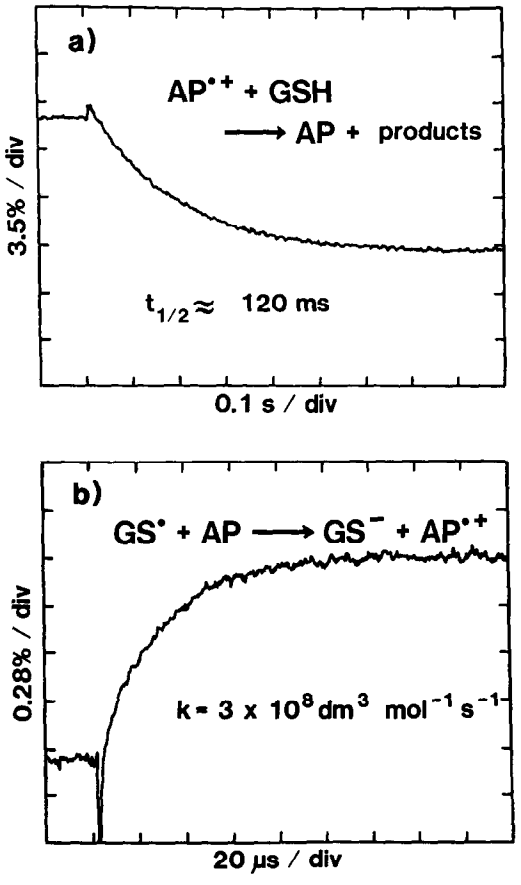

Fig. 1. Oscillograms illustrating transient absorbance changes (ordinate) vs time (abscissae) of aminopyrine cation radical at 570 nm, pathlength = 2 cm: (a) reaction of 13 μM AP^+ with 200 μM GSH in the presence of 0.5 mM AP, 4 mM sodium phosphate, pH 5.6, N_2O saturated, by stopped-flow spectrophotometry, (b) pulse radiolysis of solution containing 2 mM GSH, 100 μM AP and 4 mM sodium phosphate, pH 4.9, N_2O saturated, dose = 3 Gy (≈2 μM radicals).

These studies demonstrate the crucial, reductive role of thiols in redox-cycling oxidizable drugs, and illustrate how reactions which are apparently thermodynamically most unfavourable can be kinetically controlled. An important parallel may be made with the redox cycling of quinones, in which semiquinones (Q^-) are oxidized by O_2:

$$Q^- + O_2 \rightleftarrows Q + O_2^- \quad (9)$$

Since in many biological systems, O_2^- is removed with a half-life of the order of a few tens of microseconds via catalytic disproportionation by superoxide dismutase [10], equilibrium (9) can be driven to the right even if the reduction potential $E(Q/Q^-) > E(O_2/O_2^-)$, i.e. $K_9 \ll 1$. This is possible since the disproportionation of Q^- is much slower than the catalysed decay of O_2^- at likely steady-state concentrations of Q^-. In conclusion, kinetic rather than thermodynamic factors control these redox reactions involving radicals in biological systems.

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Generation of photoemissive species during quinone redox cycling*

The redox cycling of quinones constitutes an oxidative challenge to cells. The field of quinone-induced oxidative injury to cells and tissues has been reviewed (e.g. refs 1 and 2). The generation of reactive oxygen species is thought to be involved in the chemotherapeutic action of quinone anticancer drugs (e.g. ref. 3). We have, in recent years, been interested in the process of the generation of reactive species, taking advantage of their property of photoemissive decay by employing techniques of single-photon counting [4].

The intact hemoglobin-free perfused rat liver was shown to respond to an infusion of a model quinone, menadione (2-methyl-1,4-naphthoquinone), with an increased photoemission [5]. The bulk of the photoemission was in the red spectral region, with about 80% of the intensity being emitted at wavelengths greater than 620 nm; this and other data shown in ref. 5 point to the formation of singlet molecular oxygen during menadione redox cycling in the

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intact cells. Singlet oxygen dimol photoemission is known to occur at 634 nm and 703 nm [6].

The process of redox cycling is a one-electron process. Thus, it is concluded from the above that a certain steady-state level of the intermediate semiquinone radical of menadione is formed in the cell. A further analysis regarding variations of the steady-state level of the semiquinone was performed in liver by manipulating the operation of the two-electron reduction pathway that is also present in the cell. NADPH:quinone oxidoreductase, also known as DT diaphorase, is an enzyme capable of the two-electron reduction of menadione [7]; this two-electron reduction can be considered as an initial reaction in the pathway of quinone detoxication [8, 9]. Using the inhibitor of this enzyme, dicoumarol, we observed an increase in the steady-state level of red photoemission of the liver cells [5]; this is in line with the view that inhibition of the two-electron pathway of reduction favors flux through the one-electron pathway of reduction, which is catalyzed by flavoproteins not inhibited by dicoumarol.

