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Changes in glutathione and its metabolizing enzymes in human erythrocytes and lymphocytes with age

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The levels of glutathione (GSH) and the activities of glutathione *S*-transferases (GST) and glutathione reductase (GSR) in human erythrocytes and lymphocytes were determined in three age groups (5–12, 25–40, 65–83 years). The levels of GSH in lymphocytes increased with age, however, its levels in erythrocytes reached a maximum in the middle age group. The activity of GST in erythrocytes and lymphocytes changed as a function of age in a pattern similar to the changes found for GSH levels. GSR activity increased from young to middle age in both erythrocytes and lymphocytes, but decreased again in the old age group.

Glutathione (GSH) is a naturally occurring tripeptide isolated from animal and plant cells, including yeast, and it has been detected in other moulds (Waley 1966). It is a constituent of all cells and is almost always the major non-protein thiol compound. It is involved in different types of reactions in biological systems. One of its functions is in transhydrogenation reactions which form or maintain protein thiol groups. Oxidized glutathione is converted back to GSH by the action of glutathione reductase (GSR), an enzyme that needs riboflavin for its activation. This enzyme reduces GSSG to GSH with the concomitant oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to $NADP^+$.

GSH is also involved in the conjugation of electrophiles which is necessary for the elimination of foreign compounds in the body (Chasseaud 1980) and protects macromolecules in cells from being attacked by harmful electrophiles. The conjugates which are formed with GSH are more water-soluble and can be excreted in the bile or can be metabolized further to mercapturic acids. Conjugation reactions are catalysed by glutathione *S*-transferases (GST) which are widely distributed soluble enzymes present in hepatic and extrahepatic tissues of vertebrate species including man (Grover & Sims 1964), insects (Shishido et al 1972) and plants (Frear & Swanson 1970). They are thought to play a role in initiating the detoxication of potential alkylating agents, including pharmacologically active compounds.

The nature of the ageing process has been the subject of considerable speculation. One suggested possibility was related to free radical reaction damage (Harman 1968). Since GSH plays a role in cells as an antioxidant and since free radicals may be responsible for cellular ageing (Al-Turk & Stohs 1981; Vanella et al 1982), several studies have been conducted to correlate GSH and its metabolizing enzymes to the ageing process (Stohs et al 1980, 1982).

The object of the present investigation was to measure the levels of reduced GSH and the activities of GST and GSR in erythrocytes and lymphocytes of healthy individuals, 5–83 years old, and try to correlate any changes in GSH content and GSH metabolizing enzymes with age.

MATERIALS AND METHODS

Blood samples were collected from healthy individuals, aged 5–83 years, and divided into three age groups. These were young 5–12, middle 25–40 and old 65–83 years age groups; any variations likely to occur due to red cell size and in Hb content were minimized by excluding individuals having abnormal mean corpuscular volume and mean corpuscular haemoglobin values. Erythrocytes and lymphocytes were separated by a modification of the method of Boyum (1968). Ten mL of heparinized blood was carefully layered over 5 mL of Lymphoprep (sodium metrizoate/Ficoll solution), and centrifuged at 1700 rev min^{-1} for 30 min at 4 °C. The white ring (lymphocytes) was removed from the aqueous-Ficoll phase (erythrocytes) with a disposable pipette and transferred to a 50 mL centrifuge tube. Preliminary work had shown that the Lymphoprep did not affect GSH levels.

Glutathione determination

The reduced form of glutathione (GSH) was determined in erythrocytes and lymphocytes by the

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fluorometric method of Hissin & Hilf (1976), with the use of *O*-phthalaldehyde, at an excitation wavelength of 350 nm and an emission of 450 nm.

Galutathione S-transferase (GST) activity determination

The activity of GST in erythrocytes and lymphocytes was measured according to the spectrophotometric assay procedure of Habig et al (1974), at 340 nm and using 1-chloro-2,4-dinitrobenzene as substrate.

Glutathione reductase (GSR) activity determination

The GSR enzyme activity was measured spectrophotometrically at 340 nm using the method of Racker (1955), where in the presence of the enzyme, the hydrogen of NADPH is transferred to the oxidized glutathione.

