Occupational lead exposure in Denmark: screening with the haematofluorometer

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ABSTRACT The zinc protoporphyrin/haemoglobin (ZPP/Hb) ratio was measured in the field with a haematofluorometer. A significant increase in ZPP/Hb ratio with advancing age was found in 1295 men who denied any excess exposure to lead. Ninety-seven per cent of the results were below 110 μmol ZPP/mol Hb(Fe) (4.4 μg ZPP/g Hb). The ZPP/Hb ratio was determined in a lead-exposed population of 2275 men, and in 305 a blood lead analysis was also performed. A blood lead limit of 2.9 μmol/l (60 μg/100 ml) corresponds to about 500 μmol ZPP/mol Hb(Fe) (20 μg/g). This limit was exceeded in workers engaged in secondary lead smelting, storage battery manufacture, car radiator repair, crystal glass manufacture, storage battery repair, ship breaking, metal foundries, the ceramic industry, scrap metal handling, and PVC plastic manufacture. Other occupations caused lower lead exposures with ZPP/Hb ratios between 110 and 500 μmol ZPP/mol Hb(Fe): such ratios were found in men from shooting ranges, in leaded pane manufacturers, gunsmiths, car paint sprayers, type setters, steel rolling mill workers, shipbuilders and welders, car mechanics, lead pigment handlers, and solderers. Increased ZPP/Hb ratios and blood lead levels in 210 workers were associated with a decrease in haemoglobin concentration in the blood. Thus, the haematofluorometer has proved to be very useful for screening purposes. A blood lead determination should be performed if the ZPP/Hb ratio exceeds 300 μmol ZPP/mol Hb(Fe) (12 μg/g).

The incidence and severity of lead poisoning have decreased substantially during recent years, but much still remains to be done to eliminate lead poisoning completely as an occupational disease (World Health Organization, 1977). Lead and lead compounds are used in a very wide range of industries, and the US Public Health Service (1964) has listed 113 occupations with potential lead exposure. Cases of lead poisoning occur mainly within a limited number of occupations. Thus, in Denmark, poisoning has been notified in industries manufacturing electric storage batteries, a secondary lead smelter, metal foundries, and industries producing polyvinyl chloride plastic. In this regard Denmark resembles other countries from which official statistics have been published (Popper and Raber, 1966; Quaas and Naas, 1971; Tola et al., 1976). It has been estimated that about 1% of the workforce in Finland has a blood lead level in excess of 1.9 μmol/l (40 μg/100 ml) because of occupational lead exposure (Tola et al., 1976). Forty years ago a survey in the USA showed that about 1-5% of the workers were exposed to large quantities of lead (Bloomfield et al., 1940). The major hazard today seems to be in the small workshops, but high exposures also occur in some large companies with inadequate industrial hygiene programmes (National Academy of Sciences Committee on Biological Effects of Atmospheric Pollutants, 1972; Hernberg, 1973). Under these circumstances, extensive exposure control is necessary.

Blood lead levels are, under certain conditions, a useful indicator of the exposure to lead in groups of workers (World Health Organization, 1977). Cases of lead poisoning have, however, occurred below a safety limit for blood lead concentration of 3.9 μmol/l (Waldron, 1974; Federal Register, 1975). Alternatively, measurements of biological effects of lead have been recommended (Balogh, 1974; Tomokuni and Ogata, 1976; Beričič et al., 1977; Joselow and Flores, 1977). An international working group has recently recommended the use of protoporphyrin analysis in screening for occupational lead exposure (Zielhuis, 1977). Protoporphyrin accumu-

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lates in the erythrocytes during their maturation in the bone marrow because lead interferes with the function of ferrochelatase (Piomelli et al., 1973; Sassa et al., 1975). It has been shown that this protoporphyrin is mainly chelated with zinc (Lamola and Yamane, 1974), and a special front surface fluorometer, the haematofluorometer, has been designed to measure zinc protoporphyrin (ZPP) rapidly in the field (Blumberg et al., 1977). The ZPP measurement, however, has two drawbacks: it is not sufficiently sensitive to recent, sudden, or mild increases in lead exposure, and it is not specific for lead, as increased levels may be attributable to iron deficiency anaemia (Lamola and Yamane, 1974; Beritic et al., 1977). The purpose of the investigation described here was to evaluate the use of a haematofluorometer in a screening programme for occupational lead exposure in male workers in Denmark.

