Cilia beating frequency and percentage of normal epithelium in tracheal ring cultures after termination of a 2-h in vitro exposure to singlet oxygen

| Treatment | Cilia beating frequency[a] | | | | Mean percentage of normal epithelium[b] | |
|---|---|---|---|---|---|---|
| | 0 time | | Post-exposure | | 0 time | Post-exposure |
| | Mean | SE | Mean | SE | | |
| Control | 1238 | 4 | 1206 | 6 | 94 | 87 |
| Singlet oxygen (0.121 ppm) | 1244 | 4 | 847* | 38 | 97 | 43** |

[a] Values represent mean ± SE from 16 ring cultures (64 determinations). Cilia beating frequency measured as beats per min; [b] Mean data from 16 separate ring cultures; * Significantly different from both 0 time baseline values and the blank 2-h post-exposure ($p < 0.05$; Dunnett's test[7]); ** Significantly different from 0 time baseline values and the blank 2-h post-exposure ($p < 0.05$; $\chi^2$ distribution test).

treated cultures, the general appearance of the epithelium no longer had its sharp outline; it had a swollen appearance with some sloughing of epithelial cells. Ciliary arrest was observed in approximately 25% of the epithelium. In this area, normal cellular morphology was observed.

The singlet oxygen induced alterations in the ciliated respiratory epithelium are different from other environmental toxicants previously studied, since both cytopathology and ciliostasis were observed. Exposure of tracheal cultures to 2 ppm $NO_2$ for 1.5 h/day for 5 consecutive days caused reduced ciliary activity without significant changes in morphology[6]. Since the mucociliary escalator depends on the functioning of the ciliated epithelium and rate of mucus transport, exposure to singlet oxygen could cause ciliary dysfunction and epithelial damage resulting in an altered host defense system. Although the mechanism of $^1O_2$-induced damage remains to be elucidated, these data reflect an important physiological response that warrants further investigation. To the best of our knowledge, this is the first report on $^1O_2$-induced damage to respiratory tract epithelium.

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## Inhibition of platelet ADP and serotonin release by carbon monoxide and in cigarette smokers[1]

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*Summary.* The release of $^{14}$C-serotonin by ADP, epinephrine and arachidonic acid and the release of ADP by kaolin were measured in normal platelets in the presence and absence of carbon monoxide and in smokers' platelets. It is shown that carbon monoxide inhibits significantly the platelet release reaction. This function is also decreased in platelets obtained from heavy cigarette smokers.

The incidence of atherosclerosis is significantly enhanced in heavy cigarette smokers[2-4]. Platelet aggregation is also enhanced in this population[5-7]. This enhancement of platelet aggregation has been suggested as a possible cause for vessel wall endothelial cell damage[8-10].

Contrary to the previous findings by others, our recent work has shown that platelet aggregation is decreased in the presence of cigarette smoke and carbon monoxide (CO)[11]. Because aggregation and release reaction are not necessarily linked[12,13], we have investigated the platelet release reaction in platelets obtained from smokers compared to non-smokers and in normal platelets (obtained from non-smokers) in the presence and absence of CO.

*Methods.* Blood was obtained from healthy cigarette smokers and non-smokers who were not on any drugs known to affect platelet function. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared as reported previously[11]. Carboxyhemoglobin (CO-Hb) levels were determined on a Co-oximeter 282, Instrumentation Laboratory. Platelet counts were obtained on an Ortho ELT-8 cell counter.

Serotonin release was measured using a modified technique of Valdorf-Hansen and Zucker[14]. The PRP was incubated for 20 min at 37 °C with $5.2 \times 10^{-4}$ μCi/ml $^{14}$C-serotonin with a specific activity of 58.5 μCi/mmol. Volumes of 0.5 ml PRP were placed in glass tubes (according to the original method[14]) and incubated for 30 min at room temperature in the presence of CO or air which was bubbled through the sample. A releasing agent (1.2 μmol of ADP, 1.3 μmol of arachidonic acid (AA) or 0.022 μmol of epinephrine (EP) in 0.05 ml) or 0.05 ml of non radioactive PPP for the control sample, was then added to every tube. The remainder of the procedure was the same as described[14]. The percent of $^{14}$C-serotonin release was calculated as follows: (sample (cpm) − control (cpm))/sample (cpm) × 100. The control sample was taken as zero percent released. Samples were prepared in duplicate or triplicate and the results averaged.

Serotonin release in smokers and non-smokers was determined as above except that samples were not preincubated with CO or air.

ADP release, (by exposure to kaolin), was measured in the

presence and absence of CO by a modified method of Weiss[15]. 1 ml of PRP was incubated with CO or air for 30 min. The procedure was then carried out as described[15] with minor modifications. The extent of aggregation (measured in mm) induced by the PPP supernatant preincubated with air was taken arbitrarily as 100% ADP release.

The percent of the extent of aggregation induced by the PPP supernatant preincubated with CO was calculated as follows: (+CO sample (extent of aggregation)/−CO sample (extent of aggregation)) × 100. Samples were tested a minimum of three times and the results were averaged.

