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FACTOR ANALYSIS OF THE ACTIVITIES OF SUPEROXIDE DISMUTASE, CATALASE AND GLUTATHIONE PEROXIDASE IN NORMAL TISSUES AND NEOPLASTIC CELL LINES

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Exploratory factor analysis of reported specific activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in normal human tissues, normal mouse tissues, vertebrate red blood cells and neoplastic human cell lines shows that the activities of copper–zinc superoxide dismutase, catalase and glutathione peroxidase in normal tissues are influenced by a single factor. Catalase activity has the highest loading and correlation with this factor, suggesting a catalase- or hydrogen peroxide-related influence. The activity of manganese superoxide dismutase is influenced by a separate factor. The activities of copper–zinc and manganese superoxide dismutases in normal tissues therefore appear to be dichotomously regulated.

The activities of superoxide dismutase and glutathione peroxidase in vertebrate red blood cells are influenced by a single factor. The activity of catalase is influenced by a separate factor. The roles of glutathione peroxidase and catalase in hydrogen peroxide catabolism in red blood cells in fact differ.

In neoplastic human cell lines, two bipolar factor factors appear to influence the activities of catalase and manganese superoxide dismutase, and glutathione peroxidase and copper–zinc superoxide dismutase, respectively. The factors are, however, mainly catalase and glutathione peroxidase activity factors as the loadings and correlations of manganese superoxide dismutase on the one hand and copper–zinc superoxide dismutase on the other, with the respective factors, are relatively small. Potentially low superoxide production and intrinsically low peroxidizability of tumour cell membranes underlie the peculiar variation of antioxidant enzyme activities in tumour cells.

Factor analysis is proposed as a heuristic data reduction and hypothesis-creating technique for the variation of antioxidant and other functionally-linked enzyme activities in normal and pathological cells and tissues.

KEY WORDS: Antioxidant enzyme activities, copper–zinc superoxide dismutase, manganese superoxide dismutase, catalase, glutathione peroxidase, normal human tissues, normal mouse tissues, vertebrate red blood cells, neoplastic human cell lines, factor analysis, heuristic technique.

INTRODUCTION

The enzymes superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6) and glutathione peroxidase (1.11.1.9) are directed against the products of partial reduction of oxygen.[1,2] Superoxide dismutase (SOD) accelerates the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide. Catalase dismutes hydrogen peroxide to molecular oxygen and water. Glutathione peroxidase (GSH peroxidase) accelerates the reduction of hydrogen peroxide to water by GSH. Acting together these enzymes therefore eliminate the toxic products of one-electron and two-electron

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reduction of oxygen. This is important to prevent three-electron reduction of oxygen to hydroxyl radicals via the Haber–Weiss reaction, which is catalyzed by transition metal ions such as Fe^{3+} and Cu^{2+} and their complexes.[3]

In eukaryotes, SOD exits as copper–zinc SOD (CuZnSOD) in the cytosol and manganese SOD (MnSOD) in the mitochondria.[4] Generally, CuZnSOD activity is higher than MnSOD activity. About 90% of the CuZnSOD activity of rat liver is found in the soluble fraction. Particulate CuZnSOD has been assigned to the intermembrane space of mitochondria,[5,6] but this may represent a misidentification of CuZnSOD taken up by lysosomes and destined for degradation.[7] Virtually all the MnSOD activity of rat liver is confined to the mitochondrial inner membrane and matrix space.[5,6] In human and baboon liver, MnSOD activity has also been found in cytosol.[8]

Catalase is a typical enzymes of the peroxisomes.[9] In red blood cells it appears to exist in microgranules in the cytoplasm of maturing erythroblasts which disappear during red cell maturation, and in mature red cells catalase is entirely soluble.[10] Glutathione peroxidase exists as a cytosolic enzyme with some activity in the mitochondria.[11] In rat liver, about 73% of the total GSH peroxidase activity is found in the cytosol and about 26% is found in the mitochondria mainly in the matrix space.[12] Glutathione peroxidase is a tetrameric selenoenzyme.[13] Although it catalyzes the reduction of organic hydroperoxides to the corresponding alcohols, it does not attack esterified fatty acid hydroperoxides. A monomeric selenoenzyme active against membrane-bound phospholipid hydroperoxides, phospholipid hydroperoxide GSH peroxidase[14] found in the cytosol compensates for the lack of effect of GSH peroxidase against esterified lipid hydroperoxides. This enzyme may contribute to the total GSH peroxidase activity as ordinarily measured.

