coarsely crystalline dolomite is more widespread than the ore (*4*). Any organic-rich rock affected by this dolomitization (and hence temperatures of 75° to 100°C) will also generate H_2S. Facies F of the barrier complex contains an average of 3.75 percent organic carbon (*6*). Approximately 0.13 km^3 of facies F would contain the 16.8×10^6 metric tons of organic matter required for the generation of sulfide at Pine Point by Eq. 1. The total volume of facies F is about 5 km^3 (*4*), and thus only 2.6 percent would need to be fully altered for the generation of sufficient sulfide to account for the ore bodies. From these considerations, there appears to be no constraint regarding the availability of organic matter for sulfide generation.

The precipitation of metals by H_2S, formed by in situ thermochemical reduction of sulfate at Pine Point, is consistent with the temperature of formation of the ore bodies; the amounts, alteration, and composition of the bitumen; the presence of native sulfur; and the isotopic composition of the various sulfur species. Such reactions may have provided an important means of generating the large volumes of sulfide necessary to precipitate sulfide ore bodies in sedimentary carbonate rocks.

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Polyene Toxicity in Renal Medulla: Injury Mediated by Transport Activity

Abstract. *Polyene antibiotics such as amphotericin and nystatin increase membrane permeability and thus increase the amount of oxygen consumed in active electrolyte transport. In isolated perfused rat kidneys, the polyenes produced extensive injury to the medullary thick ascending limb, a segment of the nephron with limited oxygen supply. This damage was prevented if reabsorptive transport was inhibited by ouabain. Cell death under these circumstances thus appears to be mediated by increased oxygen demand for transport activity.*

Acute renal failure is a well-known complication of amphotericin therapy, thought to be related at least in part to the increased membrane permeability induced by polyene antibiotics (*1*), which leads to disruption of the internal electrolyte milieu and disordered volume regulation in cells (*2*). Which of the many consequences of membrane damage caused by polyenes is ultimately responsible for cell death is unknown (*3*). Increased permeability triggers a compensatory increase in the rate of active electrolyte transport, associated with a rise in oxygen demand (*4*), but the significance of these changes for the generation of injury is not generally recognized. We report that in isolated perfused rat kid-

Table 1. Effects of amphotericin ($3 \times 10^{-5} M$) and nystatin (200 U/ml) on renal function and structure in comparison with regular perfusions (control) and perfusions equilibrated with 95 percent N_2 and 5 percent CO_2 (hypoxia). Functional parameters are whole kidney measurements and include renal perfusion flow, oxygen consumption (Q_{O_2}), and tubular reabsorption of sodium (expressed as T_{Na}/Q_{O_2}). Quantitation of histological injury (evaluated without knowledge of experimental conditions) is for the mTAL only and is expressed as the proportion of tubules affected by severe damage (illustrated in Fig. 1, A and B). The polyenes led to a recruitment of all tubules by a lesion identical to that seen in control and hypoxic perfusions and shown to be derived from local oxygen deficiency (*6*) but much more extensive. Treatment with ouabain ($10^{-2} M$) 20 minutes before the addition of the polyenes essentially eliminated polyene-induced damage to the mTAL. The results are expressed as means ± standard errors, for three to seven experiments per group, at 90 minutes of perfusion, and were analyzed by a multiple comparison procedure (Walker-Duncan).

Treatment	Flow (mg/min)	Q_{O_2} (μmole/min)	T_{Na}/Q_{O_2} (μeq/μmole)	mTAL's with severe damage (%)
Control	40.9 ± 2.5	3.8 ± 0.2	18.6 ± 1.2	62 ± 12
Hypoxia	31.9 ± 2.7	0.9 ± 0.1	8.9 ± 1.3	85 ± 6
Amphotericin	15.9 ± 3.3	4.8 ± 1.3	0.5 ± 0.4*	100 ± 0*
Nystatin	26.3 ± 3.8	3.8 ± 0.4	0.7 ± 0.5*	100 ± 0*
Ouabain and amphotericin	17.5 ± 0.6	2.4 ± 0.1	1.7 ± 0.9	6 ± 3†
Ouabain and nystatin	14.8 ± 3.1	2.7 ± 0.2	2.1 ± 0.8	0 ± 0†
Furosemide and amphotericin	26.3 ± 1.0	5.7 ± 0.5	1.8 ± 0.7	100 ± 0

*Significantly different ($P < 0.05$) from control. †Significantly different ($P < 0.05$) from polyene alone.

neys, lethal cell injury induced by polyenes depends critically on transcellular transport activity in the face of limited oxygen supply.

