in none of the alcoholic animals was the liver disease seen to develop beyond the stage of fatty liver which is in agreement with the findings of Lieber [14] in this respect.

In summary, the lack of consistency in the raised alkaline phosphatase levels, the apparent effect of the liquid diet on the enzyme levels and the presence of an 'ageing' effect would indicate that, of the two enzymes investigated in the present study, GGT provides the most useful and sensitive index of alcohol consumption for use in a long term alcohol study. The fact that the GGT appears to be completely unaffected by the composition of the liquid diet also argues in favour of its use in such studies.

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Effect of cumene hydroperoxide or hypoxia-reoxygenation on glutathione status in guinea-pig heart

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Hypoxia-reoxygenation induces oxidative damage [1]. To examine the mechanism by which oxidative injury occurs, cumene-hydroperoxide (Cum-OOH) was used in the guinea-pig heart. Guinea-pig heart was selected because while it is devoid of selenium dependent glutathione peroxidase activity [2] it possesses selenium independent activity (GPD), known as glutathione transferase of class alpha [3].

Materials and methods

The hearts were excised and mounted on Langendorff apparatus as described elsewhere [4]. The perfusion medium was a modified Krebs–Henseleit bicarbonate solution at 37° and pH 7.4 gassed with 97% O_2:3% CO_2 (pO_2 650 mm Hg) and containing 5 mM glucose (normal perfusion). The experimental time course consisted of 45 min of aortic perfusion (about 6 mL/min) followed by 60 min of normal perfusion or by 30 min of perfusion with different concentrations of Cum-OOH plus 30 min of perfusion with Cum-OOH-free buffer (washout); or 30 min of perfusion with a glucose-free medium gassed with 97% N_2:3% CO_2 ($pO_2 < 50$ mm Hg) (hypoxia); then hearts were returned to the normal perfusion for 30 min (reoxygenation).

Developed and resting tension, coronary pressure and surface electrocardiogram (ECG) were measured. The lactate dehydrogenase activity (LDH) was measured in the effluent as previously described in Ref. 5.

After hypoxia-reoxygenation and Cum-OOH perfusion and washout or 60 min of normal perfusion, malondialdehyde (MDA) was measured in frozen tissue as previously described in Ref. 6.

Glutathione transferase (GST), thioltransferase (TT), GPD and glutathione reductase activities (GSSG red) were assayed in 14,000 *g* clear supernatant [7–10], respectively, with minor modification. Reduced glutathione (GSH) and oxidized glutathione (GSSG) were measured in the supernatant obtained after trichloroacetic extraction of samples [11, 12]. Proteins were determined by the method of Bradford [13].

Data (mean ± SE) were subjected to variance analysis and Bonferroni multiple comparison test. Ventricular arrhythmias were compared by chi square test. A P value of 0.05 was regarded as significant.

Results and discussion

Figure 1 shows that Cum-OOH and washout decreased developed tension and increased resting tension as does hypoxia followed by reoxygenation. The effect on devel-