Together with natural antioxidants such as vitamin E and ascorbic acid, the superoxide dismutases, catalase and GSH peroxidase form a multiple level system of protection against oxygen free-radicals and lipid peroxidation. The genes for the enzymes occur on differen chromosomes. In man, the genes for CuZnSOD, MnSOD, catalase and GSH peroxidase are located on chromosomes 21, 6, 11 and 3, respectively.[15] These enzymes act in integrated fashion to bring the pathway of univalent reduction of oxygen rapidly to water without an intermediate stage of hydroxyl radicals. Co-ordinated expression of the activities of the enzymes is expected, but the principles which underlie the regulation of the activities are not known. In this exploratory study this problem has been approached by investigating the correlation structure of the activities of CuZnSOD, MnSOD, catalase and GSH peroxidase in living cells. Different tissues, and different species make different demands on these enzymes possibly depending on the relative importance of univalent reduction of oxygen in aerobic respiration. The variation of the activities of the enzymes in normal and pathological tissues of a species and in a tissue across species may be used to build up correlation matrices of the enzyme activities, and factor analysis of the manifested variation may be used on a heuristic basis to suggest a picture of the regulation of the respective activities in living cells. This approach is obviously applicable to other groups of functionally-linked enzymes. Factor analysis is both a data reduction and hypothesis-creating procedure. It makes no other assumption than that distinct influences acting in linear fashion exist in the data. Applied to the correlations among a set of variables the object of the method is to describe each of the variables as a linear sum of a smaller number of common factors and a unique factor. The common factors account for the correlations (covariation) among the variables, while each

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unique factor accounts for the remaining variance, including error, of the corresponding variable.[16]

METHODS

Correlations among the specific activities (U/mg protein) of CuZnSOD, MnSOD, catalase and GSH peroxidase were obtained from data for 14 normal human tissues (excluding red blood cells) from two subjects,[17] eight normal mouse tissues (excluding red blood cells),[18] and 19 neoplastic human cells lines.[17] Correlations among the specific activities (U/g Hb) of SOD, catalase and GSH peroxidase in red blood cells were obtained from data for ten vertebrate species including man.[19] The existing correlations had not been reported previously. It was assumed that sample sizes available were adequate. Principal components were determined in the standard manner.[16] Factor analysis was carried by the minres (minimum residuals) method which minimizes the sum of the squares of the residuals of the off-diagonal elements in the approximation of the correlation matrix by the derived factor loadings.[16] The solutions were terminated when the maximum difference in the factor loadings between successive iteration cycles was less than 0.001 or when 60 iteration cycles had been performed, whichever was the sooner. The minres solutions were rotated to orthogonal simple structure by the varimax criterion[16] and to oblique structure ($k = 2$) by the promax criterion.[20]

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RESULTS

The intercorrelations found between CuZnSOD, MnSOD, catalase and GSH peroxidase activities are given in Table I. In the data for normal human tissues, CuZnSOD activity is significantly correlated ($P < 0.05$ is considered statistically significant here and elsewhere in this study) with MnSOD and catalase activities. Catalase activity is significantly correlated with GSH peroxidase activity, and MnSOD acitivity shows low correlations with catalase and GSH peroxidase activities. These correlations are not statistically significant. All correlations are positive.

In the data for normal mouse tissues, CuZnSOD activity is significantly correlated with catalase and GSH peroxidase activities. Catalase activity is also significantly correlated with GSH peroxidase activity. These correlations are positive. In addition, CuZnSOD activity shows a low negative correlation with MnSOD activity, which is not statistically significant. There is virtually no correlation between MnSOD and catalase and GSH peroxidase activities. Overall the correlation patterns for normal human and mouse tissues are similar except for the correlation between CuZnSOD and MnSOD activities, which is positivie for the human and negative for the mouse data.