Protein determination

Protein was determined according to the method of Lowry et al (1951) using bovine serum albumin as the standard. All protein determinations were performed spectrophotometrically at 750 nm.

Cell counting

This was performed using a Coulter Counter on suspensions of erythrocytes and lymphocytes.

Statistical analysis

Multiple group comparisons were made by using analysis of variance (ANOVA). Two-way comparisons of data utilized Student's *t*-test. A P value less than 0·05 was considered significant.

RESULTS

Glutathione (GSH) concentrations in human erythrocytes and lymphocytes as a function of age

GSH concentrations in human erythrocytes and lymphocytes (expressed in μg (mg protein)$^{-1}$) as a function of age are shown in Table 1. The GSH content of erythrocytes from the middle age group was higher than that of the young and old groups. This increase was statistically significant with respect to both age groups. GSH levels in lymphocytes increased by 3-fold in going from young to middle age. The difference in GSH content between the young and the old age groups was not significant. Table 2 represents the levels of erythrocyte and lymphocyte GSH expressed in μg/10^6 cells. The differences in erythrocyte GSH content were similar to the results obtained when GSH levels were expressed per mg protein (Table 1). GSH levels in lymphocytes increased with age, and the increase in the middle age group was statistically significant with respect to the young and old age groups. These results are comparable with the data obtained for lymphocytes when GSH content is expressed per mg protein (Table 1).

Table 1. Reduced glutathione concentrations in human erythrocytes and lymphocytes as a function of age.

	Glutathione (GSH) concn in μg (mg protein)$^{-1}$	
Age group	Erythrocytes	Lymphocytes
Young	1·62 ± 0·29 (13)	0·55 ± 0·11 (15)
Middle	2·17 ± 0·55* (15)	1·69 ± 0·76* (15)
Old	1·46 ± 0·25 (10)	0·71 ± 0·42 (10)

*$P < 0·05$ with respect to the young and old age groups. The young group was 5 to 12 years old, the middle group 25 to 40 years old and the old group 65 to 83 years old. Each value is the mean ± s.d. of the number of samples given in parentheses.

Table 2. Reduced glutathione concentrations in human erythrocytes and lymphocytes as a function of age.

	Glutathione (GSH) concn in μg/10^6 cells	
Age group	Erythrocytes	Lymphocytes
Young	0·07 ± 0·02 (21)	0·43 ± 0·07 (22)
Middle	0·16 ± 0·04* (23)	1·36 ± 0·21* (22)
Old	0·07 ± 0·02 (10)	0·64 ± 0·33* (20)

*$P < 0·05$ with respect to the young and old age groups. Each value is the mean ± s.d. of the number of individual samples given in parentheses.

Glutathione S-transferase and glutathione reductase activities in human erythrocytes and lymphocytes as a function of age

GST activity in the erythrocytes of the old age group was approximately 29% lower compared with the middle age group (Table 3). However, the difference

Table 3. Glutathione *S*-transferase activity in human erythrocytes and lymphocytes as a function of age.

	Glutathione-*S*-transferase activity (units)	
Age group	Erythrocytes	Lymphocytes
Young	2·84 ± 1·11 (26)	82·45 ± 25·5 (26)
Middle	4·03 ± 0·66 (21)	109·87 ± 18·45* (20)
Old	2.88 ± 0·81 (20)	68·43 ± 19·06* (19)

*$P < 0·05$ with respect to the young group. One unit is defined as the amount of enzyme catalysing the formation of 1 μmol product min^{-1}/10^6 cells. Each value is the mean ± s.d. Values in parentheses are the number of individuals in each group.

was not significant due to the large standard deviations. No difference existed in erythrocyte GST activity when the young age group was compared with the other two groups. GST activity in lymphocytes was found to decrease as a function of age, and, as shown in Table 3, the decrease was statistically significant with respect to the middle age group.

GSR activity in erythrocytes and lymphocytes as a function of age is presented in Table 4. The activity of GSR in both erythrocytes and lymphocytes was linear in the young age group compared with the middle age group, and decreased again in the old age group. The increased activity of the erythrocyte GSR from the middle age group was statistically significant with respect to both young and old age groups, while that of the lymphocytes was significant only with respect to the young group. A 49% decrease in erythrocyte GSR activity occurred in going from middle to old age, and a 24% decrease in lymphocyte GSR activity was observed over this same age span.

