involved in the cellular uptake of all transferrin-transported metals is currently under investigation in this laboratory.

The transferrin binding characteristics for various 'foreign' metals are being studied and Sephadex gel-chromatographic methods are being used to measure the stability constants of the metal–transferrin complexes. Preliminary studies with iron(III) and hafnium(IV) have yielded overall stability constants of $\log \beta = \sim 30$.

In vitro uptake studies with a human lymphoblast cell line (WIL-2) have shown (Table I) that when compared to that from physiological concentrations of citrate, the uptake of iron, gallium and hafnium from serum-free medium is enhanced by transferrin. The uptake is dependent on the metal–transferrin ratio and is highest with a molar ratio of 1:1 for iron and 1:10 or 1:30 for the other metals.

TABLE I. The Uptake of Iron, Gallium and Hafnium by WIL-2 Cells *in vitro*.

Metal	Metal: TF ratio	% Uptake per 10^6 cells per 2 h	
		Transferrin	0.1 m*M* citrate
Iron	1:1	2.78 ± 0.12	0.38 ± 0.05
Gallium	1:10	0.29 ± 0.003	0.033 ± 0.002
	1:30	0.19 ± 0.005	
Hafnium	1:10	1.03 ± 0.09	0.087 ± 0.018
	1:30	1.84 ± 0.11	

Analysis of the subcellular distribution of gallium and hafnium after *in vitro* uptake indicates that, as expected from *in vivo* studies [2, 8], the incorporated metal is associated with lysosomal structures.

The uptake data for the metals studied suggest that a metal–transferrin–transferrin–receptor mechanism is involved. However, the pattern of uptake observed with iron is not identical with that of gallium or hafnium, nor probably with that of plutonium.

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S3

Inhibition of Lipid Peroxidation by Manganese

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The peroxidation of unsaturated lipids of biological membranes is a degenerative process usually sparked by free radicals or by singlet oxygen. $MnCl_2$ is an inhibitor of lipid peroxidation [1] but its mechanism of action is still unknown.

This report summarizes some results on the inhibitory effect of $MnCl_2$ on peroxidizing systems such as ascorbate/$FeSO_4$, NADPH/$FeCl_3$/ADP, cumene hydroperoxide (CHP), hematin and rose bengal/light. 1 m*M* $MnCl_2$ efficiently inhibited all the systems tested, with the exception of rose bengal/visible light where lipid peroxidation is not initiated by a free radical mechanism but by singlet oxygen which directly forms a hydroperoxide through an 'ene' reaction [2]. The lipid peroxidation induced by CHP was independent of iron ions since it occurred to the same extent even in the presence of 1 m*M* EDTA. Since $MnCl_2$ also strongly inhibited the CHP-induced lipid peroxidation, a competition of $MnCl_2$ for the binding sites of iron ions, which are well known promotors of lipid peroxidation, can be excluded.

Figure 1 illustrates the formation of lipid hydroperoxides (LOOH) after photoactivation in the

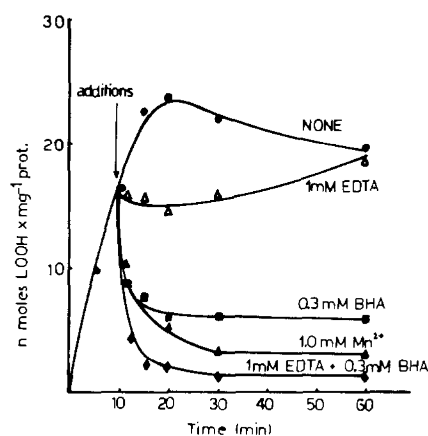

Fig. 1. Time course of LOOH formation in microsomes incubated with rose bengal. Microsomes (1 mg/ml) were incubated with 15 m*M* HEPES and 6.5 m*M* Tris pH 7.4 at 25 °C in the presence of 10 μg/ml of rose bengal and visible light. The reaction was stopped after 10 min and, at the same time, the indicated substances were added. Aliquots were withdrawn and LOOH content was estimated with KSCN [3] after extraction with $CHCl_3/CH_3OH$ (3/2, v/v).

presence of rose bengal. In this case the formation of new hydroperoxides occurred also when the photo-activation was ended because of the branching reactions initiated by decomposition of the lipid hydroperoxides previously formed by rose bengal. The chelating agent EDTA lowered the rate of lipid hydroperoxide formation due to branching reactions, but did not decrease the amount of preformed lipid hydroperoxides. On the contrary, Mn^{2+}, like the antioxidant agent butylated hydroxyanisole (BHA), decreased them.