In the data for vertebrate red blood cells, SOD activity shows a moderate positive correlation with GSH peroxidase activity and a low positive correlation with catalase activity, while catalase activity shows a low negative correlation with GSH peroxidase activity. These correlations are not statistically significant, and only the correlation between SOD and GSH peroxidase activities follows the trend for normal human tissues.

In the data for neoplastic human cell lines, CuZnSOD activity shows a low negative

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TABLE I
Correlations among superoxide dismutase, catalase and glutathione peroxidase activities

Normal human tissues				
Enzyme	CuZnSOD	MnSOD	Catalase	GSH peroxidase
CuZnSOD	1			
MnSOD	0.466	1		
Catalase	0.748	0.301	1	
GSH peroxidase	0.455	0.321	0.489	1
Normal mouse tissues				
Enzyme	CuZnSOD	MnSOD	Catalase	GSH peroxidase
CuZnSOD	1			
MnSOD	−0.295	1		
Catalase	0.875	−0.057	1	
GSH peroxidase	0.880	−0.088	0.994	1
Vertebrate red blood cells				
Enzyme	SOD	Catalase	GSH peroxidase	
SOD	1			
Catalase	0.191	1		
GSH peroxidase	0.444	−0.224	1	
Neoplastic human cell lines				
Enzyme	CuZnSOD	MnSOD	Catalase	GSH peroxidase
CuZnSOD	1			
MnSOD	0.052	1		
Catalase	0.082	−0.225	1	
GSH peroxidase	−0.139	0.013	0.364	1

correlation with GSH peroxidase activity and virtually no correlation with MnSOD and catalase activities. Manganese SOD activity shows a low negative correlation with catalase activity and virtually no correlation with GSH peroxidase activity. Catalase activity shows a moderate positive correlation with GSH peroxidase activity. No significant correlation is present in the data, and only the moderate correlation between catalase and GSH peroxidase activities approaches the trend for normal human tissues.

Principal components for the enzyme activities are given in Table II. The first two principal components account for large proportions of the variance of the activities of CuZnSOD, MnSOD, catalase and GSH peroxidase in normal human tissues (79.1%), normal mouse tissues (96.6%), vertebrate red blood cells (86.3%) and neoplastic human cell lines (62.1%). This justified the analysis into two factors.

Table III shows the minres factors (F_1, F_2) obtained from two principal components (P_1 and P_2; Table II) and the rotations to varimax factors (M_1, M_2). Any factor can be reflected in the origin, changing the sign of its coefficients or loadings. With this proviso, the minres solution for the enzyme activities in normal human tissues equates the standardized CuZnSOD activity of a tissue to

$$0.819\,F_1 + 0.147\,F_2 + 0.554\,Y_{\mathrm{CuZnSOD}}$$

having regard to the value of the communality, and so on for the other enzyme activities. Y_{CuZnSOD} is the unique factor for the CuZnSOD activity.

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TABLE II
Principal components for superoxide dismutase, catalase and glutathione peroxidase activities

Normal human tissues				
Enzyme	P_1	P_2	P_3	P_4
CuZnSOD	0.885	0.071	−0.311	0.339
MnSOD	0.633	−0.767	0.019	−0.099
Catalase	0.848	0.341	−0.257	−0.314
GSH peroxidase	0.719	0.186	0.668	0.040
Variance	2.420	0.745	0.609	0.225
Per cent	60.5	18.6	15.2	5.6
Normal mouse tissues				
Enzyme	P_1	P_2	P_3	P_4
CuZnSOD	0.953	−0.103	−0.284	0.001
MnSOD	−0.226	0.971	−0.073	0.002
Catalase	0.975	0.178	0.127	−0.052
GSH peroxidase	0.979	0.148	0.133	0.051
Variance	2.868	1.007	0.12	0.005
Per cent	71.7	25.2	3	0.1
Vertebrate red blood cells				
Enzyme	P_1	P_2	P_3	
SOD	0.829	0.39	0.401	
Catalase	−0.082	0.955	−0.285	
GSH peroxidase	0.867	−0.283	−0.41	
Variance	1.446	1.144	0.41	
Per cent	48.2	38.1	13.7	
Neoplastic human cell lines				
Enzyme	P_1	P_2	P_3	P_4
CuZnSOD	−0.132	0.856	0.446	0.226
MnSOD	−0.430	−0.352	0.792	−0.253
Catalase	0.828	0.276	0.138	−0.469
GSH peroxidase	0.734	−0.363	0.389	0.421
Variance	1.427	1.064	0.997	0.513
Per cent	35.7	26.6	24.9	12.8

