Increased concentrations of haemoglobin X and Y in the erythrocytes of workers in a chemical plant in Japan

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When aromatic compounds such as aminophenols and nitrobenzenes are absorbed they cause oxidation of the intracellular haemoglobin of circulating erythrocytes and sometimes induce severe methaemoglobinemia. We have also found that half oxidised haemoglobins are greatly increased in the erythrocytes of patients with hereditary methaemoglobinemia owing to a deficiency of NADH methaemoglobin reductase. This prompted us to analyse the oxidised haemoglobins in circulating erythrocytes of workers in chemical plants. We found that half oxidised haemoglobins such as \((\alpha^+\beta^0)\) and \((\alpha^0\beta^+)_2\) were significantly increased in the erythrocytes of workers of some chemical factories of the Kansai area of Japan (A Tomoda et al, unpublished data). When analysing the haemoglobin patterns of workers from these factories by isoelectric focusing electrophoresis on polyacrylamide gel plates, we found that two unusual haemoglobins, which migrate to anodic positions on electrophoresis, were significantly increased in the erythrocytes of all the workers studied in a chemical plant producing 4-chloroaminophenol. One of these haemoglobins moved to an anodic position consistent with that of haemoglobin X, and another moved to the position between haemoglobins X and A. The present report deals with cases with a pronounced increase in the concentrations of haemoglobins X and Y, half oxidised haemoglobin, and methaemoglobin in the erythrocytes of workers in a chemical plant.

Materials and methods

Blood samples were drawn from 21 production workers handling aromatic compounds such as p-nitrophenol, o-nitrophenol, 4-chlor-2-aminophenol, and 4-chloro-o-aminophenol after their consent. We added 0-1 ml aliquots of blood to 5 ml distilled water and CO gas was bubbled through the haemolysates. The haemolysates were subjected to isoelectric focusing electrophoresis on polyacrylamide gel plates (LKB-Pharmacia, pH 3.5-9.5) within five hours. After electrophoresis, the gels were treated with a fixing solution and analysed by gel-scanner. The percentages of haemoglobins X, Y, and A, half oxidised haemoglobins such as \((\alpha^+\beta^+)_2\) and \((\alpha^0\beta^+)_2\), methaemoglobin, and haemoglobins E and A were estimated by cutting out and weighing the chart paper as described previously.

Results

The results are illustrated in figs 1 and 2. Figure 1 shows the gel-scanning patterns of erythrocyte lysates obtained from a worker who showed a specific haemoglobin pattern. Half oxidised haemoglobins such as \((\alpha^+\beta^0)\) and \((\alpha^0\beta^+)_2\) and methaemoglobin were significantly increased in the erythrocytes. Unexpectedly, the results showed that two anodic components of haemoglobin (one like haemoglobin X and another which migrated to a position between haemoglobins X and A on electrophoresis) were

Fig 1 Gel-scanning patterns of haemolysates of a worker handling chemical compounds after performance of isoelectric focusing electrophoresis.
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Fig 2  Percentages of haemoglobin components to total haemoglobin in haemolysates obtained from 21 production workers of a chemical plant. (Broken lines in columns show normal values of each component in human erythrocytes.)

present in large amounts. These unusual haemoglobins are not present in normal controls. From the gel-scanning analysis of the sample, the percentages of each haemoglobin in the total haemoglobin were: haemoglobin X, 7%; haemoglobin Y, 22%; haemoglobin A + haemoglobin F, 57%; (α2β+2), + (α+β2), 8%; methaemoglobin, 3%; and haemoglobin A2, 3%.

Figure 2 shows the percentages of haemoglobins X and Y; haemoglobin A + haemoglobin F; half oxidised haemoglobins such as (α2β+2)1 + (α+β2); methaemoglobin; and haemoglobin A2 in the total haemoglobin in each erythrocyte lysate. The broken lines in each column show the normal value for each component in the controls. Haemoglobins X and Y, half oxidised haemoglobins, and methaemoglobin were found to be greatly increased in the erythrocytes of every worker.

Discussion

Our results suggest that oxyhaemoglobin in the cells may be oxidised by chemical compounds absorbed at work. Since the chemical plant produced aromatic compounds such as p-nitrophenol, 4-chlor-2-amino-phenol, monochlorbenzene and 4-chlor-nitrophenol,
the derivatives of which cause methaemoglobinemia, these compounds may have induced oxidation. We unexpectedly, however, found two unusual haemoglobins. As shown in fig 1, two anodic components were found in the erythrocytes of workers at the chemical plant in varying concentrations (fig 2).

Carrell et al discovered a new haemoglobin when normal haemoglobin solutions were treated with copper and they named it haemoglobin X. One of the two anodic haemoglobins found here was in good agreement with haemoglobin X in terms of its electrophoretic mobility and we have called it such in the present report (figs 1 and 2). We also found another anodic component of haemoglobin to be present in large amounts and this unusual haemoglobin moved between haemoglobins X and A on isoelectric focusing electrophoresis. We have named this haemoglobin, haemoglobin Y, since no description of it exists at present. We have recently found that haemoglobin Y was produced in the circulating erythrocytes of a woman who drank creosol solutions to commit suicide. We are now trying to purify it from the erythrocytes.

The appearance of two new anodic components of haemoglobin in the erythrocytes of workers at a chemical plant may result from the oxidation and modification of intracellular haemoglobin A by chemicals absorbed at work. Some of these compounds are likely to become active after being metabolised in the liver.

References