tested with the [^{3}H] leucine uptake. Pinosylvin at 30 µg/ml almost completely suppressed the [^{3}H] leucine uptake (fig. 4). The effect of all these compounds at the same concentrations on normal human peripheral blood lymphocytes was tested and at 72 h showed no effect on viability.

Discussion. Many of the antineoplastic agents now in use are derived from plants. Continual search for new biosynthetic substances with possible antineoplastic action must be maintained if progress in therapy of malignancies is to continue. Of the compounds tested all, except hordatine M and lubimin showed significant growth inhibitory action at at least the higher concentration tested. It should be noted, however, that dehydroloroglossol could only be tested at a concentration of 10 µg/ml while the others were tested at 30 µg/ml. With pinosylvin a cytotoxic action was noted at a concentration of 30 µg/ml. Pinosylvin was also shown to have a marked inhibitory action on protein synthesis as assessed by the [H^3] leucine uptake (fig. 4) and a similar effect though to a lesser degree on the [^{3}H] thymidine uptake (fig. 3). Dehydroloroglossol at 10 µg/ml had a slight but significant inhibition of both [H^3] leucine and [H^3] thymidine uptake (figs 3, 4). It is interesting to note that hordatine M was the only constituent tested at high concentrations which was not growth inhibitory or cytotoxic. Hordatine M is the only plant constituent tested which was not stress induced. No toxicity was noted on the normal mononuclear cells tested.

The phytoalexins and related substances open up a vast new field of biosynthetic substances that should be explored in search of new antineoplastic agents.

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Mitochondrial activity: A possible determinant of anoxic injury in renal medulla

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Summary. In brain[1], heart[2] and kidney[3], cell work in the absence of oxygen has been thought to precipitate anoxic damage by increasing the rate of depletion of cellular energy stores. In the medullary thick ascending limb of isolated perfused rat kidneys, however, reduction of ATP synthesis by a variety of mitochondrial or metabolic inhibitors caused ATP depletion comparable to that produced by oxygen deprivation but did not reproduce the lesions of anoxia. In these cells, unrestrained mitochondrial activity may be an important source of anoxic injury.

Key words. Anoxic injury; mitochondrial respiration; renal medulla; acute renal failure; renal metabolism.

Cell work under hypoxia has recently been shown to accelerate anoxic damage in isolated neurons[1], in myocytes[2], and in the medullary thick ascending limb of Henle's loop (mTAL) of isolated perfused rat kidneys[3]. Increased energy expenditure under these circumstances might hasten the depletion of cellular energy stores, generally considered an important element in anoxic cell injury[4]. The present study suggests that apart from its role in depleting cellular ATP, mitochondrial activity per se may contribute to the generation of cell injury during anoxia.

Severe hypoxic injury develops rapidly and consistently in isolated rat kidneys perfused with bovine albumin in Krebs-Ringer-Henseleit medium equilibrated with oxygen[5]. The lesion is located in the mTAL which, because of its strategic location, may play an important role in the pathogenesis of acute renal failure[6]. The selective vulnerability of the mTAL to anoxia results from its high transport activity combined with a meager oxygen supply[5]. The damage can be prevented by reducing reabsorptive transport and oxygen demand (adding ouabain or furosemide)[7] or can be intensified by increasing active electrolyte transport (adding a polyene antibiotic)[3]. Cell death under these circumstances thus appears to be mediated by increased energy demand for transport.

To evaluate the role of cellular energy depletion in the genesis of this injury, we examined the morphology of the mTAL in kidneys in which ATP production was reduced by inclusion in the perfusate of various mitochondrial or metabolic inhibitors, as follows. 1) Rotenone, antimycin (inhibitors of electron transfer at different proximal sites of the mitochondrial respiratory chain and known to deplete cellular ATP)[8] or oligomycin (inhibitor of oxidative phosphorylation). 2) Monofluoroacetate, malonate (blockers of the citric acid cycle)[9] or 2-deoxyglucose (which inhibits ATP production by interfering with the metabolism of glucose)[10]. 3) A combination of rotenone or antimycin with 2-deoxyglucose (to suppress both aerobic and anaerobic production of ATP).

For comparison, oxygen deprivation was achieved either by equilibrating the perfusion medium with nitrogen or by adding potassium cyanide (which prevents the binding of oxygen to the mitochondrial cytochrome a; a_3). Under these conditions, extensive injury occurs in the mTAL[11].

The effects of these probes were monitored by measuring tissue ATP content and oxygen consumption by the isolated perfused kidney. As expected, the various inhibitors effectively depleted the ATP content of the renal medulla (table) and significantly reduced oxygen uptake by the isolated kidney (fig. 1).

