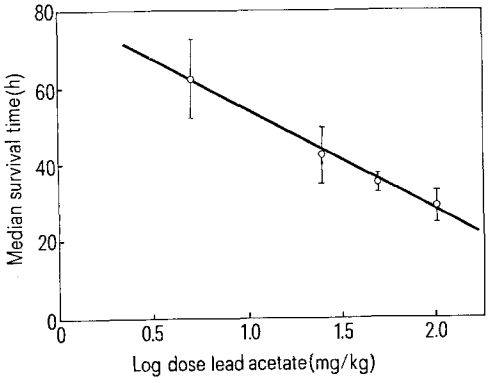
Synergism in the Toxicities of Lead and Oxygen

Although oxygen is one of the most abundant elements in nature, and is essential for most life processes, it can also be highly toxic. Therefore, any interaction between oxygen and potential environmental contaminants is of interest; especially, if that interaction results in enhanced toxicity. One such possible contaminant is lead, and this report demonstrates that its administration significantly shortens the survival time of rats exposed to increased partial pressures of oxygen.

Under light ether anesthesia femoral cutdowns were performed on male Sprague-Dawley rats weighing 180–220 g, and then either 20 mg of lead acetate dissolved in 0.5 ml deionized water, or deionized water alone was injected i.v. The rats were next placed in specially constructed chambers in groups of 8 each, and exposed to 100% oxygen (1 atm) at a flow rate of 1 l/min. The oxygen concentration in each chamber reached 100% after 2.5 h, and remained at that level for the duration of the experiment. The humidity was maintained at 100%, and the temperature at 23 °C. The animals were given free access to food and water, and were observed at hourly intervals. The median survival time was determined for each group of 8 rats. The mean median survival time in hours was 29.0 $\pm$ 4.4, and 79.0 $\pm$ 15.7 for 3 groups of lead, and 3 groups of non-lead treated rats, respectively ($p < 0.01$). Furthermore, this effect of lead acetate on survival time is dose dependent (Figure). No deaths were observed in any animals treated with lead acetate and exposed to air under otherwise identical conditions, nor did the administration of equimolar acetate in the form of the sodium salt have any effect on survival in 100% oxgyen.



Logarithmic dose response curve for the effect of a single i.v. injection of lead acetate on the median survival time of rats in 100% oxygen at 1 atm. The logarithm of the dose of lead acetate given in mg/kg of body weight is plotted on the abscissa. Each point represents the mean $\pm$ the standard error of the median survival times from 2 separate experiments involving 8 rats each.

The in vivo toxicity of oxygen at 1 atm has been attributed primarily to pulmonary damage[1]. In order to ascertain if the effect of lead was simply an acceleration of this process, some lead, and non-lead treated animals were autopsied after 24 h of oxygen exposure. Sections of brain, heart, lung, liver, kidney, and adrenal gland were taken for histological examination. No significant pathological changes were observed in any of these organs. Still, it is possible for functional changes to occur prior to histological ones. Therefore, in order to measure pulmonary function blood gas determinations were made on samples taken from lead, and non-lead treated rats after exposure to either air, or 100% oxygen. No significant differences were observed after 24 h of exposure, even though the lead treated, oxygen exposed animals were within a few hours of death at the time the samples were taken. On the other hand, non-lead treated rats exposed to oxygen for 68 h, and also near death, were severely hypoxic, acidotic, and hypercarbic ($p < 0.005$).

The central nervous system toxicity of high partial pressures of oxygen is impressive, and has been well documented[2]. In the present study, rats were subjected to 4 atmospheres of oxygen (Table). When lead acetate was given 1 h prior to oxygen exposure, there was no change in the time of exposure required to produce convulsions, or in the survival time. However, when it was given 18 h prior to exposure, while there was still no significant change in the time of appearance of convulsions, the survival time was significantly shortened ($p < 0.001$).

Thus, it is apparent that, under the conditions described, lead and oxygen are capable of acting synergistically to hasten death. The mechanism of this interaction has not been delineated. However, two of the most commonly measured parameters of oxygen toxicity, pulmonary damage, and convulsive activity, are not significantly affected by lead administration. It has been previously demonstrated that copper and iron, which are also heavy metals, enhance oxygen toxicity in vitro[3]. Unlike lead,

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[2] J. W. Bean, in *Oxygen in the Animal Organism* (Eds. F. Dickens and E. Neil; Pergamon Press, London 1963), p. 455.
[3] N. Haugaard, Physiol. Rev. *48*, 311 (1968).

Effect of lead pretreatment on convulsions and survival in hyperbaric oxygen

Lead acetate injection (100 mg/kg)	Time to exposure (h)	Time to convulsions (min)	Survival time (min)
+	1	152 $\pm$ 31	216 $\pm$ 61
–	1	131 $\pm$ 55	270 $\pm$ 95
+	18	123 $\pm$ 47	146 $\pm$ 35
–	18	136 $\pm$ 45	265 $\pm$ 76

Rats were maintained in air at ambient pressure with free access to food and water until the beginning of exposure. Exposure was begun at the time indicated by placing the rats in groups of 5 each in a small animal hyperbaric chamber (Bethlehem Corp., Bethlehem, Pa.) containing soda lime to prevent carbon dioxide accumulation. The chamber was pressurized to 4 atm absolute of oxygen. The animals were observed continuously, and the times at which the first convulsion, and at which death occurred, were recorded. Values are given as the mean $\pm$ the standard error for groups of 10 rats each.

these metals undergo reversible oxidation-reduction reactions under physiological conditions, and are capable of catalyzing autoxidations. Our observations with lead indicate that this capability is not necessary for the enhancement of oxygen toxicity. In addition, they raise the possibility that lead may interact with other oxidative agents in ways that are harmful to living organisms.

