), rice bran oil and wheat germ (Liu et al., 1976; Deprez et al., 1990; Sun et al., 1997; Newmark, 1997). Squalene extracted commercially from olive oil is marketed as vegetable squalene having purity of around 97.5% while squalene from shark liver oil can be processed up to 99.9% purity.

## Occurrence of squalene in humans

Squalene is a key intermediate in the biosynthesis of cholesterol. Over 60% of the ingested squalene is absorbed from the small intestine and then transported to the lymph in the form of chylomicrons into the circulatory system. In the blood, squalene combines with low density lipoproteins and is transported to various body tissues. A major portion of the absorbed squalene is distributed to the skin. Studies conducted on squalene in human adipose tissues show that fat tissues contain over 80% of the total squalene present and upto 10% in microsomal membranes (Koivisto & Miettinen, 1988; Stewart, 1992). Scientific evidence suggests that only microtonal membrane bound squalene (around 10% of total squalene) present in human body, is metabolically active and stimulates immune cells in the inner and outer coat of our body (Owen et al., 2004). Squalene is present in important human tissues. The endogenous synthesis of squalene in animal body begins with the production of acetate from glucose. Acetate is converted to 3-hydroxyl-3-methylglutaryl coenzyme A (HMGCoA). The HMGCoA is reduced to mevalonate. The mevalonte is then phosphorylated in a three stage process and then decarboxylated to form delta-3-isoprenyldiphosphate. This is followed by successive additions of prenyl groups with formation of 15-carbon farnesyl phosphate. Two molecules of farnesyl diphosphates are then enzymatically joined and reduced to form squalene by an enzyme called squalene synthetase (Kelly, 1999; Reddy & Couvreur, 2009). Various steps involved in the biosynthesis of squalene are given in Fig. 2.

## Synthesis of cholesterol from squalene

Cholesterol is synthesized in human body from squalene (Fig. 3). Squalene is cyclized by an enzyme called squalene cyclase to form cholesterol (Liu et al., 1975). Steps involved in the process are

cyclization and carbocation. Squalene epoxide formed is converted to lanosterol by the enzyme catalase. This is followed by the removal of 3 methyl groups from lanosterol to form cholesterol (Clayton & Bloch, 1956; Popják et al., 1961).

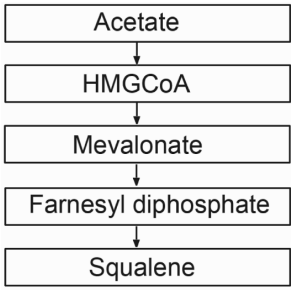


Fig. 2. Biosynthesis of squalene from acetate

## Synthesis of steroids from squalene

All steroids are derivatives of cholesterol. Cholesterol is hydroxylated by enzyme cytochrome P-450 (removes 6 member carbon side chain from cholesterol), to form pregnenolone (Fig. 4). This hormone is secreted by uterus to control ovum implantation. Pregnenolone is the precursor of androgens, estrogens and glucocorticoids. Testosterone is an androgenic steroid hormone produced mainly in the gonads and in smaller quantities by adrenal cortex.

## Effect of squalene on cholesterol metabolism

Amounts of squalene present in human tissues vary, being rich in areas which have high sebaceous glands (face, forehead) and low in areas having poor sebaceous glands. Secretion of squalene by sebaceous glands of the skin amounts to 125-475 mg $day^{-1}$ $person^{-1}$ (Kelly, 1999). Although cholesterol is