Further support for this thesis came from experiments in which the two-electron pathway was, conversely, increased in its capacity. This was achieved by inducing the amount of NADPH:quinone oxidoreductase. Feeding mice with an inducer of the enzyme, 2(3)-(tert-butyl)-4-hydroxyanisole (BHA), for two weeks, the level of activity is increased about 10-fold [10]. In hepatic postmitochondrial fractions of such pretreated mice, menadione redox cycling was associated with significantly less photoemission as compared to untreated controls [11]. The protection was abolished when dicoumarol was added.

The metabolism of the hydroquinone, menadiol, includes reoxidation to the semiquinone and further back to the quinone, and also it includes the conjugation reactions, sometimes called Phase II reactions. In liver, a major Phase II reaction is the glucuronidation reaction, leading to the menadiol glucuronide at the expense of UDPGA; the glucuronide then is eliminated into the biliary space. Interfering with glucuronidation, using salicylamide, was associated with a substantial increase in menadione-elicited photoemission [12]. This is interpreted as favoring the back-oxidation pathway to the semiquinone by diminishing the rate of menadiol conjugation. Likewise, the limitation of sulfation reactions by perfusing for a time interval without sulfate in the medium led to an increase in photoemission in a reversible fashion. Thus, the impairment of Phase II metabolism may increase the steady-state level of reactive species formed during quinone redox cycling. This could have implications for clinical use, e.g. in conditions in which cellular energy supply is low, so that UDPGA and/or PAPS levels may become rate-limiting for conjugation reactions.

An important further conjugation reaction is that forming the glutathione conjugate. With menadione, the product is the menadione GSH conjugate, 2-methyl-3-glutathionyl-1,4-naphthoquinone, also called thiodione [13]. We have studied some of the properties of this conjugate in biological terms, starting out from an apparent paradox. This consisted of the observation that in liver depleted of glutathione (following pretreatment with phorone) the intensity of photoemission elicited by menadione was significantly lowered as compared to normal glutathione-containing controls [5]. In similar experiments using t-butyl hydroperoxide instead of menadione, glutathione depletion increased photoemission, explainable as a weakening of the glutathione-dependent defense [14].

To resolve this apparent paradox, we synthesized the menadione GSH conjugate and studied its properties in redox cycling using isolated rat liver microsomes. It was found [5] that the glutathione conjugate was capable of redox cycling similar to menadione itself; this may not be surprising, since the quinone structure remains. Thus, the occupancy of the 3-position of menadione has little influence on the redox properties, as supported by a similar redox potential, −203 mV and −192 mV for the midpoint potential of menadione and thiodione, respectively (P. Wardman, personal communication). The conjugation of menadione with GSH, therefore, is not considered to be a detoxication as such [5, 15]; in fact, it may serve to facilitate transport across the canalicular membrane into the biliary space, carrying a redox cycler away from the hepatocyte.

Using the menadiol GSH conjugate and studying its autoxidation, we found that photoemission attributable to singlet oxygen was observed only when GSH was added to the cuvette [16]. Likewise, generating $O_2^{\cdot-}$ from the xanthine oxidase reaction, we found singlet oxygen formation only when GSH was present [16]. Thus, GSH reacts with $O_2^{\cdot-}$ or its protonated form, $HO_2^{\cdot}$, to yield reactive species which are suggested to derive from the GS· and, aerobically from GSOO·. A final end-product, glutathione sulfonate, was detected in addition to GSSG [16]. Thus, the autoxidation of the hydroquinone or the xanthine oxidase reaction, both yielding $O_2^{\cdot-}$, do not lead to singlet oxygen, but will do in the presence of GSH. Recently, the lack of singlet oxygen formation in the xanthine oxidase reaction was confirmed [17]; no GSH was added in that study.

In summary, photoemissive species were detected during the redox cycling of menadione and they were attributed to singlet oxygen. Conditions favoring a rise in the steady-state level of the semiquinone, such as an inhibition of the 2-electron reduction by DT diaphorase or an inhibitor of the glucuronidation or sulfation of the hydroquinone, increased the level of photoemissive species. Conversely, conditions favoring a decrease in the semiquinone level, such as an induced amount of cellular activity of DT diaphorase following induction with BHA, decreased photoemission. Glutathione conjugate formation is not in itself a detoxication reaction, since the 1- and 4-positions of the quinone are not blocked; in fact, the level of photoemissive species is higher in GSH-containing cells than in GSH-depleted cells. Finally, it may be noted that in a recent study on the oxygen dependence of menadione-elicited O_2 uptake, it was found that more than 90% of the extra O_2 uptake due to menadione can be attributed to the shunting of electrons in the mitochondrial respiratory chain, whereas the remaining minor portion of O_2 uptake can be attributed to redox cycling [18]; this smaller portion of O_2 uptake, however, may be the critical one regarding biological effects such as toxicity or carcinogenic and therapeutic effects.

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