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REDUCED GLUTATHIONE CONTENT OF ERYTHROCYTES IN LEAD EXPOSED WORKERS

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ABSTRACT

This work was carried out with the aim of evaluating reduced glutathione content of erythrocytes (GSR) as a test for detection of lead affection of exposed workers.

Twenty-five lead exposed workers and twenty control workers were subjected to a clinical examination and to the estimation of lead in blood (Pb-B), lead in urine (Pb-U) delta--aminolevulinic dehydratase activity of erythrocytes (ALAD), haemoglobin in blood (Hb), GSR and delta-aminolevulinic acid in urine (ALAU).

The results showed that GSR was more reduced in lead workers than in control workers. The values of GSR correlated significantly with the parameters of lead exposure and with those of lead response. GSR was affected in a greater number of lead workers and at a lower Pb-B levels than other parameters.

It is concluded that GSR can be considered to be a sensitive early test of lead affection and that it can be used for routine monitoring of lead workers. The mechanisms of reduction of GSR and its effects are discussed.

Glutathione is the most abundant non-protein thiol in animal and plant tissues[7]. Through its sulphhydryl group (-SH) glutathione plays an important role in the oxidation and reduction reactions in the cell. A number of enzymes specially require glutathione as a co-enzyme. The erythrocyte membrane is continuously exposed to oxygen and GSR helps maintain the integrity of the membrane[14]. Reduced GSR therefore promotes haemolysis. GSR is also known to be involved in the incorporation of iron by protoporphyrin for the formation of haem[11].

In 1944 Albahary suggested that lead may affect glutathione[1]. It is thought that lead binds to the erythrocyte membrane by forming complexes with -SH groups including GSR[9]. In literature reduced GSR values have been reported in cases of lead poisoning[2,12].

This investigation was carried out with the aim of evaluating GSR determination as a test for early detection of lead affection and of comparing this test with other known parameters of lead exposure (Pb-B and Pb-U) and those of lead response (ALAD, Hb and ALAU).

SUBJECTS AND METHODS

Twenty-five lead exposure workers (LW) undergoing a routine periodical medical examination in Ain-Shams Faculty of Medicine, employed in a printing shop and in an accumulator factory, together with twenty control workers (CW) never exposed to lead in their work were the subjects of this study. Both groups were of comparable age and all were males. Workers of both groups were clinically examined but no specific relevant findings were detected. At the end of the clinical examination 10 ml of blood was taken in lead-free test tubes and CaEDTA was used as anticoagulant. A sample of urine was also taken in a polythene bottle.

Pb-B and Pb-U were estimated by the method of Keenan and co-workers[8]. Hb was measured as cyanmethaemoglobin. ALAD was estimated by the method of Bonsignore and co-workers[3], GSR content of erythrocytes according to the method of Stevenson and co-workers[13] and ALAU by the method of Grabecki and co-workers[5].

All urine analyses were made on spot samples. No correction factors were applied but samples with a specific gravity of less than 1.010 were discarded.

Coefficients of correlation (r) and regression lines were calculated according to standard statistical methods.

RESULTS

Table 1 shows the mean values of the parameters studied in CW and in LW. The mean Pb-B, Pb-U and ALAU values are significantly higher in LW than in CW. On the other hand, the mean Hb ALAD as well as GSR values are significantly lower in LW than in CW. The mean values of all parameters (except those of GSR) of LW fall in the categories of slight to moderate lead exposure[14].

The next step was to correlate the levels of GSR with the levels of other parameters of lead exposure and with those of lead response. The correlation

TABLE 1
Comparison of the parameters studied in control and lead exposed workers.

Parameter	Control workers		Lead workers		t
	Mean	± S.D.	Mean	± S.D.	
Pb-B (μmol/l)	1.54	0.4	2.96	0.8	6.764*
Hb (g/100 ml)	14.5	0.9	11.8	1.4	7.317*
ALAD (unit/mlE)	88.8	10.7	63.0	19.2	5.257*
GSR (mmol/l)	29.0	3.6	17.7	2.8	11.578*
Pb-U (mol/l)	0.17	0.06	0.37	0.1	11.219*
ALAU (μmol/l)	37.4	12.9	61.0	24.4	3.827*

*P < 0.001.

coefficients and the regression equations are shown in Table 2. The highest correlation was observed between GSR and parameters of lead exposure. GSR values showed also statistically significant correlations with parameters of lead response.

TABLE 2
Correlation coefficients and regression equations between the values of CSR and other parameters in LW.