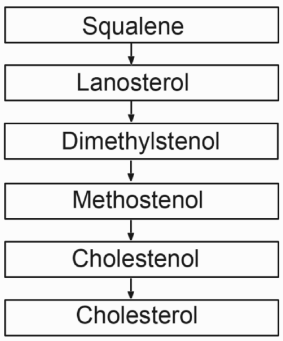


Fig. 3. Biosynthetic pathway of cholesterol from squalene

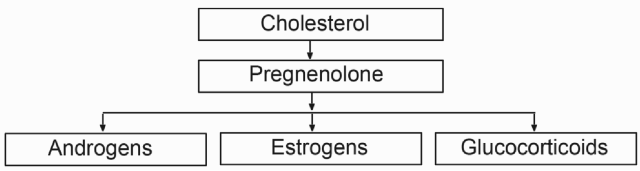


Fig. 4. Biosynthetic pathway of steroid hormones from cholesterol

synthesized from squalene in human body, dietary intake of squalene does not elevate serum cholesterol level. Only microsomal bound squalene is actively involved in this process and rest of the squalene is stored in the lipid droplet (Tilvis & Miettinen, 1983). Strandberg (1990) has shown that when human volunteers were fed with squalene (90 mg $day^{-1}$ $person^{-1}$) for 7-30 days, serum squalene levels increased up to 17-fold, but no significant changes were noticed in serum triglycerides and cholesterol contents. But squalene feeding produced significant increase in fecal excretion of cholesterol, its non polar derivatives and bile salts suggesting that although cholesterol synthesis has increased by as much as 50%, faecal elimination was up-regulated. Because of high faecal elimination, no increase in serum cholesterol was noticed. Oral administration of squalene also results in the reduction of low density lipoprotein cholesterol (LDL), triglycerides and increase in high density lipoprotein (HDL).

## Squalene and cancer

Olive oil is the staple source of dietary fat among people of Greece and many European countries. Epidemiological studies in the latter part of the $20^{th}$ century showed that people who eat the so called Mediterranean diets (Gjonca & Bobak, 1997; Buckland et al., 2011) have lower incidence of major disease like cancer and cardiovascular diseases. Consumption of olives and olive oil containing squalene is found to be responsible for these beneficial effects. Those women who consume large quantities of olive oil have lesser risk of breast cancer (Kelly, 1999; Newmark, 1997; Owen et al., 2004). It is now well established that olive oil contains squalene and the beneficial effect of resistance to cancer may be due to the squalene present in olive oil. The studies conducted on animals also support this observation of decreased incidence of breast cancer (Kate et al., 1992). Animal studies have shown that squalene is effective in inhibiting lung tumors. Squalene is also found to have chemo-preventive action against

colon cancer and enhanced immune function (Nakagawa et al., 1985; Ikikawa et al., 1986; Rao et al., 1998). Hence, squalene is now being used as a prophylactic agent after irradiation treatment of cancer patients.

Squalene carries oxygen to cells (Strandberg, 1990; Wefers et al., 1991) and thereby enhances the function of several organs like liver and kidney (Kelly, 1999). It also prevents acidotic cell syndrome disease in which the cells become acidic and die due to absence of oxygen. Squalene is an excellent scavenger of free radicals and thereby protects cells and organs from auto-oxidative damages. Animal studies in recent times have shown that squalene in sebum played a protective role against formation of hydrocarbon carcinogens in skin and prevented damages caused by radiation therapy (Wefers et al., 1991; Strandberg, 1990). Being a natural product used by man from time immemorial, its consumption is harmless and has no side effects.

## Cytoprotective activity of squalene

One of the major limitations of cancer chemotherapy is the indiscriminative injury of normal tissues, leading to multiple organ toxicity and consequent dose limitation/treatment failure (Das et al., 2003). The resultant problems are myelo-suppression, renal toxicity and neuropathy having profound effects on adults and children with long term remission that affects not only therapy but also overall quality of life (Bukowski, 1999). Often, free radicals produced by chemicals and radiation therapy are the major problems. Scavenging these free radicals is important to minimize the toxicity of chemotherapy. Antioxidants are extensively used for this purpose. Several experimental studies conducted using squalene had demonstrated that it is an excellent detoxifying agent against toxic and carcinogenic chemicals like hexachlrobiphenyl, hexachlorobenzene, arsenic, theophilline, phenobarbital and strychnine (Kamimura et al., 1992; Richter & Schafer, 1982; Fan et al., 1996). Squalene has also been reported to have protective activity against nicotine-derived nitrosamineketone (NMK) -induced lung cancer (Smith et al., 1998).