In an interacting biochemical system any two or more influences are unlikely to be unrelated and precisely orthogonal. Oblique factors were therefore determined with the results shown in Table IV. For normal human tissues, for instance, the correlation between the oblique factors (T_1, T_2) is −0.373. From this correlation it follows that the reference axes, representing the common factors, are separated by an angle of 112° (the angle whose cosine is − 0.373). This is illustrated in Figure 1 in which it is apparent that the points given by the minres solution, with respect to the orthogonal axes F_1 and F_2, fall into two distinct groups with respect to the oblique axes T_1 and T_2. The points representing the activities of CuZnSOD, catalase and GSH peroxidase are aggregated about the axis T_1, and the point representing MnSOD activity is close to the axis T_2. The factor structures in Table IV represent the correlations between the enzyme activities and the oblique factors (denoted $r(T_1)$ and $r(T_2)$ in the Table).

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TABLE III
Two-factor solutions for superoxide dismutase, catalase and glutathione peroxidase activities (minres and varimax)

	Minres		Varimax		
Normal human tissues					
Enzyme	F_1	F_2	M_1	M_2	Communality
CuZnSOD	0.819	0.147	0.779	−0.293	0.693
MnSOD	0.698	−0.716	0.232	−0.973	1.000
Catalase	0.842	0.399	0.927	−0.088	0.868
GSH peroxidase	0.543	0.08	0.507	−0.209	0.301
Normal mouse tissues					
Enzyme	F_1	F_2	M_1	M_2	Communality
CuZnSOD	0.249	0.936	0.433	0.868	0.937
MnSOD	−0.555	−0.167	−0.578	−0.051	0.337
Catalase	−0.189	0.982	−0.014	1	1.000
GSH peroxidase	−0.142	0.982	0.059	0.991	0.985
Vertebrate red blood cells					
Enzyme	F_1	F_2	M_1	M_2	Communality
SOD	0.512	0.392	0.596	0.245	0.416
Catalase	−0.201	0.748	0.001	0.775	0.600
GSH peroxidase	0.910	−0.055	0.865	−0.29	0.832
Neoplastic human cell lines					
Enzyme	F_1	F_2	M_1	M_2	Communality
CuZnSOD	−0.037	0.173	0.085	0.155	0.031
MnSOD	−0.136	0.186	−0.225	−0.051	0.053
Catalase	0.839	0.544	0.99	−0.142	1.000
GSH peroxidase	0.809	−0.587	0.223	−0.975	1.000

For the orthogonal solutions (minres and varimax; Table III) the correlations are the same as the factor loadings.

The communalities in Table III represent the portion of the unit variance of each standardized enzyme activity ascribable to the common factors. The sums of the communalities show that the two-factor solutions accounted for 71.5%, 81.5%, 61.6% and 52.0% of the total variance of the activities of CuZnSOD, MnSOD, catalase and GSH peroxidase in normal human tissues, normal mouse tissues, vertebrate red blood cells and neoplastic human cell lines, respectively. The percentage value for neoplastic human cell lines was not improved by a three-factor solution, which in fact accounted for 47.2% of the total variance. The reproduction of the total variance refers to the self-correlations of the variables. The intercorrelations were well reproduced by two factors. The value of the root-mean-square of the residuals was 4.444×10^{-4}, 2.563×10^{-3}, 1.217×10^{-8} and 3.419×10^{-2} for the fit of the correlations between the enzyme activities in normal human tissues, normal mouse tissues, vertebrate red blood cells and neoplastic human cell lines, respectively.