ADP release in smokers and non-smokers was measured as above except that samples were not incubated with CO or air. To correct for differences in platelet counts between smokers and non smokers, the extent of aggregation was calculated in mm/$10^5$ platelets.

*Results.* Serotonin release is significantly decreased in smokers' platelets compared to non-smokers' platelets (control) when EP and ADP are used as aggregating agents. This decrease in release is also present when non-smokers' platelets are incubated with CO. When AA is used as an aggregating agent, the release of serotonin is not affected whether the platelets are obtained from smokers or non-smokers with or without CO incubation. Figure 1 shows that the weighted mean of the percent of $^{14}$C-serotonin released using EP is 41.92 ± 8.35 in non-smokers, 24.18 ± 6.70 in non-smokers in the presence of CO and 9.31 ± 1.78 in smokers' platelets. Using ADP the percent of $^{14}$C-serotonin released is 34.72 ± 4.63 in non-smokers, 24.32 ± 5.35 in non-smokers' platelets exposed to CO and 13.58 ± 4.08 in smokers. When AA is used the percent released in non-smokers' platelets is 45.25 ± 2.87, 46.61 ± 2.78 in non-smokers in the presence of CO and 48.56 ± 4.89 in smokers. ADP release from normal platelets was measured in the presence and absence of CO by exposure of platelets to kaolin. The extent of aggregation of normal platelets induced by the ADP which was released from the platelet samples exposed to CO is 25.76 ± 4.61% less than that of samples exposed to air (control) (fig. 2).

The release of ADP from smokers' platelets was measured in 7 smokers and 8 non-smokers. The extent of aggregation induced by the ADP released from smokers' platelets is 4.3 ± 0.3 mm/$10^5$ platelets, whereas in non-smokers it is 6.6 ± 1.1 mm/$10^5$ platelets (fig. 3).

*Discussion.* Figures 1 and 2 demonstrate that platelets exposed to CO release significantly less serotonin and ADP except when AA is used in serotonin release. Our previous work has shown that when CO is present, platelet aggregation using AA is significantly inhibited, while ADP induced aggregation is unaffected[11].

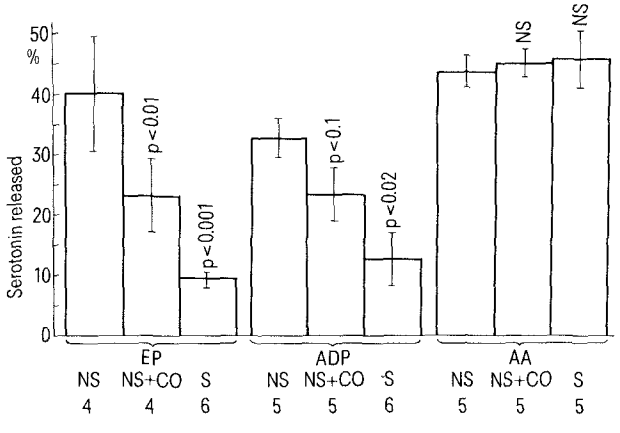


Figure 1. Platelet serotonin release in smokers (S), non-smokers (NS) and in non-smokers in the presence of CO (NS + CO). Percent serotonin released in PPP supernatant using epinephrine (EP), adenosine 5'-diphosphate (ADP) and arachidonic acid (AA) as releasing agents. Results are expressed as weighted mean and SE. Numbers below are number of experiments; numbers above columns indicate probable significance after analysis by paired T-test (NS + CO compared to NS) and students T-test (S compared to NS).

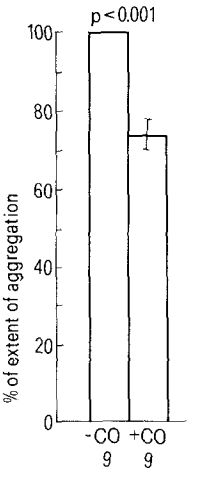


Figure 2. Extent of aggregation of normal (non-smokers') platelets induced by the ADP which was released from another platelet sample (same donor) preincubated with or without CO and then exposed to kaolin. The percent of the extent of the +CO sample is a percentage of the −CO sample (taken as 100% arbitrarily). Results are expressed as weighted mean and SE. Numbers below are number of experiments; numbers above columns indicate probable significance after analysis by Student's t-test.

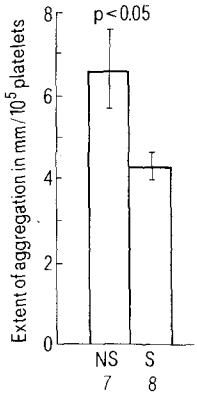


Figure 3. Extent of aggregation of normal (non-smokers') platelets induced by the ADP which was released from smokers' (S) and non-smokers' (NS) platelets by exposure to kaolin. Results are expressed as weighted mean and SE. Numbers below represent number of experiments; numbers above columns indicate probable significance after analysis by Student's t-test.