Most antioxidants used today have side effects and thus fail to get approval from certifying agencies like the USFDA or WHO. Hence, there is an urgent need to develop new generation of antioxidants having essential characteristics like better safety profile, good oral availability and meeting basic qualities required for a cytoprotective agent (Das et al., 2003). Squalene being a naturally occurring cytoprotective agent, toxicity of this compound has not been reported so far.

## Squalene as antioxidant

Most healthy diets rich in good lipids contain squalene as a naturally occurring constituent (Rowland & Robson, 1986), especially one containing olive oil and deep sea shark liver oil (Heller et al., 1963). Squalene has unique ability to carry oxygen throughout the body without the help of haemoglobin. Being highly unsaturated, it contains 6 double bonds, and is extremely reactive to get into an oxidized state. Squalene extracts hydrogen from water and releases oxygen by the following reaction:

$$C\ 30\ H\ 50 + 6\ H_2\ O \rightarrow C\ 30\ H\ 62 + 3\ O_2$$

The released oxygen enhances cellular metabolism giving vitality and vigour and also prevents acidosis of cell. Rajesh & Lakshmanan (2009) reported antioxidant effect of dietary squalene on sodium arsenite-induced oxidative stress in rat myocardium. Squalene is not very susceptible to peroxidation and appears to protect skin from lipid peroxidation due to exposure of UV radiation and other oxidative damage (Wefers et al., 1991). Human body produces a number of peroxides generated by autoxidation of fat present in foods consumed. These peroxides which are free radicals produce a large quantity of carbonyl compounds harmful to human body. But cells possess their own defense mechanisms consisting of antioxidants. Antioxidants are very vital for health and they get oxidized at their expense and prevent damages by carbonyls. One of the well known antioxidant present in human body is vitamin E. Squalene is the best naturally occurring antioxidant which have no known side effects. A comparison of properties of squalene and vitamin E is given in Table 2.

Low ionization threshold of squalene makes it an efficient donor of electrons without undergoing molecular disruption. This capacity makes squalene an excellent antioxidant. Oxidative damage of the skin starts at the surface induced by pro-oxidants which brings about oxidation of skin lipids (Ohsawa et al., 1984; Wefers et al., 1991). Due to its presence in skin squalene can effectively scavenge free radicals (Kohno et al., 1995; Atkins, 2002; Das, 2005) from skin, one reason why squalene finds application as a skin moisturizer in cosmetics.

Table 2. Comparison of properties of squalene and vitamin E

| Squalene | Vitamin E |
|---|---|
| A hydrocarbon, 30-carbon polyprenyl compound having 6 isoprene units | An alcohol having 3 isoprene units called alpha- tocopherol (5,7,8-trimethyltocol). |
| Produced by human body, present in many tissues, rich in skin (McKenna, 1950). Squalene is available to human body both exogenously and endogenously. | Depend on dietary sources, has to be supplied through food. |
| Once it enters human body, squalene abstracts hydrogen from water in cells and releases oxygen to cells (Atkins, 2002). | Vitamin E has no such property |
| One of the strongest anti-oxidant found in nature (Kohno et al., 1995) and relatively stable to attack by free radicals as compared to Vitamin E | Powerful anti-oxidant but unstable to attack by free radical compared to squalene. |
| Increases male potency and vitality by increasing production of male hormones. | Has only the capacity to improve health. |
| Strongly attracted to the hydrophobic bond between the two lipid layers of the biomembrane, where lipid per-oxidation is the greatest. Can move freely through the biomembrane without altering the properties of the biomembrane | Limited integration into the biomembranes and cannot move freely. |
| Can be used as an antidote to reduce drug toxicity (Kamimura et al., 1989) | Cannot be used |
| Squalene is stable after neutralization with free radicals and hence can be recycled. | Requires the use of other endogenous antioxidants like squalene for recycling |
| Have capacity to increase good cholesterol (HDL) and to reduce bad cholesterol (LDL). | No such properties are reported. |
| Can tolerate uptake of up to 5g $day^{-1}$ without any toxic effect. | No side effects at low dosages |
| Unlike other antoxidants like Lycopene, Vitamin A & E, squalene can be stored in high concentration in body (skin and adipose tissues) | Cannot be stored in high concentration |