The interpretation of factors is guided by the loadings and correlations of the variables with the factors. The rotation to oblique factors enhanced the interpreta-

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TABLE IV
Two-factor pattern and structure for superoxide dismutase, catalase and glutathione peroxidase activities (promax)

Normal human tissues				
	Factor pattern		Factor structure	
Enzyme	T_1	T_2	$r(T_1)$	$r(T_2)$
CuZnSOD	0.758	−0.161	0.819	−0.444
MnSOD	0.034	−0.987	0.402	−1
Catalase	0.96	0.085	0.928	−0.273
GSH peroxidase	0.49	−0.124	0.536	−0.307
Correlation among factors = −0.373				
Normal mouse tissues				
	Factor pattern		Factor structure	
Enzyme	T_1	T_2	$r(T_1)$	$r(T_2)$
CuZnSOD	0.394	0.813	0.547	0.887
MnSOD	−0.585	0.028	−0.58	−0.082
Catalase	−0.039	1.007	0.149	0.999
GSH peroxidase	0.007	0.991	0.193	0.993
Correlation among factors = 0.187				
Vertebrate red blood cells				
	Factor pattern		Factor structure	
Enzyme	T_1	T_2	$r(T_1)$	$r(T_2)$
SOD	0.573	0.273	0.584	0.296
Catalase	−0.066	0.775	−0.035	0.772
GSH peroxidase	0.887	−0.250	0.887	−0.215
Correlation among factors = 0.040				
Neoplastic human cell lines				
	Factor pattern		Factor structure	
Enzyme	T_1	T_2	$r(T_1)$	$r(T_2)$
CuZnSOD	0.109	0.166	0.071	0.141
MnSOD	−0.236	−0.072	−0.22	−0.019
Catalase	0.986	−0.056	0.999	−0.279
GSH peroxidase	0.087	−0.977	0.308	−0.996
Correlation among factors = −0.226				

tions in this study. This account of the results is therefore based on the promax solutions (Table IV). Considering the solution for normal human tissues (Table IV; Figure 1), it is seen that CuZnSOD, catalase and GSH peroxidase activities have relatively high loadings, while MnSOD activity has a relatively small loading on the first factor (T_1). The order of the high loadings (and correlations in the factor structure) is catalase > CuZnSOD > GSH peroxidase. This appears to identify a factor globally influencing catalase, CuZnSOD and GSH peroxidase activities, in this order, in normal human tissues. The second factor (T_2) appears to influence mainly the activity of MnSOD since only this enzyme activity has a high loading and correlation with the factor (the signs can of course be reversed).

The promax solution for normal mouse tissues (Table IV) shows a factor (T_1) with a moderately high loading and correlation for MnSOD activity and a second factor (T_2) with high loadings and correlations for CuZnSOD, catalase and GSH peroxidase

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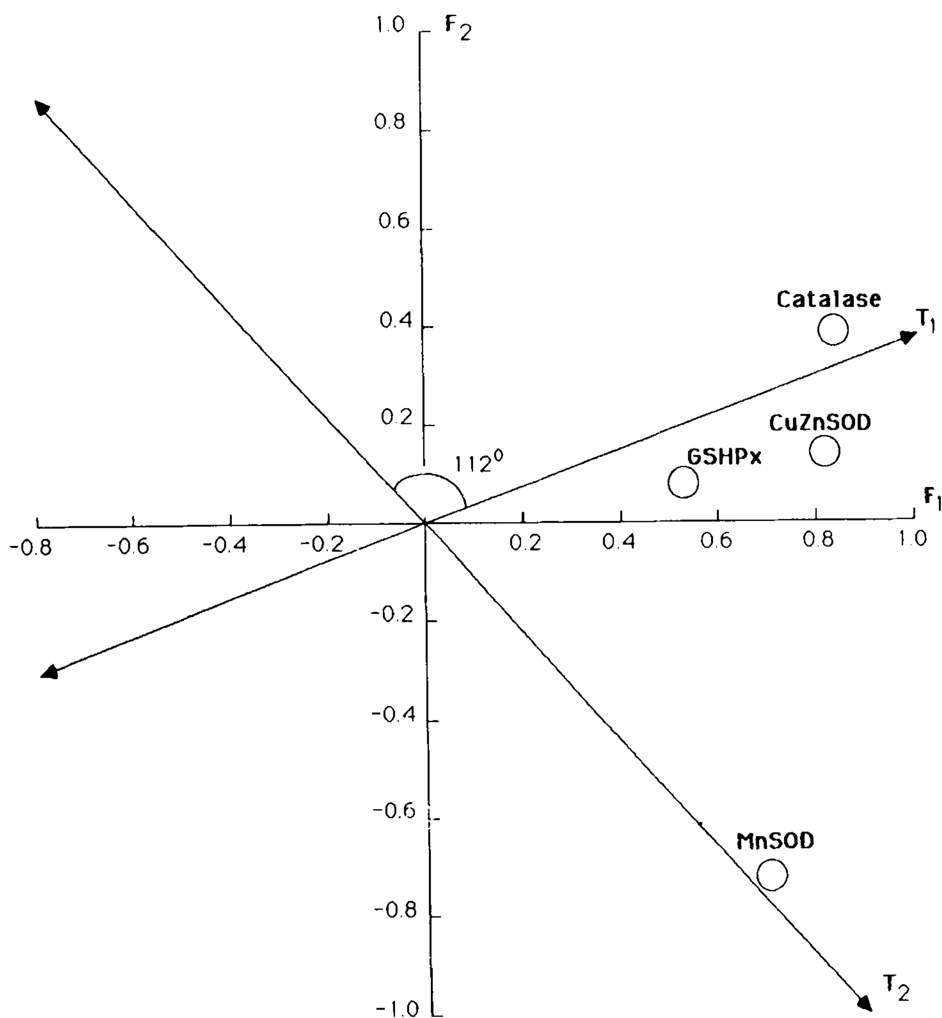