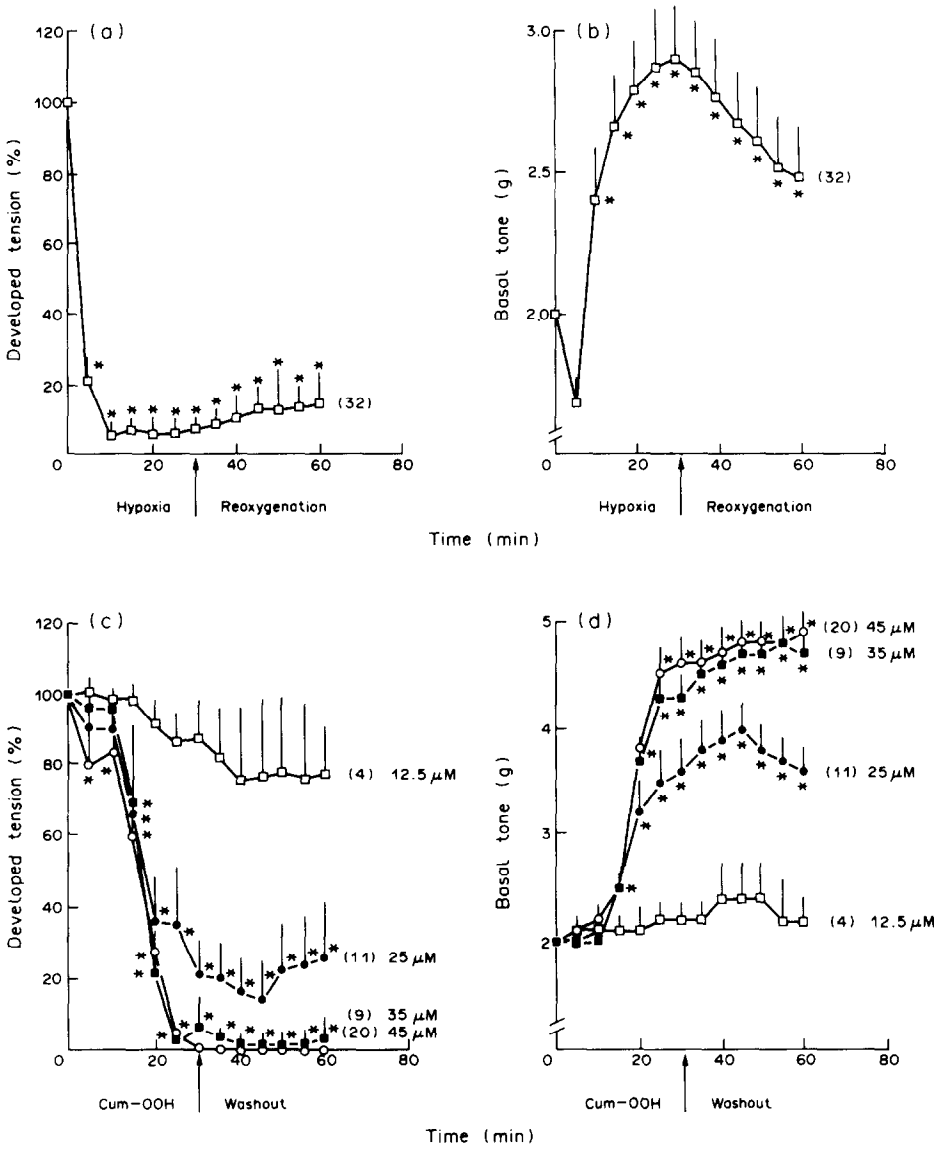

Fig. 1. Effects of hypoxia and reoxygenation or Cum-OOH treatments on mechanical activity of isolated perfused guinea-pig heart. Each point is the mean ± SE, the number of experiments is given in parentheses. * Indicates significant differences from control.

oped and resting tension is parallel and an irreversible phenomena. After 5 min of hypoxia, coronary pressure decreased from 51 ± 1 to 36 ± 2 cm H_2O ($N = 32$, $P < 0.05$). Then it increased and reached 67 ± 3 cm H_2O ($N = 32$) at the end of reoxygenation. Cum-OOH did not significantly change coronary pressure.

The ECG changes mediated by hypoxia-reoxygenation and Cum-OOH perfusion plus washout were different. In the former, during hypoxia, atrio-ventricular block was always present as ventricular arrhythmias classified according to Lambeth conventions [14]. Reoxygenation induced an increase in the incidence (%) of ventricular fibrillation from 29 to 48 ($P < 0.05$); ventricular tachycardia from 22 to 25 and ventricular premature beats from 55 to 100 ($N = 32$, $P < 0.05$).

Cum-OOH (45 μM) produced an enlarged QRS complex but ventricular arrhythmias were rare both during Cum-OOH perfusion and washout. Cum-OOH dose-dependently increased LDH release (Fig. 2).

Cum-OOH induced heart failure and LDH release were manifested after a lag time. This may be caused by several factors, including the time required to overwhelm the cell's ability to metabolize peroxide or that required to accumulate a sufficient amount of lipid peroxides to induce membrane permeability changes. However, the lag time is longer for LDH release in comparison with functional damage. While the level of functional damage is dependent on the dose of Cum-OOH, the lag time is not.