Zusammenfassung. Nachweis, dass i.v. Injektion von Bleiacetat die Überlebenszeit von Ratten, die reinen Sauerstoff atmen, dosisabhängig um 50% verkürzt, was sowohl bei 1 als auch bei 4 atm Sauerstoffpartialdruck beobachtet wird.

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1β-Hydroxylation of D-Norgestrel and Norethisterone by *Botryodiplodia malorum*

In the course of metabolic studies of the progestational agents norgestrel[1] (DL-13β-ethyl-17α-ethynyl-17β-hydroxygon-4-en-3-one, DL-I) and norethisterone (D-19-nor-17α-ethynyl-17β-hydroxyandrost-4-en-3-one, II) it was suggested that the 1β-hydroxy derivatives might be possible metabolites[2,3]. This led us to consider the preparation of D-1β-hydroxynorgestrel (D-1β, 17β-dihydroxy-13β-ethyl-17α-ethynylgon-4-en-3-one, III) and 1β-hydroxynorethisterone (D-19-nor-17α-ethynyl-1β,17β-dihydroxyandrost-4-en-3-one, IV).

Microbiological 1β-hydroxylation of 19-norsteroids has been reported with *Aspergillus ochraceus*[4] and *Botryodiplodia malorum*[5–7]; we felt, therefore, that this microbial transformation might give ready access to the desired compounds III and IV. Since 1-hydroxylated Δ^4-3-one-19-norsteroids are easily converted to their ring A aromatic congeners by base[4,5], we found through analysis of base treated solvent extracts by thin layer chromatography that *B. malorum* was the culture of choice. The experiments were designed primarily for the isolation of III and IV; other products were investigated only if they were isolated in the course of achieving this objective.

Incubation of D-norgestrel (D-I) with *B. malorum* CBS 134.50 gave the 1β-hydroxy analog III as the major product. With norethisterone (II) as steroid substrate, the 1β-hydroxy derivative IV was a very minor product, while the major product was hydroxylated at C-11β (D-19-nor-17α-ethynyl-11β,17β-dihydroxyandrost-4-en-3-one, V).

For the conversion of D-I, the inoculum was grown in shaken flasks containing a corn steep liquor: peptone: dextrose medium for 72 h at 28°. Mycelial transfers were made to a 14 l fermentor with 8 l of growth medium. After 24 h incubation the cells were filtered off and suspended in distilled water. The steroid D-I, 1.4 g added in ethanolic solution, was incubated for 71 h before harvest. The mycelium was filtered off, and the filtrate was extracted with ethyl acetate. The dried solvent extracts, dissolved in acetone, afforded 1.08 g of a crude mixture containing 5 products.

This material was chromatographed on preparative silica gel thin layer plates in chloroform: ethanol: acetone (8:1:1). The area containing the desired product was eluted and yielded, after charcoal treatment and recrystallization from acetone, 601 mg of product III, m.p.

[1] Norgestrel is a racemate (DL-I); D-norgestrel (D-I) corresponds in absolute configuration to the natural form of steroids.
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[9] U.S. Patent No. 2, 744, 122 (1956).

NMR assignments and optical properties of transformation products

	$[\alpha]_D$	$OH\Delta M_D^{OH}$ or $OAc\Delta M_D^{OAc}$	C-13 or C-18 Me Signal[a]	C-2 Protons	C≡CH Proton	1α or 11α Proton	C-4 Proton	Acetoxy Me Signal
D-1β-Hydroxynorgestrel (III)	−145.1[b]	−344[c]	0.95 *t* (7)	2.50 d (4.8)	2.52 *s*	4.40 *m*	5.85 *s*	
D-1β-Acetoxynorgestrel (III a)	−144.0[d]	−400[c]	1.02 *t* (7)	2.60 d (4)	2.62 *s*	5.61 *m*	5.98 *s*	2.03 *s*
1β-Hydroxynorethisterone (IV)	−153.7[b]	−397[e]	0.93 *s*	2.50 d (4)	2.52 *s*	4.45 *m*	5.88 *s*	
1β-Acetoxynorethisterone (IVa)	−135.8[d]	−399[e]	0.91 *s*	2.55 d (4)	2.57 *s*	5.53 *m*	5.88 *s*	2.01 *s*
11β-Hydroxynorethisterone (V)	− 5.8[b]	+ 76[e]	1.00 *s*		3.33 *s*	4.18 *m*	5.90 *s*	
11β-Acetoxynorethisterone (Va)	0.0[b]	+ 94[e]	1.05 *s*		2.62 *s*	5.32 *m*	5.89 *s*	2.07 *s*

[a] All NMR spectra were recorded in deuterated chloroform with the exception of V, which was recorded in deuterated dimethyl sulfoxide. All signals are given in ppm downfield from tetramethylsilane; the letters *s*, t, *m*, designate singlet, triplet, and multiplet, respectively. Numbers in parenthesis are J values; [b] Solvent was chloroform: methanol (1:1); [c] Parent compound: D-norgestrel[8]. [d] Solvent was chloroform. [e] Parent compound: norethisterone[9].