## Squalene and Coronary Heart Disease (CHD)

The widespread occurrence of coronary heart disease is chiefly attributed to modern life style and food habits. Dietary squalene has been found to lower cholesterol levels in blood and also to reduce LDL and to increase HDL (Ikikawa et al., 1986). This effect is due to efficiency of squalene to down-regulate the HMGCoA reductase which in turn enhances the capacity of liver to filter bad cholesterol. These findings are supported by epidemiological correlation studies of squalene rich olive oil consuming population having low incidence of CHD. The cholesterol lowering property of squalene has prompted pharmacologists to combine squalene with statin drugs (used to lower cholesterol levels) and to use in human therapeutic applications. This leads to lower doses of drug formulations and reduction in potential side effects of statins. This can also reduce in the long term, therapeutic costs of patients having hypercholesterolemia.

A clinical trial conducted by Chan et al. (1996) showed the effectiveness and safety of squalene alone and in combination with pravastatin in lowering the cholesterol levels. This double blind placebo-controlled, 20-week trial was conducted on a randomized selection of 102 elderly people, all suffering from high cholesterol levels. They received 10 mg pravastatin and/or 860 mg squalene daily either separately or in combination. The results showed that both pravastatin and squalene effectively reduced levels of total cholesterol and LDL cholesterol and increased levels of HDL cholesterol. The study concluded that (i) co-administration of pravastatin and squalene combined the effects of the two drugs on lipoprotein concentrations; (ii) the combination may be useful and cost-effective in elderly patients with hypercholesterolemia, who might have a higher incidence of side effects when using larger doses of pravastatin alone. Farvin et al. (2009b) reported the cardioprotective effect of squalene against isoprenaline-induced myocardial infarction in rats.

Although squalene is an intermediate in the cholesterol biosynthesis in animals, intake of squalene by man has not shown increase in serum cholesterol levels. On the contrary, squalene is seen to reduce cholesterol levels in man. This makes it safe for human consumption. In Greece and Italy, average intake of squalene is 200-400 mg $day^{-1}$ while in United States, the consumption is only 30 mg $day^{-1}$ (Yokota, 1997).

## Effect of squalene on testosterone level, obesity and High Blood Pressure (HBP)

Obesity is a resilient and complex chronic disease. Leptin, an adipocyte-secreted hormone level has been shown to be responsible for obesity with several other hormones (Mantzoros, 1999). Leptin, a product of the *Lep* gene was discovered through the positional cloning technique used to determine the genetic defect resulting in obesity on ob/ob mice (Zhang et al., 1994). The ob gene encodes a peptide from 167 amino acids named leptin (from Greek word *leptos*, means thin), whose crystal structure suggests that it belongs to cytokine family (Zhang et al., 1997). This hormone has to be associated with weight loss, cachexia, inflammatory process, oxidative stress, arterial hypertension and aging. Another potential causative factor in obesity syndrome is leptin resistance (Mantzoros, 1999). Zhang et al. (2009) found that squalene regulates leptin, a compound controlling obesity in rats. Liu et al. (2009) found that feeding squalene may counteract increase in body fat, blood pressure, levels of plasma leptin, glucose, cholesterol, triglycerides and also effect increase in testicular weight and testosterone levels in rats. Similar results were seen in male red panda (Li et al., 2003), boar (Zhang et al., 2008) and also in chicken (Li et al., 2010).