FIGURE 1 Plot of copper–zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), catalase and glutathione peroxidase (GSHPx) activities of normal human tissues with respect to orthogonal (F_1, F_2) and oblique factor axes (T_1, T_2).

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activities. The order of the loadings and correlations with the second factor is catalase > GSH peroxidase > CuZnSOD. It is seen that there is essentially the same separation of the enzyme activities as in the solution for normal human tissues, with a factor (now T_2) influencing mainly catalase, GSH peroxidase and CuZnSOD activities, in this order, and a factor (now T_1) influencing mainly MnSOD activity.

The promax solution for vertebrate red blood cells (Table IV) shows a factor (T_1) influencing mainly the activities of SOD and GSH peroxidase and a second factor (T_2) influencing mainly the activity of catalase. The same result was obtained when the

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SOD activities of the red cells of the species having nucleated red cells in the circulation were reduced by 10% as a correction for possible presence of non-particulate (or very loosely bound) MnSOD in the cytoplasm, as in carp red cells.[21] Carp red cells were not included in the analysis because they have a very low SOD and a disproportionately high GSH peroxidase activity compared to other vertebrate red cells, which produced an anomalous negative correlation ($r = -0.724$) between SOD and GSH peroxidase activities when combined with the data for other vertebrate red cells. The effect of this on the two-factor solution was to give a factor (T_1) with loadings of opposite instead of the same sign for SOD and GSH peroxidase activities. The second factor (T_2) remained the same.

The promax solution for neoplastic human cell lines (Table IV) shows two bipolar factors. The loadings in the factor pattern and the correlations in the factor structure suggest that the first factor (T_1) influences the activity of catalase and to a lesser extent that of MnSOD, while the second factor (T_2) influences the activity of GSH peroxidase and to a lesser extent that of CuZnSOD. The signs of the loadings suggest a contrast between catalase and MnSOD activity and between GSH peroxidase and CuZnSOD activity. This reflects the low negative correlations between catalase and MnSOD and between GSH peroxidase and CuZnSOD activities in the original data (Table I). Thus the three-enzyme factor influencing the activities of CuZnSOD, catalase and GSH peroxidase supported by the solutions for normal tissues was not seen, but separate catalase and GSH peroxidase activity factors, with opposite influences on MnSOD and CuZnSOD activities, respectively, were observed.

