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COMMENTARY

ALBUMIN—AN IMPORTANT EXTRACELLULAR ANTIOXIDANT?

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The nature of antioxidants

The importance of free radicals in Biology and Medicine is becoming increasingly recognised [1], and there is a growing interest in mechanisms of antioxidant protection, with potential relevance for therapeutic use [1, 2]. Much attention has been paid to enzymes which scavenge radicals (superoxide dismutase) or prevent their formation (catalase, glutathione peroxidase) as important antioxidants that stop free radical reactions [3 *loc cit*]. Also important are chain-breaking antioxidants such as vitamin E, which interrupts the radical chain reaction of lipid peroxidation [4]. The vitamin E radical that results can be converted back to vitamin E employing reduction by ascorbic acid [4, 5], so vitamin E is a *renewable* chain-breaking antioxidant. Ions of such transition metals as copper and iron are very important in accelerating free-radical reactions, such as the decomposition of lipid peroxides to peroxy and alkoxy radicals and the formation of highly reactive hydroxyl radical from H_2O_2 in the presence of superoxide or ascorbate. Hence, another aspect of preventative antioxidant defence is to keep such metal ions safely sequestered in proteins or to otherwise prevent them from participating in free radical reactions [6–8]. Proteins such as transferrin, lactoferrin and caeruloplasmin are important antioxidants in plasma at physiological pH [6–10] by this mechanism.

If a reactive oxidant is formed in a biological system and can attack a key target (such as DNA or α_1-antiprotease), any other molecule (X) that intercepts that oxidant may be regarded as an antioxidant, provided that X is less important than the target. The molecule X, unlike vitamin E or such enzymes as superoxide dismutase, need not have evolved specifically as an antioxidant. If it has not, then X may be regarded as a *sacrificial antioxidant* [6], but only in that particular situation, i.e. X is destroyed to protect the target. Two examples will illustrate this principle. Tracheobronchial mucus has many functions, including an ability to react with H_2O_2 and hydroxyl radical [11]. Hence, toxic oxidant gases will have to penetrate through this mucus layer to attack the epithelial cells underneath. The mucus is degraded [11] and, while it lasts, it can protect the cell surfaces below it. Hyaluronic acid in the inflamed rheumatoid joint may be degraded by hydroxyl radical attack [2, 12], but hyaluronic acid is easily replenished and its presence may stop the hydroxyl radicals from attacking something more important. This hyaluronic acid degradation is usually regarded as "damage" [2, 12], but it is also possible to think of the hyaluronic acid as being sacrificed to protect more important targets. Uric acid also appears to be an important antioxidant in humans, and part of its action is thought to be a scavenging of singlet O_2, peroxy radicals and hydroxyl radicals, the uric acid being irreversibly degraded into several fragments [13, 14].

Albumin

Albumin is a small, highly-soluble, protein (M_r 69,000) that is present in human plasma at concentrations around 40 mg/ml. Its functions include regulation of osmotic pressure, and transport of a wide range of substances, including fatty acids, bile pigments and some drugs. Its half-life is about 20 days [15]. Since plasma volume is approximately 3 litres, the liver must therefore synthesise about 3 g of albumin per day. Human albumin has specific binding sites for copper ions.

As mentioned above, copper ions are able to accelerate damaging radical reactions. Most plasma copper is attached to the protein caeruloplasmin, which has antioxidant properties [6–9], but there is some non-caeruloplasmin copper, although how much is debatable [16]. A high percentage of plasma non-caeruloplasmin copper may exist bound to albumin. Table 1 shows that albumin, at concentrations less than physiological, is able to inhibit markedly copper-stimulated peroxidation and haemolysis of erythrocyte membranes. A similar inhibitory effect of albumin has been reported in other copper-dependent lipid peroxidation systems [21]. Albumin also inhibits generation of *free* hydroxyl radicals from systems containing copper ions and H_2O_2. The copper ions bind to albumin, some of them being rendered less available [22] for reaction. Some of the bound ions are still accessible to H_2O_2, superoxide and ascorbate, and they can still cause formation of hydroxyl radicals. However, these radicals immediately attack the albumin molecule itself and are not released into free solution [18]. The albumin molecule will be damaged [18, 22–24], but there is so much albumin in the plasma that damage will be biologically insignificant. Damaged albumin may be proteolytically degraded [25] and quickly replaced. Hence, the albumin is protecting targets from copper-dependent hydroxyl radical damage by keeping the copper ions bound to itself. This has been called [6] a "biologically-insignificant site-specific free radical reaction". Albumin does not normally inhibit iron-ion-dependent hydroxyl radical formation, a