A comparative cross-sectional study conducted (Mendoza-Nunez et al., 2006) on 70 healthy elderly persons *viz*., 46 women (mean age: 67±5.8) and 24 men (mean age: 73±7.5), and another group of 91 elderly persons with HBP composing 62 women (mean age: 67±8.2) and 29 men (mean age: 70±0.3) showed that elderly subjects with high blood pressure had significantly higher levels of leptin than healthy elderly subjects.

## Squalene and skin care

Today, squalene is widely used in pharmaceutical formulations as an effective skin moisturizer and also to prevent formation of lines and wrinkles in skin associated with aging process or cancer. A variety of squalene incorporated cosmetic products are available in the markets all over the world. Besides being odourless and colourless, its properties such as high spreadability, light consistency, stability at all ambient temperatures, non greasy texture, rapid transdermal absorption, restoration of suppleness to skin, creation of moisture barrier, healing of chapped and cracked skin, antibacterial properties and ability to promote cell regeneration make squalene an excellent skin protector. Areas of application of squalene include anti-aging, wrinkle protection, eczema, damaged hair, dry scalp and brittle nail.

## Squalene as adjuvant in vaccines

Immunological adjuvants are substances, administered in conjunction with vaccines that stimulate the immune system and increase response to vaccines (WHO, 2008). Squalene contact tests indicate that it is not a significant contact allergen or irritant and both squalene and its hydrogenated form, squalane are safe as cosmetic ingredients (IJT, 1982). MF59 is the propriety adjuvant name of squalene patented and in use by Novartis (Anon, 2009). This compound is added to human influenza vaccine to stimulate human body's immune response through production of CD4 memory cells. It is the first oil-in-water influenza vaccine commercially used. WHO

and US Defense Department have both published extensive reports which emphasize that squalene is a chemical naturally occurring in human body (Asano et al., 2002). According to the WHO, squalene has been present in over 22 million flu vaccines given to patients in Europe since 1997 and significant vaccine related adverse events were not reported (Roscoe, 2009).

## Toxicity and dosages

Animal studies conducted have proved that there are no side effects or toxic signs in plasma, biochemical and hepatic functional tests (Kamimura et al., 1989). Dosages depend on area of application, *viz*., for lowering cholesterol, 860 mg $day^{-1}$ was given along with pravastatin without any side effects. A dose of 500 mg $day^{-1}$ was safe and had normalizing effect on lipid profile (Kelly, 1999). For cancer treatment in human beings, a dose of 2-5 g $day^{-1}$ appears to be of therapeutic use. Since most olive oil consuming populations in the world, particularly in the Mediterranean area, who consume 500-800 mg squalene $day^{-1}$, have not experienced any ill effects for centuries, it can be concluded that a daily consumption of 500 mg squalene is safe and provide health benefits.

## Conclusion

Available research findings have shown that substantial amount of dietary squalene is absorbed, a major part of which is used to synthesize cholesterol. This increase is not associated with elevation of plasma cholesterol in humans as a result of concomitant faecal elimination. Hence, the theory, that increased consumption of squalene is likely to enhance serum cholesterol is misplaced. A dietary supplementation of 500 mg of squalene $day^{-1}$ appears to normalize plasma cholesterol levels, LDL and HDL cholesterol values. Squalene also confers many protective benefits to patients exposed to radiation treatment. Squalene assists in maintaining white cell counts during radiation treatment of cancer patients. Besides, it also protects the skin from many ill effects of UV radiation. The most exciting application of squalene for human health is its use as a safe and naturally occurring antioxidant. However, its availability in nature cannot meet the growing demand. The most abundantly available source of squalene is deep sea sharks which is not an inexhaustible resource. Another major source of squalene is olive oil but the content is very low. Besides, after extraction of squalene, the residual oil cannot be used for food purposes and hence is not an economically viable source. The need of the day is to synthesize squalene by organic hemi-synthesis.

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