DISCUSSION

The demonstration of factors concomitantly influencing the variation of the activities of CuZnSOD, catalase and GSH peroxidase in normal tissues (T_1 for human and T_2 for mouse tissues in Table IV) supports the idea of a functional balance between these enzymes. A common denominator in this balance is hydrogen peroxide. The action of SOD requires that of catalase and GSH peroxidase to prevent accumulation of hydrogen peroxide inside the cell. Catalase activity had the highest loading and correlation with the corresponding three-enzyme factor for normal human and mouse tissues (Table IV). This suggests a catalase- or hydrogen peroxide-related factor affecting not only the variation of catalase but also that of CuZnSOD and GSH peroxidase activities. Hydrogen peroxide is the most stable of the oxygen reduction intermediates, and it may conceivably exert a common influence on the expression of catalase, CuZnSOD and GSH peroxidase activities in normal tissues. Catalase and CuZnSOD also appear to be intimately related becasue catalase may act to protect CuZnSOD from inactivation by hydrogen peroxide,[22] while CuZnSOD may act to protect catalase from inactivation by superoxide.[23] Correlation of the enzymatic activities of catalase and CuZnSOD may be expected on this basis. Experimentally the levels of catalase and CuZnSOD enzyme protein, determined immunochemically, are also correlated in normal human tissues. This may be seen from the measurements of Hartz *et al.*[24] which show a significant positive correlation ($r = 0.496$) between the catalase and CuZnSOD levels (μg/mg protein) in 17 tissues from two normal subjects, excluding red blood cells.

A factor concomitantly influencing the activities of catalase, CuZnSOD and GSH peroxidase was not found for vertebrate red blood cells. In these cells the variation

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of catalase activity appears to be influenced by a separate factor (T_2; Table IV). This is not a discordant observation. The apparent discrepancy with other tissues is interpretable. One consideration is the pathway of elimination of hydrogen peroxide. Although catalase breaks down high concentrations of hydrogen peroxide very rapidly, it is almost ineffective at decomposing low concentrations because of its low affinity (high effective K_m) for hydrogen peroxide.[25] Calalase is also restricted by peroxisomal compartmentation.[26] Thus GSH peroxidase is often regarded as more important than catalase in the elimination of hydrogen peroxide. At submicromolar concentrations, reduction by GSH, catalyzed by GSH peroxidase, appears to be the main pathway of hydrogen peroxide elimination in red blood cells. At higher concentrations, catalatic decomposition of hydrogen peroxide becomes increasingly important.[27] In other cells, the fate of hydrogen peroxide largely depends on its site of formation. Thus in perfused rat liver, catalatic decomposition of hydrogen peroxide is observed on infusion of substrates of peroxisomal oxidases,[28] while reduction of hydrogen peroxide by GSH is observed on infusion of benzylamine as a substrate of mitochondrial monoamine oxidase.[29]

Determination of the variation of CuZnSOD and GSH peroxidase activities by one factor and determination of catalase activity by a separate factor in red blood cells may be related to the demonstrable importance of GSH peroxidase in the elimination of relatively low concentrations of hydrogen peroxide[27] and to the relative importance of superoxide and hydrogen peroxide in the oxidation of haemoglobin. Superoxide oxidizes oxyhaemoglobin and reduces methaemoglobin. The activity of CuZnSOD in normal red blood cells is sufficient to suppress these reactions[30] and GSH peroxidase presumably eliminates the hydrogen peroxide produced by this activity under normal conditions. However, hydrogen peroxide may compete with superoxide for the oxidation of oxyhaemoglobin so that under conditions of excessive hydrogen peroxide production, CuZnSOD may have no effect[31] or may accelerate[32,33] the formation of methaemoglobin and degradation of haemoglobin. It is therefore reasonable to expect independent regulation of catalase activity or, in some cases, an anomalously high activity of GSH peroxidase, as in carp red cells[21] and the red cells of some newborn infants.[34] Interestingly, increase in GSH peroxidase activity is the response observed when CuZnSOD activity in red blood cells is elevated as a result of the gene dosage effect of trisomy 21 in Down's syndrome.[35]