After 90 min of control perfusion, an anoxic lesion[5] was obser-

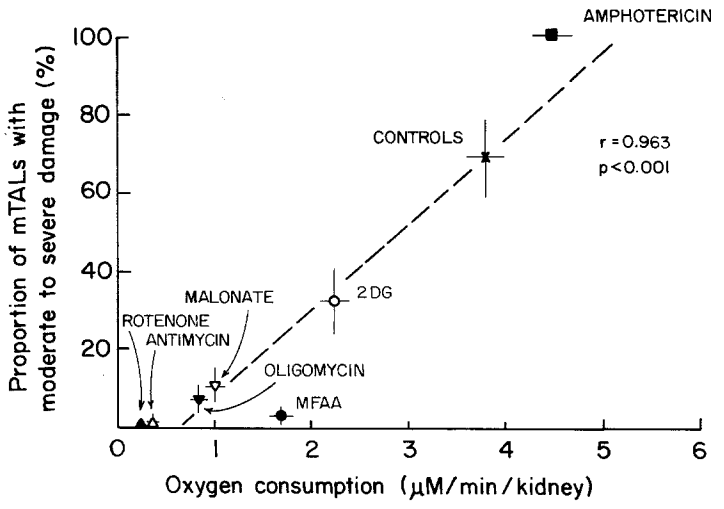

Figure 1. Correlation between mitochondrial activity (estimated by the rate of oxygen consumption of the whole kidney) and the extent of injury to the mTALs. The number of experiments ranged from 5–8 for each group, and the results are expressed as means ± standard error. The morphological quantitation was performed by one of us (S.R.) without knowledge of experimental conditions and as previously described[5]. The data for the combination of 2-deoxyglucose with either rotenone (n = 6) or antimycin (n = 4) were essentially the same as those of rotenone and antimycin alone. The linear regression is significant with or without the point representing data for amphotericin (taken from a previous study[3]). The concentrations of the inhibitors are indicated in the table. Reversal of metabolic inhibition by alpha-ketoglutarate or lactate (to bypass the blockades of monofluoroacetate or 2-deoxyglucose, respectively) reestablished the lesion[13], further indicating a link between substrate-supported mitochondrial respiration and mTAL injury. Abbreviations: 2DG, 2-deoxyglucose; MFAA, monofluoroacetate; mTAL, medullary thick ascending limbs.

ved in the mTAL consisting of mitochondrial swelling, nuclear pyknosis and cell fragmentation (fig. 2a). However, when a mitochondrial inhibitor such as rotenone was added, this damage was no longer seen (fig. 2b). Comparable protection from injury was achieved when rotenone or antimycin was combined with 2-deoxyglucose (fig. 2c). Intermediate degrees of protection were obtained with the metabolic blockers malonate, oligomycin, and 2-deoxyglucose alone (fig. 1). Indeed, a correlation was found between the rate of oxygen consumption and the intensity of damage in the mTAL. In no instance could the anoxic injury be made worse by these metabolic or mitochondrial blockades. Only the inhibition of the final step of respiration (electron transfer to oxygen at cytochrome $a;a_3$) by hypoxia or cyanide, generated severe injury. More proximal blocks (before or along the mitochondrial electron chain) did not.

Thus, in the renal medulla, ATP depletion achieved by inhibition of intramitochondrial activity does not reproduce damage from anoxia. Severe anoxic injury to the cells of the mTAL appears to correlate with mitochondrial activity (fig. 1). The fact that energy utilization for transport activity intensifies this an-

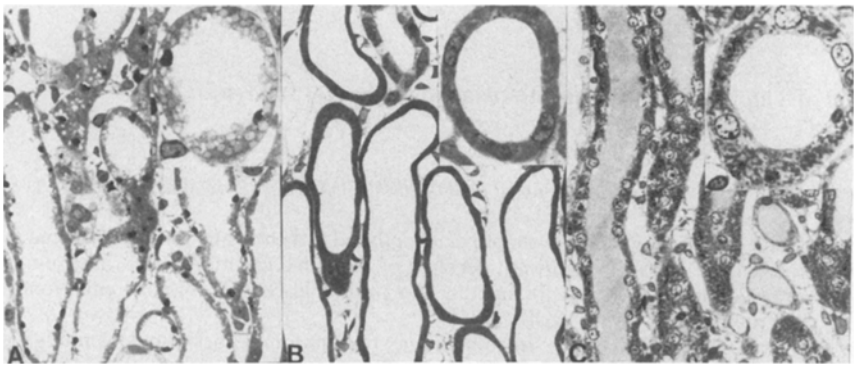

Figure 2. Outer medulla from isolated perfused rat kidneys (× 350, × 875). After 90 min of control perfusion (A) approximately 60% of medullary thick ascending limbs (mTALs) show cell fragmentation, nuclear pyknosis and highamplitude mitochondrial swelling. If rotenone (10^{-5} M) is included in the perfusate (B), cell integrity is maintained and nuclear pyknosis is not observed (cytoplasmic organelles are closely approximated in these dilated tubules, so that individual mitochondria are not sharply delineated). When both antimycin (10^{-5} M) and 2-deoxyglycose (50 mM) are present (C), cellular integrity is likewise preserved with normal sized mitochondria, but chromatin margination and cytoplasmic edema are noted. The lesion observed in (A) is seen neither in (B) nor in (C). Examination by electron microscopy confirmed the relative structural integrity of the mTALs with metabolic inhibitors compared to the cell disruption observed in control perfusions. The kidneys were fixed by perfusion with 1.25% glutaraldehyde at the end of all experiments. While cell viability might have been affected by metabolic inhibition, more importantly the characteristic pattern of irreversible injury seen in hypoxic mTALs[5], including nuclear pyknosis and cell fragmentation, could not be reproduced by any of these inhibitors despite comparable ATP depletion. By contrast with the present results in medullary tubules, inhibition of ATP synthesis did produce anoxic-like damage in the proximal tubule[14]. Greater capacity for anaerobic glycolysis in the mTAL is an unlikely explanation for the difference, since the addition of 2-deoxyglucose did not alter the results.