It has been reported that aggregation is not necessarily a consequence of secretion[13]. Arachidonic acid can cause platelets to release without aggregation[12]. Therefore, our results are consistent with previous work in showing the lack of dependency of aggregation on secretion or vice versa.

Figures 1 and 3 show that serotonin and ADP release, respectively, is also decreased in smokers' platelets but not when AA is used. To insure that CO was the prime factor in cigarette smoke which caused a decrease in serotonin release we measured the reaction in the presence of various concentrations of nicotine. Nicotine had no effect on serotonin release (not shown here).

There was no good correlation between the blood CO-Hb level and the release reaction since smoker's blood CO-Hb level depends on the time of smoking and blood collection whereas the platelet abnormality persists for at least several hours[11].

Mechanistically, CO may inhibit thromboxane synthesis by binding to certain heme enzymes, such as cyclooxygenase, which in turn inhibit prostaglandin synthesis[16]. Carbon monoxide may also react with cytochrome P-450 to inhibit its peroxidase activity in the transformation of AA to endoperoxides and thromboxane $A_2$[17]. In this respect, Moroff and Kosow[18] have shown that the inhibition of a platelet membrane NADH dependent reductase (cytochrome C reductase) which transfers reducing equivalents to cytochrome P-450 and NADH cytochrome $b_5$ reductase[17], inhibited aggregation.

Carbon monoxide may also bind to copper containing enzymes[19] which may be involved in prostaglandin synthetase activity[20].

According to previous workers[21] aggregation is dependent on

thromboxane synthesis in the following manner: ADP < EP < AA. Our previous work[11] agrees with this, if in fact CO inhibits thromboxane synthesis, since CO inhibits platelet aggregation as follows: ADP < EP < AA.

Our in vitro experiments may, however, not be directly comparable to the in vivo situation possibly due to the synergistic actions of many aggregating agents with ADP[22] since ADP release, although inhibited in the presence of CO and in smokers is not absent. Also important is that serotonin release by AA is unaffected. Rao and White's finding[23] that drug-induced defects in platelet prostaglandin synthesis may be compensated for by membrane modulation and receptor cooperativity also complicates the extrapolation of in vitro obtained data to in vivo situations.

The presence of deaggregating agents as well as the alteration of platelet sensitivity towards them is another complication. Recently Pittilo et al.[24] have shown that prostacyclin production in vitro is decreased in smoking rats. Diminished prostacylin formation has also been shown in umbilical arteries of babies born to smoking women[25]. Our data showing that serotonin release is inhibited in smokers may support this, since serotonin can act as a coenzyme for prostaglandin biosynthesis[26]. Recent data from other workers have suggested that smokers' platelets may be less sensitive to prostacyclin[27].

Therefore, although platelet aggregation and the release reaction are generally decreased in the presence of CO and in smokers, they may not reflect the in vivo situation. Atherosclerosis in smokers probably occurs due to a combination of interacting factors for which no one in vitro experiment can give a clear understanding.

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## Metabolic implications in the elevation of serum activity of intestinal alkaline phosphatase in chronic renal failure

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*Summary.* The activity of intestinal isoenzyme of serum alkaline phosphatase was evaluated in 21 non-dialyzed patients with advanced renal failure and in 52 patients on regular hemodialysis. In patients without hepatopathy, a significant inverse correlation was found between the enzyme activity and serum calcium levels. Hepatopathy was the most significant variable influencing the enzyme activity in patients on dialysis. Secondary hyperparathyroidism and a decreased rate in enzyme elimination should be assessed for the above-normal activities of intestinal ALP in serum in chronic renal failure.

Elevation in the serum activity of intestinal isoenzyme of alkaline phosphatase (EC 3.1.3.1) has been described in patients on regular hemodialysis[1-3]. The present investigations were designed to determine the factors which influence the changes in the enzyme activity in chronic renal failure.

*Patients and Methods.* Measurements were made in 21 non-dialyzed patients with advanced renal failure (creatinine clearance mean ± 2 SD range, 35 ml/min, and 13–85 ml/min, respectively) and in 52 patients on regular hemodialysis. No patients received phosphate binders, vitamin D or its derivatives, anticonvulsants or corticosteroids (except for the transplant patients and patients with an active hepatitis). In 37 patients (35 on dialysis), a chronic hepatopathy was documented by a previous history of acute hepatitis and serial evaluation of liver tests. The dialysis calcium concentration was 2.3 mmol/l. Nine patients had a previous history of a non-successful renal transplantation. The control group consisted of 40 adults without evidence of renal, hepatobiliary, or bone disease. Blood samples were drawn in the morning after a fasting period of approximately 8 h, in patients on dialysis before the dialysis. Informed consent was obtained from all the patients.

The activity of the intestinal, liver and bone isoenzyme of serum alkaline phosphatase (ALP) was determined with 4-nitrophenyl phosphate as substrate[4]. The total concentration of calcium in the serum was determined with methylthymol blue and