The observations of determination of the variation of CuZnSOD and MnSOD activities by separate factors in normal tissues is of considerable interest. Dichotomous regulation of the activities of CuZnSOD and MnSOD has already been apparent from the preferential induction of MnSOD by hyperoxia in neonatal rat pulmonary macrophages.[36,37] In yeast, MnSOD is synthesized in the cytoplasm as a precursor of larger molecular weight, and is incorporated into the mitochondria by a process which appears to depend on oxidative phosphorylation. The oxygen-dependent pathway responsible for the synthesis of active MnSOD does not appear to involve haem.[38] In brine shrimp embryos treated with ethidium bromide there is decrease of MnSOD activity. Ethidium bromide inhibits mitochondrial gene expression, but the respiratory deficiency of the mitochondria which is produced does not allow distinction between involvement of products of oxygen metabolism and products of mitochondrial protein synthesis in the regulation of active MnSOD synthesis.[39] Apart from metal availability,[40,41] ability to appropriately poise the redox state of manganese may be important in this process.

Up till now it is the range of variation rather than the covariation of the activities

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of CuZnSOD, MnSOD, catalase and GSH peroxidase in tumour cells that has received attention.[42] Measurements of SOD activity in tumour cells have tended to show depression of MnSOD activity and less strict depression of CuZnSOD activity.[43] There are, however, discordant observations in human tumours and neoplastic cell lines.[17,44–46] In the data of Westman and Marklund[44] for 29 malignant human tumours there is a significant positive correlation between CuZnSOD and MnSOD activities ($r = 0.532$), which did not appear in the data for neoplastic human cell lines. As for catalase and GSH peroxidase activities, the general view is that catalase activity may be low in tumour cells[17,47,48] but not always,[17] while GSH peroxidase activity may be either low[42,48,49] or high[50,51] or within the range of normal tissues.[17]

In neoplastic human cell lines the variation of MnSOD activity appears to depend on a factor which mainly determines the variation of catalase activity. The correlations of catalase and MnSOD activities with the factor have opposite signs. A second factor which mainly determines the variation of GSH peroxidase activity also appears to determine in opposed fashion CuZnSOD activity. The interpretation of these observations is qualified by the low correlations of MnSOD and CuZnSOD activities with the respective factors (Table IV). Unexpected negative correlations between MnSOD and catalase and between CuZnSOD and GSH peroxidase activities are present in the original data (Table I), though the correlations are not statistically significant. Inverse variation of MnSOD and catalase activities represents a breakdown of the normal co-induction of MnSOD and catalase by oxygen.[52] More to the present point, it represents a bigger failure of MnSOD than of catalase synthesis in some neoplastic cell lines, and the same may be said of CuZnSOD and GSH peroxidase synthesis.

There is no adequate explanation for loss of SOD activity in tumour cells. Loss of MnSOD activity has been associated with the cancer phenotype.[43] In point of fact, it is only the converse of this hypothesis that has been observed experimentally. In normal rat kidney (NRK) cells, in NRK cells transformed by Kirsten sarcoma virus and in revertants of these cells, paraquat-inducible SOD activity, which can be attributed to the manganese enzyme,[53] is associated with the normal as opposed to the tumour phenotype.[54] Tumour cell mitochondria have the capacity to produce superoxide.[55,56] It is not certain, however, to what extent superoxide production occurs in intact tumour cells. Low xanthine oxidase activity, as compared to normal adult rat liver, is a biocheminal discriminant of rapidly growing rat Morris hepatoma.[57] The potential to produce superoxide (and hydrogen peroxide) in the cytoplasm, as exemplified by xanthine oxidase activity , may therefore be low in tumour cells and SOD synthesis may be repressed. An analogous situation can be seen to exist in neonatal rat liver. Here, low xanthine oxidase activity[57] is associated with low SOD activity.[58–60] Another relevant feature of tumour cells in relation to their antioxidant enzyme activities is the low intrinsic peroxidizability of tumour cell membranes,[61,62] which occurs early in the malignant transformation.[63] This may determine in part the low antioxidant enzyme activities. As regards catalase and GSH peroxidase, it is not clear why the variation of the activity of these enzymes should in contrast to normal tissues be determined by separate factors in neoplastic cell lines (Table IV). Catalase and GSH peroxidase activities were not significantly correlated in the data for neoplastic cell lines (Table I). Alterations in hydroperoxide metabolism consequent to loss of lipid peroxidation may explain the observed influence of catalase and GSH peroxidase activities by separate factors.

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