Effects of hypoxia and metabolic (or mitochondrial) inhibitors on ATP content of renal medulla in isolated perfused rat kidneys. ATP levels were measured in separate experiments, using kidneys perfused for 20 min, rapidly frozen by immersion in liquid nitrogen, medulla separated from cortex by free hand dissection while frozen, and assayed by an enzymatic method.[12] These short perfusions were designed to obtain ATP levels as an early parameter of metabolic inhibition (presumably less affected by loss of cellular ATP resulting from later secondary injury). In additional determinations at 90 min of perfusion (not shown), rotenone or 2-deoxyglucose compared to controls produced ATP depletion similar to that observed at 20 min of perfusion. While the level of ATP in frozen kidney tissue may not correspond precisely to the ATP content of cells of the medullary thick ascending limb, rotenone and antimycin have been shown to deplete ATP in isolated proximal tubules[8] and to stop respiration in isolated in TAL cells from rabbit kidney (Lear, S. and Silva, P., unpublished observations). The results are expressed as means ± SE and were analyzed by a multiple comparison procedure (Walker-Duncan)

	Medullary ATP (μM/100 mg protein)	Number of kidneys perfused
Control perfusions	1.48 ± 0.08	9
Hypoxic perfusions	0.69 ± 0.03*	6
Cyanide (2.5 mM)	0.76 ± 0.05*	4
Rotenone (10^{-5} M)	0.72 ± 0.06*	6
Antimycin (10^{-5} M)	0.70 ± 0.04*	4
Oligomycin (10^{-4} M)	0.88 ± 0.07*	5
2-deoxyglucose (50 mM)	0.88 ± 0.05*	4
Malonate (25 mM)	1.09 ± 0.07*	5
Monofluoroacetate (5 mM)	0.98 ± 0.07*	6
Antimycin and 2-deoxyclucose[a, b]	0.50 ± 0.02*†	5
Rotenone and 2-deoxyglucose[a, b]	0.48 ± 0.01*†	5

[a]Glucose was excluded from the perfusion medium; [b]Same concentrations as in previous experiments; *Significantly lower ($p < 0.05$) than control perfusions; †Significantly lower ($p < 0.05$) than hypoxic perfusions, rotenone and antimycin.

oxic damage[3] may not simply relate to a more rapid exhaustion of cellular energy stores but possibly to further stimulation of mitochondrial activity. This mode of injury appears to depend on continued mitochondrial electron flow in the face of limited oxygen supply, a situation which may conceivably lead to aberrant energy biotransformation such as the production of electron-dependent free radicals. Metabolic arrest, by hypothermia or inhibition of cell activity, which confers protection from ischemia in various tissues[1,2,7,15], may be mediated at least in part by a reduction in hypoxic mitochondrial respiration.

While it is clear that anoxia can damage cells by depletion of energy stores[4,14], some cells such as the mTAL and cultured hepatocytes[16] withstand mitochondrial inhibition far better than they do anoxia. Mitochondrial activity in the face of oxygen deprivation may be in itself an important determinant of anoxic injury.

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The protective effect of Thiola against the genotoxic action of benzo(a)pyrene

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Summary. The protective effect of Thiola against the genotoxicity, induced by benzo(a)pyrene, in vitro and in vivo, was investigated. By association of Thiola to benzo(a)pyrene a significant decrease of the numerical and structural chromosome aberrations and a reduction of the incidence of c-mitoses has been obtained in human diploid cells, i.e. human embryonic lung fibroblasts of the cell-line ICP-23, and C_{56}Bl/6 mouse bone marrow cells.

Key words. Chemoprophylaxis; cancer prevention; benzo(a)pyrene; chromosomal aberrations; genotoxicity; Thiola; anticarcinogens.

It is well known that benzo(a)pyrene (B(a)P), a widespread environmental precarcinogen, is converted in animal and human tissues by the P-450 dependent monooxigenase system[1] into electrophilic species which represent the active genotoxic metabolites[2,3]. The activation takes place simultaneously with the detoxication which transforms some B(a)P intermediates into hydrophilic derivatives which are subsequently excreted as conjugates[4].

The implication of SH-physiological compounds (i.e. glutathione, cysteine, etc.) in the detoxication processes was demonstrated and widely accepted[5,6].

The aim of the present study was to demonstrate the protective effect of a synthetic thio-compound: Thiola (N-2-mercaptopropionyl glycine) against the genotoxic action produced by B(a)P in vitro and in vivo systems.

Materials and methods. Cell culture: human diploid cells (HDC) i.e. human embryonic lung fibroblasts of the cell line ICP-23[7,8], at their 19th and 20th passages were cultivated as a monolayer in