Reticulocyte haem synthesis in occupational exposure to trinitrotoluene

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Trinitrotoluene (TNT) causes cataracts in workers even at low exposure levels.¹ The changes in the lens may be virtually irreversible and be caused by a peroxidative denaturation of the lens fibre protein suggested by the brownish colour of the cataract.² TNT is known to initiate lipid peroxidation through its cyclic reduction and oxidation by mitochondrial nitroreductases giving rise to superoxide anion.³

Because of the refined ophthalmological equipment and skills required to detect the changes in the lens we wondered whether a biochemical test could be developed for the effects of absorbed TNT. Our earlier experiment suggested that mitochondrial damage was an initial toxicological effect of TNT in rats.³ The rate limiting enzymes of haem synthesis in mammals, δ -aminolaevulinic acid synthase, and haem synthase are of mitochondrial location so that their activity in circulating reticulocytes that still contain mitochondria may be assayed. Subsequently, the acute effects of TNT could be shown in reticulocytes of rats exposed to TNT,⁴ and in the present study we have applied the test to workers with known exposure to TNT.

Subjects and methods

Nine workers (3 women, 6 men) exposed to TNT in a factory producing the explosive took part in the study (table 1). Four worked in the packing department and five in the synthesis process room. The mean TNT concentration in the process room air was 0.35 mg/m³ (range 0.31-0.39) and in the packing department 0.10 mg/m³ (range 0.02-0.19). None of the air samples exceeded the current Finnish hygienic limit level of 0.50 mg/m³.

The clinical state of the participants was unremarkable with the exception of binocular cataracts of varying severity in seven cases (table 1). The opacities were never noted in the central parts of the lens so that they did not interfere with the visual fields. Their demonstration required a maximal pupillary dilatation.¹ The blood samples (50 ml) were drawn from the cubital vein into heparinised tubes and immediately delivered to the laboratory in an ice bath. The buffy coats were removed by centrifugation and the remaining red cells were suspended in cold 0.9% NaCl solution to achieve $(2-3) \times 10^{12}$ cells per litre. The number of reticulocytes was determined and δ -aminolaevulinic acid synthase (EC 2.3.1.37) and haem synthase (EC 4.99.1.1) activities were determined.⁴ The results were com-

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Table 1 Subjects exposed to trinitrotoluene

Case	Sex	Age (years)	Task	Current occupation (years)	Cataract
1	F	54	Packing	20	Faint peripheral haze
2	F	45	Packing	18	Continuous equatorial opacity
3	M	58	Process	18	Spoke like equatorial opacities
4	M	21	Process	1	None
5	M	44	Process	13	Faint peripheral haze
6	M	28	Packing	19	Spoke like equatorial opacities
7	F	53	Packing	27	Continuous equatorial opacity
8	M	25	Process	3	None
9	M	36	Process	7	Faint peripheral haze

Table 2 Trinitrotoluene induced effects on reticulocyte haem synthesis

Case	δ -Aminolaevulinic acid synthase (pmol/h $\times 10^6$ reticulocytes)	Haem synthase (pmol/h $\times 10^6$ reticulocytes)
1	0.11	8.6
2	0.09	3.2
3	0.30	9.7
4	0.11	6.6
5	0.13	17.1
6	0.19	18.6
7	0.36	5.6
8	0.23	2.7
9	0.18	6.4
Controls:		
Mean	0.50	14.0
5th percentile	0.15	6.0

pared with a control range (mean $\pm$ 2 SD) of 25 individuals of both sexes with no occupational exposure to chemicals. Statistical evaluation was performed by the Student's *t* test as modified by Cochran.⁵

Results and discussion

The activity of δ -aminolaevulinic acid synthase in the exposed workers was smaller than that of controls (0.19 ± 0.09 , $n = 9$ v 0.50 ± 0.18 , $n = 25$, $\pm$ SD; $p < 0.01$) and four subjects had values below the fifth percentile (mean -2 SD) of controls (cases 1, 2, 4, 5; table 2). The mean haem synthase activity of the exposed workers (8.7 ± 5.7 , $n = 9$, $\pm$ SD) was also somewhat smaller than that of controls (14.0 ± 4.0 , $n = 25$, $\pm$ SD; $p < 0.05$). Three subjects had values below the fifth percentile of controls (cases 2, 7, 8; table 2).

None of the exposed workers had clinical anaemia, which may indicate that the haemoglobin synthesis in the bone marrow is not affected. Examination of bone marrow by electron microscopy in experimentally exposed rats failed to show morphological damage of mitochondria.⁴ This might indicate that the TNT induced effects in reticulo-

cytes take place in the circulation after the oxygenation of cells in the lungs as molecular oxygen is needed as the acceptor of electrons from the TNT radical.³

It is not known whether the changes in the haem synthesis predict the cataract formation which is only found after at least one year in the trade.¹ The cataracts probably represent cumulative effects of TNT absorption whereas the effects on circulating blood cells must result from a recent exposure as the cells are short lived by comparison with lens components that are maintained the whole life.²

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