Hypoxia-reoxygenation led to a light increase in MDA production (22.2 ± 3.9 nM TMP/g wet wt, $N = 8$). Cum-OOH (35 μM) plus washout doubled MDA formation (Table 1). Possibly the MDA formation may be a significant indicator of lipid peroxidation only when the flux of peroxide is high. In the rat heart, 300 μM Cum-OOH increased MDA formation but no increase was observed after reoxygenation [15]. GSH, expressed as μmol/g tissue fell from 1.53 ± 0.05 ($N = 8$) to 1.21 ± 0.08 ($N = 8$, $P < 0.01$) in the reoxygenated heart. Cum-OOH (12.5, 35, 45 μM) plus

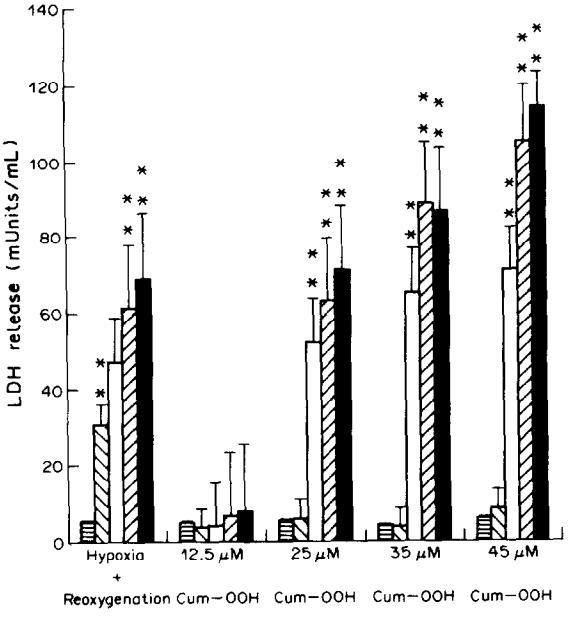

Fig. 2. Changes in LDH release in the perfusate during last 5 min of stabilization (▤), 10–15 min (▧) and 25–30 min (□) of hypoxia and Cum-OOH treatment, 5–10 min (▨) and 15–20 min (■) of reoxygenation and washout. Values are mean of at least four experiments. **P, compared to each stabilization period was <0.01.

Table 1. MDA formation in ventricle obtained by isolated guinea-pig heart perfused with Cum-OOH for 30 min and with normal saline solution for 30 min

Treatment		nM TMP/'g wet wt
None	(6)	17.7 ± 2.6
Cum-OOH 25 μM + washout	(4)	22.8 ± 3.6
Cum-OOH 35 μM + washout	(4)	34.1 ± 4.2*

Each value is the mean ± SE; the number of experiments is given in parentheses.
* $P < 0.05$.

washout dose-dependently decreased GSH from 1.53 ± 0.05 (N = 8) to 1.35 ± 0.02 (N = 4), 1.00 ± 0.07 (N = 5, $P < 0.01$) and 0.98 ± 0.05 (N = 7, $P < 0.01$), respectively. GSSG expressed as nmol/g tissue was decreased from 40.1 ± 4.4 to 25.8 ± 1.8 ($P < 0.05$) after reoxygenation. Cum-OOH (12.5, 35, 45 μM) plus washout reduced it to 29.9 ± 4.0, 26.6 ± 4.5 and to 24.1 ± 1.5 ($P < 0.05$), respectively.

The enzymatic activities, measured in eight normal perfused hearts expressed as nmol/mg protein/min were: GPD 28.2 ± 3.3, GSSG red 36.8 ± 3.7, TT 14.9 ± 3.0, GST (using CDNB and ethacrynic acid as substrate) 253 ± 24 and 15.7 ± 2.6, respectively. These values are similar to those reported in the rabbit heart [16] but GPD was lower and GSSG red was higher than in the rat heart [17]. The enzymatic activities were not changed by both experimental procedures.