Table 4. Glutathione reductase activity in human erythrocytes and lymphocytes as a function of age.

	Glutathione reductase activity (units)	
Age group	Erythrocytes	Lymphocytes
Young	0·47 ± 0·18 (17)	4·18 ± 1·75 (14)
Middle	0·97 ± 0·15* (21)	6·64 ± 1·49** (21)
Old	0·50 ± 0·16 (8)	5·06 ± 1·20 (8)

*$P < 0.05$ with respect to the young and old age groups. **$P < 0.05$ with respect to the young group. One unit of activity is defined as the amount of enzyme which will oxidize 1 μmol of NADPH $min^{-1}/10^6$ cells. Each value is the mean ± s.d. of the number of individual samples given in parentheses.

DISCUSSION

Alterations in the metabolism of drugs and foreign chemicals with ageing not only pose several pharmaceutical and therapeutic problems, but may have far-reaching implications for immune response and carcinogenesis.

Tissue glutathione levels reflect one of the major pathways of drug metabolism that have been shown to be age-related (Al-Turk & Stohs 1981). Changes in GSH levels and/or its metabolizing enzymes with age may be related to impaired immunological responsiveness, increased susceptibility to drugs and free radicals, and enhanced susceptibility to some diseases.

Harman (1975) has proposed that the ageing process involves an increased susceptibility to free radicals. The ability of antioxidants to retard the ageing process as well as chemical carcinogenesis, suggests that GSH (as an antioxidant) may exert an inhibitory effect on these processes (Stohs et al 1982). Since GST and GSR play roles in GSH metabolism and in detoxication processes, the GSH content and the activities of GST and GSR were examined in human erythrocytes and lymphocytes as a function of age. Red blood cells offer an interesting model for studying the process leading to ageing because they have limited life span and cannot divide (Vanella et al 1982). It was also of interest to determine whether peripheral lymphocytes, as a part of the immune system and a readily accessible human tissue, could be used as a good model for studying the ageing process.

In this work, the GSH concentrations in human erythrocytes, expressed in μg (mg protein)$^{-1}$, were found to increase significantly (1·3-fold) in going from the young to the middle age group, and to decrease by 1·5-fold in going from the middle to the old age group. When erythrocyte GSH was expressed in terms of μg/10^6 cells, the age-related changes were similar. These results are in agreement with those obtained by Stohs et al (1980) where the GSH level in whole blood of mice increased in young and middle age animals and subsequently decreased again in old mice. Abraham et al (1978) have also found a decrease in GSH concentration in erythrocytes as a function of both increasing cell age and mouse age. However, Eng et al (1973) found an increase in GSH content in erythrocytes of the newborn compared with adults. The workers did not measure GSH in young individuals and did not specify the age of the adult group.

The human lymphocyte GSH content also changed as a function of age, comparable to erythrocytes. The decrease with age was significant with respect to the middle age group when the GSH content was expressed either in μg (mg protein)$^{-1}$ or μg/10^6 cells.

The activity of human GSG in both human erythrocytes and lymphocytes changes as a function of age in a pattern similar to the changes found for GSH levels. Large inter-individual variability within the age groups lead to large standard deviations. GST in erythrocytes increased in going from the young to the middle age group and then decreased by 29% when going from the middle to the old age group. However, these changes were not significant. Carmagnol et al (1981) observed a decrease in human erythrocyte GST during the first weeks of life (one week versus three months). The GST activity

remained constant from three months to 15 years, and increased slightly but significantly in individuals over 75 years. These observations are in contrast to the results obtained in this work for erythrocyte GST. The reason for these differences is not known.

GSR activity in both human erythrocytes and lymphocytes increased in going from the young to middle age groups, and decreased by 49 and 24% in the erythrocytes and lymphocytes, respectively, when the middle and old age groups were compared. The differences were significant between the latter two groups only with erythrocytes.

Despite the fact the GST and GSR activities are lower in erythrocytes than in lymphocytes, it can be concluded that erythrocytes may be a good model for studying the ageing process and the contributions which alterations in GSH and its metabolizing enzymes may make to the ageing process.

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