Table 1. Effect of albumin on oxidant-generating systems

System studied	Final concentration of albumin present (mg/ml)	% Inhibition by albumin
Copper-ion-dependent generation of ·OH from O_2^- and H_2O_2 [17]	0.2	15
	0.67	44
Copper-ion-dependent generation of ·OH from H_2O_2 only [18]	0.1	57
	0.5	79
Damage to α_1-antiprotease by HOCl [19]	3.3	81
	10.0	95
	16.7	97
Haemolysis accelerated by copper ions plus ascorbate [20]	12 μM*	90

* A 1 μM solution of albumin corresponds to 0.069 mg/ml, assuming M_r to be 69,000.

function that can be performed by transferrin, caeruloplasmin and lactoferrin [6–10]. However, at a concentration of 40 mg/ml (approx. 0.58 mM), it might be able to scavenge some hydroxyl radicals [21].

Activated neutrophils release the enzyme myeloperoxidase, which uses H_2O_2 to oxidize Cl^- into a powerful oxidant that has been identified as hypochlorous acid, HOCl [26, 27]. An important biological target that can be inactivated by HOCl is probably α_1-antiprotease, permitting uncontrolled elastase activity. However, albumin also reacts with HOCl. Table 1 shows that concentrations of albumin less than those normally present in plasma are able to prevent completely inactivation of α_1-antiprotease by HOCl, by preferentially scavenging this molecule. The albumin is damaged by the HOCl, but this is again probably biologically insignificant in view of albumin's high concentration and rapid turnover. This HOCl-scavenging activity may account for the ability of albumin to influence the luminol-dependent chemiluminescence produced by activated neutrophils [28].

Another aspect of the antioxidant action of albumin may be its ability to scavenge peroxy radicals [29], which may partly account for its reported ability to decrease lipoxygenase activity [30]. Peroxy radicals can arise as a result of hydrogen atom abstraction from biological molecules by hydroxyl radical, followed by reaction of the resulting carbon-centred radical with oxygen. Albumin can also bind free fatty acids and protect them from peroxidation [30], although it has little effect on iron-stimulated peroxidation in intact membranes [21].

Other extracellular fluids: is damage always damage?

The albumin concentrations of cerebrospinal fluid, aqueous humour, synovial fluid and lung bronchoalveolar lining fluid are normally far lower than in plasma [15, 31] and so the "antioxidant protection" of albumin is absent. Hence, copper ions are extremely damaging to the eye [32] and stimulate inflammation [33]. Both iron and copper ions may be very damaging to the central nervous system [2], since cerebrospinal fluid appears to lack all forms of metal-binding antioxidant protection [6, 7].

An early event in tissue damage is increased vascular permeability. At sites of inflammation this can be mediated by chemicals secreted by neutrophils [34], that might include oxygen-derived species [35, 36]. An early event in "oxygen toxicity" in lung is alteration of capillary endothelial cells, resulting in alveolar-capillary leaks [37, 38] and an increased protein content in lung extracellular fluids [31, 38]. The synovial membrane shows increased permeability in inflammatory joint disease.

Endothelial modification and increased permeability are often regarded as "damage", and severe biological consequences can certainly follow if too much modification occurs, as when exposure to high O_2 is too prolonged, or in the adult respiratory distress syndrome [37, 39]. Neutrophil infiltration plays a part in the tissue alterations produced in the lung in hyperoxia and in most forms of adult respiratory distress syndrome, and also at sites of inflammation. Activated neutrophils produce superoxide, H_2O_2 and HOCl. If little albumin is present in the fluids surrounding the neutrophils, these species can do damage by generating hydroxyl radical in metal-dependent reactions [2, 40] and by inactivating α_1-antiprotease [26, 27].

One *beneficial* effect of increased vascular permeability will be to increase the extracellular fluid content of proteins such as albumin, transferrin and caeruloplasmin. For example, lung lavage fluids from patients exposed to 95% O_2 contain increased transferrin and albumin [38]. Synovial fluid from inflamed joints contains significant concentrations of transferrin, caeruloplasmin and albumin [21]. Allowing more protein, such as albumin, to cross a membrane barrier may help to prevent excessive damage by oxidants. Hence, as can be argued for hyaluronic acid (see above), apparent "damage" may have some beneficial effect. Perhaps we should not forget that, overall, acute inflammation is a useful process that normally resolves naturally. Increased release of proteins such as albumin and transferrin into the

inflamed site may play some part in the normal self-limiting nature of neutrophil-mediated "damage".

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Note added in proof: Another aspect of the antioxidant action of albumin is raised by recent reports [41, 42] that albumin-bound bilirubin might act as an inhibitor of lipid peroxidation.

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