A hypoxic lesion develops rapidly and consistently in the isolated rat kidney perfused with bovine albumin in a Krebs-Ringer-Henseleit medium equilibrated with oxygen (*5, 6*). The lesion is located in the medullary thick ascending limb of Henle's loop (mTAL), which, because of its strategic location, may play an important role in the pathogenesis of acute renal failure in vivo (*7*). The selective vulnerability of the mTAL to anoxia results from its high transport activity combined with meager oxygen supply (*6*). The damage can be prevented either by increasing oxygen supply (adding erythrocytes or hemoglobin to the perfusate) or by reducing active transport and oxygen demand (adding ouabain or furosemide) (*5*). The lesion therefore appears to result from an imbalance between oxygen supply and demand, suggesting that the increase in transport work might itself accelerate anoxic cell injury.

To further explore this hypothesis, we added to the perfusion medium the polyene antibotics amphotericin (10^{-4} to $10^{-5}M$) or nystatin (200 U/ml). These substances greatly increase the permeability of cell membranes to electrolytes (*8*) and maximize oxygen consumption on behalf of active sodium transport in proximal tubules (*4*) or isolated mTAL cells (*9*). In the isolated kidney, amphotericin or nystatin produced a marked decrease in glomerular filtration and effective sodium reabsorption, with no change or some increase in the rate of oxygen consumption (Table 1). These changes were associated with marked intensification of the lesion seen in the mTAL's after 90 minutes of regular perfusion (Table 1 and Fig. 1) (*10*). All of the tubules showed extensive mitochondrial swelling, advanced nuclear pyknosis, and widespread cytoplasmic disruption (Fig. 1, A and B). Comparison of the histological damage to the mTAL resulting from polyenes to that of perfusion without oxygen (oxygen content of the medium reduced tenfold from control perfusions) is shown in Table 1. Thus, the polyenes intensify the effect of moderate local hypoxia (control) and simulate the consequences of severe generalized hypoxia (medium equilibrated without oxygen) (see Table 1) (*11*).

Polyene-induced histological alterations could result from nonspecific membrane damage. Alternatively, polyenes could intensify anoxic injury by increasing active transport, thereby augmenting energy consumption and oxygen demand in the hypoxic cells of the mTAL, as suggested earlier (*5, 6, 12*). We tested this possibility by adding ouabain at the concentration ($10^{-2}M$) necessary to inhibit rat kidney Na- and K-dependent adenosinetriphosphatase completely (*13*) before adding the polyenes to the perfusate. Stopping active transport with ouabain reduced the rate of oxygen consumption by the whole kidney, even in the presence of the polyenes. Ouabain afforded striking protection from the toxic effect of the polyenes; the integrity of the mTAL was essentially restored (Table 1 and Fig. 1, C and D). Although some exogenous sterols bind polyenes and may blunt their toxicity (*2*), ouabain probably does not have such a property, since it does not prevent the electrophysiological effect of amphotericin in the toad bladder (*14*).

To show that the protective effect of ouabain was not the simple consequence of an increase in urine flow, we used a different diuretic, furosemide ($10^{-4}M$), which reduces transport at the mTAL under control conditions by inhibiting a chloride cotransporter (*15*). When inward flux of electrolytes to the cell is no longer limited by specific channels as a result of polyene-induced abnormal membrane permeability, furosemide cannot be expected to slow transport activity or to reduce oxygen demand. Furosemide, accordingly, did not reduce