On this ground we suggest that Mn^{2+} could directly interact with the oxy and peroxy radicals ($RO^{\bullet}$, $RO_2^{\bullet}$) originating from lipid hydroperoxide decomposition. Consequently these free radicals cannot spark new radical chains, and lipoperoxidation is blocked.

Recently Mn^{2+} was shown to act as a scavenger of $O_2^{\bar{\bullet}}$ [4] and $^{\bullet}OH$ radical [5] and this supports the hypothesis of a direct antioxidant action of this ion.

The inhibitory action of Mn-superoxide dismutase (SOD) on lipoperoxidation was also studied. The enzyme inhibits by 25–30% the lipid peroxidation sparked by CHP in experiments carried out in the presence of reduced glutathione.

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S4

Amino Acid Compositions of Carp Kidney Metallothionein

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In fish, homology on amino acid compositions of metallothionein (MT) between liver and kidney is not recognized. We previously reported the amino acid composition of carp hepato-pancreas MT [1]: in the present study amino acid composition of kidney MT was determined, and homology between them discussed. Twenty carps were injected intraperitoneally with 2 mg/kg body weight of Cd as $CdCl_2$ solution, daily for 3 days. On the fourth day the kidney was excised, treated successively by homogenization, ultracentrifugation, heating treatment, centrifugation, gel filtration (Sephadex G-75, Bio Gel P-10) and ion exchange chromatography (DEAE Sephadex A-25). By the gel filtration using Sephadex G-75, the Cd peak was observed, and it was estimated that this peak contained MT. By ion exchange chromatography this peak was separated into two fractions, having high concentration of Cd, and high absorption at 254 nm and no absorption at 280 nm respectively. The former fraction was named MT-I and the latter MT-II. By gel filtration using Bio-Gel P-10, a single Cd peak was observed in MT-I and MT-II, respectively. The molar ratio of Cd:Zn was 36:1 in MT-I and 27:1 in MT-II. Their purity was shown by polyacrylamide DISC-gel electrophoresis. The absorption at 254 nm in both MT fractions disappeared when they were brought to acidic condition. The amino acid compositions of carp kidney and hepato-pancreas are given in Table I. Cysteic content was about 30% in each organ. The contents of Lys, Glu, Pro and Met residues in carp MT were close to the values in mammal MT, but those of Thr and Ala residues were different. In kidney MT, some Leu residue was observed. Arginine residue was detected only in kidney MT-II. No aromatic amino acids and histidine residues were detected in carp MT. The amino acid compositions in kidney MT were little different from those of hepato-pancreas MT. Between MT-I and MT-II in kidney the

TABLE I. Amino Acid Compositions of Carp Kidney and Hepato-pancreas.

Amino acid	Mol % (number)				
	Carp				Horse [2]
	Hepato-pancreas		Kidney		Kidney
	MT-I	MT-II	MT-I	MT-II	
Lys	11.37(7)	10.09(6)	10.06(6)	10.09(6)	11.1(7)
His	– (0)	– (0)	– (0)	– (0)	– (0)
Arg	– (0)	– (0)	0.51(0)	0.81(1)	1.9(1)
Asp	8.47(5)	9.10(6)	8.04(5)	7.53(4)	5.0(3)
Thr	7.10(4)	8.47(5)	6.68(4)	9.48(6)	1.8(1)
Ser	11.23(7)	10.13(6)	6.99(4)	11.78(7)	11.9(8)
Glu	3.11(2)	3.50(2)	5.40(3)	4.29(3)	4.7(1)
Pro	4.89(3)	4.16(3)	3.55(2)	3.85(2)	2.9(2)
Gly	10.64(6)	11.13(7)	9.30(6)	11.51(7)	9.2(5)
Ala	5.82(3)	5.59(3)	8.43(5)	6.05(3)	11.6(7)
Cys	34.24(20)	31.90(20)	32.70(21)	29.39(18)	33.9(20)
Val	1.98(1)	2.35(1)	2.95(2)	2.98(2)	4.5(3)
Met	1.01(1)	1.68(1)	0.96(1)	0.50(1)	1.5(1)
Ile	0.11(0)	0.99(1)	1.67(1)	0.70(1)	– (0)
Leu	– (0)	– (0)	2.74(2)	1.14(1)	– (0)
Tyr	– (0)	– (0)	– (0)	– (0)	– (0)
Phe	– (0)	– (0)	– (0)	– (0)	– (0)