The fall in the GSH level was constant in hypoxia-reoxygenation and ischemia-reperfusion [15–17] and Cum-OOH treatment [18]. But a depletion in GSSG was never seen in the rat heart; in fact, GSSG was increased by ischemia-reperfusion and hydroperoxide treatment [17, 18]. In the rabbit heart, GSSG was reduced by ischemia-reperfusion although not statistically [16]. The enzymatic pattern of the guinea-pig heart may explain the decrease in GSSG. The alteration of membrane permeability could increase the release of GSSG and GSH, capable of inducing a reduction in tissue content. The GSSG may be transported outside the cell by a saturable process [9]. In summary, hypoxia and reoxygenation and μM Cum-OOH administration induced heart failure and an increase in membrane permeability. Both treatments induced a depletion in GSH and GSSG. Hypoxia-reoxygenation produced more severe arrhythmias but did not induce any significant changes in MDA.

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Antimalarial activity of substituted anthraquinones

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Most antimalarial agents can be divided into two classes based on their speed of action [1]: (a) cinchona alkaloids, aminoquinoline, 9-aminoacridine derivatives, and (b) sulfa compounds of pyrimidine and biguanine derivatives which interfere with the conversion of dihydrofolic acid (FH_2) to tetrahydrofolic acid (FH_4), and thus inhibit the synthesis of purines and pyrimidines.

It has been suggested that in addition to these two classes, substantial evidence clearly indicates that malaria parasites may impair oxidant defence and repair functions of host erythrocytes through a number of mechanisms [2–7]. According to this view it seems likely that oxidant agents or alternatively agents that generate reactive oxygen, could damage parasitized red cells. This phenomenon seems to be related to the interference of malaria parasites with cellular oxidant repair mechanisms [2–7].

Aldehydes [8], diketones [9, 10], nitro compounds [11, 12] and apparently also substituted aminoalkylamino anthraquinones may generate reactive oxygen and thus inactivate malaria parasites (*Plasmodium falciparum, P. vinckei* and *P. berghei*). It has already been demonstrated that aminoalkylamino-anthraquinones exhibit a broad spectrum of activities such as anticancer [13–17], antiviral [18], antibacterial [13], antileishmania [19] and antiamebic [20] activity, together with their inhibitory effect on bovine serum amine oxidase [21]. Aminoalkylamino-anthraquinones intercalate [22–24] with DNA or form free radicals [25–28]. In addition the products formed by oxidizing naturally occurring polyamines by bovine serum amine oxidase [29, 30] or the purified aminoaldehyde products exhibit antimalaria activity [31].

Thus it is most likely that anthraquinone skeletons composed of aminoalkylamino or polyamino side chains should represent promising antimalarial model compounds.

In the light of the growing problem of drug resistant malarial parasites, the development of new drugs is of primary importance. In the present study we tested the effect of various anthraquinone derivatives on the growth and DNA synthesis of the malarial parasite (*P. falciparum*).

Materials and methods

Table 1 shows the structure of the substituted anthraquinones investigated in this study. The preparation of these compounds has been described elsewhere [18].

Parasites. Plasmodium falciparum (FCR 3TC) was cultured according to the candle jar method [32]. Parasites were synchronized by the combination of sorbitol lysis [33] and gelatin sedimentation [34]. Parasitemias and stage distribution were estimated from Giemsa stained smears by counting 5×10^3 erythrocytes and 5×10^2 infected cells, respectively.

Hypoxantine incorporation. Cultures of *P. falciparum* with an initial parasitemia of 10–12% ring form stage, were distributed into 96 microculture trays (100 μL/well) incubated with the drugs dissolved in RPMI-1640 medium (GIBCO) at final concentrations of 1, 10 and 100 μM. After 22–24 hr [34] hypoxanthine was added to each well (1 μCi per well sp. radioactivity 10 Ci/μmol, New England Nuclear, Boston, MA) and incubation continued for another 7 hr. Thereafter, cultures were harvested by a Microtiter Dynateck Autowash cell Harvester, using 934-H glass filters. Filters were dried and radioactivity was determined by liquid scintillation counting.

Distribution of different stages. P. falciparum parasites were grown as above with an initial parasitemia of 1.5–2.0%. When cultures reached the mature trophozoite stage, drugs at final concentrations of 10 and 100 μM were added to each microwell. After 24 hr, the distribution of different