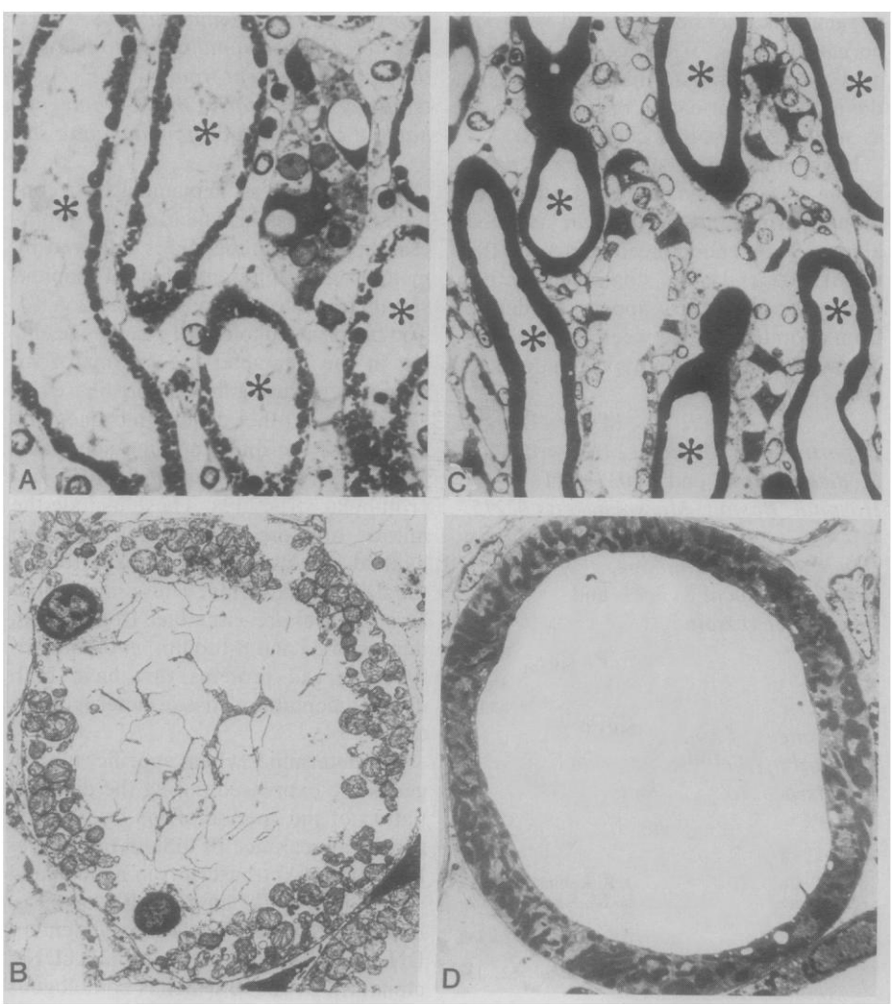

Fig. 1. Outer medulla from isolated rat kidneys perfused for 90 minutes with a polyene alone (left) or with ouabain and polyene (right). (A) Low-power microscopy (1-μm section, ×300) showing the effects of amphotericin alone ($3 \times 10^{-5}M$). The epithelium of the medullary thick ascending limb (mTAL) (asterisks) is extremely fragmented, producing a diffuse ragged appearance of the luminal surface of the tubules. A collecting duct (upper right, cut tangentially) is better maintained; the plasma membrane can be defined and the nuclei show minimal changes. (B) Electron microscopy (×1700) of the mTAL epithelium after treatment with amphotericin alone shows nuclear pyknosis, mitochondrial swelling with membrane loss and disarray. (C) Low-power microscopy (1-μm section, ×300) showing the effects of treatment with ouabain ($10^{-2}M$) before perfusion with amphotericin ($3 \times 10^{-5}M$); the mTAL (asterisks) shows no injury. (D) Electron microscopy (×1700) shows normal appearance. The kidneys were fixed by perfusion with 1.25 percent glutaraldehyde at the end of all experiments.

oxygen consumption and did not ameliorate the damage to the mTAL induced by amphotericin (Table 1).

These results support the view that anoxic injury in cells of the mTAL is strongly conditioned by the rate of active ion transport. The traditional view of anoxic injury emphasizes the role of oxygen deprivation. However, in situations like those reported here, the consequences of anoxia may depend more on the rate of energy demand than on the degree of limitation of oxygen delivery. The polyenes did reduce oxygen delivery because of renal vasoconstriction (see Table 1). However, ouabain did not improve renal flow, and its protective effect was therefore presumably mediated entirely by a decrease in oxygen demand for active transport. In analogous experiments, we showed that mTAL injury produced by hypoxia or potassium cyanide in isolated perfused kidneys is prevented by decreasing active transport with ouabain or furosemide or by halting glomerular filtration (*12*).