Parameter	r	t	Regression equations
GSR versus Pb-B	− 0.828	7.082*	GSR = 25.573 − 0.129 Pb-B Pb-B = 154.515 − 5.274 GSR
GSR versus Hb	0.735	5.396*	GSR = 0.101 + 1.489 Hb Hb = 5.396 + 0.362 GSR
GSR versus ALAD	0.716	4.919*	GSR = 11.163 + 0.103 ALAD ALAD = 4.965 GSR − 24.666
GSR versus ALAU	− 0.796	7.732*	GSR = 23.246 − 0.698 ALAU ALAU = 24.019 − 0.907 GSR
GSR versus Pb-U	− 0.887	4.279*	GSR = 27.247 − 0.123 Pb-U Pb-U = 190.629 − 6.329 GSR

*P < 0.001.

To evaluate the sensitivity of GSR as an indicator of lead affection the mean values of the control group were used to establish upper normal (mean + two standard deviations) and lower normal (mean − two standard deviations) limits for all parameters used. The number and per cent of LW with values outside these limits are shown in Table 3. It is clear that 92% of LW had GSR values lower than normal, whereas only 72%, 52%, and 40% of them had abnormal values for Hb, ALAD, and ALAU respectively.

TABLE 3
Normal limits of various parameters number and per cent of LW with values outside these limits.

Parameter	Normal limits	LW with extreme	
		No.	%
Pb-B (μmol/l)	2.33	20	80.0
Hb (g/100 ml)	12.7	18	72.0
ALAD (unit/ml E)	67.4	13	52.0
GSR (mmol/l)	21.8	23	92.0
Pb-U (mmol/l)	0.3	20	80.0
ALAU (μmol/l)	63.3	10	40.0

DISCUSSION

The finding that the GSR content of erythrocytes is significantly lower in LW than in CW confirms the previous findings of Albahary[1] and those of Secchi and co-workers[12]. A significant negative correlation between the levels of GSR and those of Pb-B is again evidence that lead is responsible for reduction of GSR among LW. Thus reduced GSR, ALAD, ALAU and Hb are considered as proper parameters of body response to increasing lead absorption. A significant correlation between these parameters and GSR stresses further the possibility that in LW reduced GSR values may be related to the effects of lead.

It is interesting to note that in LW GSR was affected in more workers than other parameters of lead response. This may be an indication of a higher sensitivity of this test. Taking Pb-B as the base it was found that in all cases with a Pb-B value higher than the upper normal limit used in this study (2.33 mmol/l) the GSR was lower than normal. Three LW had Pb-B within normal limits but their GSR values were below normal. This finding may indicate an early affection of GSR or it may be a non-specific response. However, none of the CW showed abnormally low GSR values indicating that non-specific responses to this test are rare. GSR is known to be diminished in certain types of hereditary anaemia[15] but no worker in this study suffered from such a condition. Thus it may be concluded that GSR is reduced early in cases of lead exposure, that therefore it is a sensitive parameter and that it may be altered earlier than other parameters used for the assessment of lead response.

Several explanations had been speculated in connection with reduced GSR in cases of lead exposure. Jackson[6] showed that glutathione is formed in red cells from glutamic acid, glycine and cysteine. Lead has a high affinity for -SH group. It thus combines with the -SH group of cysteine and in this way it interferes with the synthesis of glutathione reducing its level in erythrocytes. Passow and co-workers[9] suggested that lead may combine directly with the -SH group of GSR blocking its activity.

Reduction of GSR may explain various abnormalities commonly found in cases of lead poisoning. GSR is essential for the incorporation of iron in haem[11]. Reduction of GSR is therefore expected to be associated with anaemia. The finding in this study that GSR is affected at lower blood levels than Hb supports this view. Again, reduced GSR is known to promote haemolysis[14]. It is generally accepted that in lead poisoning the fragility of reticulocytes, stippled cells and other red cells is increased and this accounts for a certain degree of haemolysis[2]. The finding of reduced GSR in LW may therefore explain this increased fragility. Another effect of reduced GSR is the inhibition of ALAD activity of erythrocytes. Gibson and Goldberg[4] demonstrated experimentally that ALAD activity was reduced by lead but that it could be recovered by addition of glutathione since this enzyme needs -SH group for its action. Our finding that GSR was reduced in a larger number of workers and at a lower Pb-B concentrations than ALAD, together with the significant positive correlation between the two may support the possibility that reduction of GSR may result in a lack of restoration of ALAD activity. Of course this will be followed by increased excretion of ALAU.

In conclusion it may be said that GSR is reduced in cases of lead exposure, and this reduction may explain the anaemia, increased fragility of erythrocytes and diminshed ALAD activity that are known to occur in cases of lead poisoning. GSR is affected earlier than other parameters of lead exposure and lead response and measurement of GSR can be considered to be a sensitive test for monitoring lead exposed workers.

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