A similar phenomenon in neurons has been described, in which synaptic activity potentiates anoxic damage (*16*). Persisting mitochondrial activity and continued electron flow in the absence of an oxygen sink may be associated with abnormal handling of charges in the process of energy transformation, leading to the increased formation of free radicals, as suggested for other cells (*17, 18*).

In summary, polyene toxicity is not a simple consequence of altered cell membrane permeability, since in isolated kidneys, polyene-induced injury to the mTAL depends on continued active transport. This injury appears to derive from an imbalance between limited oxygen availability and high oxygen demand (*19*).

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10. At 15 and 30 minutes of perfusion, while moderate to severe damage was already extensive, involving 95 ± 5 percent of mTAL's, other nephron segments showed only minimal to mild injury. Specifically, small apical vesicles were observed in proximal tubules that were otherwise normal. At 90 minutes, while damage was severe in 100 percent of mTAL's, limited cell injury was seen in some proximal tubules, involving brush border disarray and mitochondrial swelling in parts S_1 and S_2 of the proximal tubules and focal cell fragmentation in part S_3. Thus, although limited injury was apparent in the renal cortex, by far the most extensive and essentially irreversible damage was found in the mTAL's.
11. This contrasts with the paucity of morphological damage from amphotericin in vivo (*1*). Although drug concentrations, time periods, and modes of tissue fixation were different, a more likely explanation is the special conditions created by isolated perfusion. Thus, the small oxygen-carrying capacity of the perfusate (*6*) and the probable blunting of neurohumoral signals (for feedback regulation of glomerular filtration) may act in concert to expose the vulnerability of the mTAL to continued transport work during limited oxygen supply (*5, 6*). In vivo, decreased glomerular filtration and reabsorptive work would attenuate or prevent tubular necrosis from anoxia in this area of the kidney (*5, 12*).
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Haploid Expression of a Mouse Testis α-Tubulin Gene

Abstract. *A complementary DNA clone for an α-tubulin has been isolated from a mouse testis complementary DNA library. The untranslated 3′ end of this complementary DNA is homologous to two RNA transcripts present in postmeiotic cells of the testis but absent from meiotic cells and from several tissues including brain. The temporal expression of this α-tubulin complementary DNA provides evidence for the haploid expression of a mammalian structural gene.*

Spermatogenesis in mammals is a continuous process in which mitotic proliferation of spermatogonia is followed by meiosis and differentiation of haploid spermatids into mature spermatozoa (*1*). The major morphological changes resulting in the characteristic spermatozoan shape occur during the haploid phase (*1*).

As seen in other cellular morphogenic processes, the differentiating spermatogenic cells contain several distinct microtubular structures. These include the mitotic and meiotic spindles and two haploid structures, the manchette and the flagellar axoneme. These microtubular structures are assembled from heterodimers of α- and β-tubulin, evolutionarily conserved proteins that have both developmental and tissue heterogeneity (*2–4*).

To determine when specific tubulin genes are expressed during the differentiation of the spermatozoon, we looked for the appearance of tubulin messenger RNA (mRNA) transcripts in meiotic and postmeiotic testicular cells. We have isolated from a mouse testis complementary DNA (cDNA) library an α-tubulin cDNA clone, the 3′ end of which is homologous to at least two different α-tubulin RNA transcripts. These transcripts are detectable only during the haploid phases of spermatogenesis.

A cDNA library was derived from mouse testis by priming polyadenylated [poly(A)$^+$] RNA with oligothymidilic acid. The resulting DNA fragments were cloned into the Sal I and Eco RI sites of plasmid pUC8 by means of linkers (*5*). After screening the cDNA library by colony hybridization (*6*) with a ^{32}P-labeled 1650–base pair (bp) insert of a clone containing the coding sequence and 3′ untranslated sequences from rat brain α-tubulin mRNA (designated pILαT1) (*7*), we obtained a colony containing an insert approximately 1000 bp long (called pRDαTT1). Plasmid pRDαTT1 proved homologous by Southern hybridization (*8*) to much of the rat brain clone pILαT1 but showed no detectable hybridization to the 3′ untranslated region of the rat α-tubulin sequence (the 3′ untranslated region of pILαT1 is hereafter called pILαTIII). On the basis of observations by others that the 3′ untranslated regions of tubulins hybridize to specific transcripts (*4, 9–11*), we sub-