Material and methods

REFERENCE POPULATION

Free ZPP tests were offered to the visitors at the Working Environment Foundation exhibitions in Copenhagen and in 33 provincial and country towns. An experienced occupational health nurse noted the name, age, occupation and possible sources of lead exposure in each case. A total number of 1295 men without known excess exposure to lead were examined.

EXPOSED POPULATION

At the same exhibitions, 340 men with potential occupational exposure to lead were tested for ZPP (21% of the total number examined at the exhibitions).

In co-operation with the Danish Labour Inspectorate, 171 enterprises were visited, and 1935 male workers with lead exposure lasting for at least six months were examined. Not all employees could be included in the study because of shift work, sickness or other reasons for absence during our visits. It is probable, however, that the workers examined are representative of the individual firms, but that the firms are not necessarily representative of the respective branches of industry. The companies were identified from the register of notified cases of lead poisoning and from the files of the district offices of the Labour Inspectorate. It is believed that all Danish secondary lead smelters, storage battery manufacturers, ship breakers, crystal glass manufacturers, paint manufacturers and steel rolling mills were visited. Moreover, because of a recent report on high lead exposure among car workers (Claussen and Rastogi, 1977), special emphasis was laid on garages.

ANALYTICAL METHODS

ZPP was measured with an Aviv Haematofluorometer, Model ZPP meter. The instrument has proved to be reliable and rapid, and the measured ratio between zinc protoporphyrin and haemoglobin concentrations is expressed in μmol ZPP/mol Hb(Fe) (Grandjean and Lintrup, 1978). This unit corresponds to approximately 25 μg ZPP/g Hb.

If the ZPP/Hb molar ratio was in excess of 300, a venous blood sample was drawn into a 10 ml Vacutainer tube for lead analysis. Previous investigations have indicated that blood lead levels higher than 2.9 μmol/l (60 μg/100 ml) rarely occur below this ZPP limit (Beritic et al., 1977; Blumberg et al., 1977; Grandjean et al., 1978). Blood samples were also drawn from individuals at their workplaces, if their birth dates were the 2nd, 15th, or 24th of the month. Of these blood samples, 305 were analysed by the National Institute of Occupational Hygiene, Copenhagen, by the method described by Hessel (1968). This laboratory has participated in several comparative programmes, and its performance has proved to be reliable with a coefficient of variation less than 10% (Grandjean, 1978).

Haemoglobin was measured in 210 of the venous blood samples by means of a Coulter S or a Hemalog 8/90, both standardised by comparison with a cyanhaemoglobin standard (Baker 3061) in a Zeiss spectrophotometer. One mmol Hb(Fe)/l blood corresponds to 1.6 g/100 ml.

Results

REFERENCE INTERVAL FOR ZPP

The normal ZPP/Hb ratio in the reference population was found to be age-dependent (Fig. 1). The Spearman rank correlation coefficient was 0.23 (p < 0.001). The total median was 71 μmol ZPP/mol Hb(Fe). The increase in ZPP with advancing age was apparently linear (Fig. 1). For practical purposes the upper cut-off limit of the reference interval was set at 110 μmol ZPP/mol Hb(Fe). This level is the 97th percentile; it was exceeded by 22 of the subjects aged over 50 years (7%), and by 19 aged 15–49 years (2%). Excess exposure to lead could explain a few high results found in the reference group, although such exposure was denied by all of them.

VALIDITY OF ZPP ANALYSIS

Because of the cumulative characteristics of lead, the ZPP level may depend on the length of exposure. Ninety-four employees from a company which manufactures electric storage batteries comprised the largest exposed group from the same firm. Thus, the possible association between ZPP level and exposure time could be evaluated. After the
first six months of exposure, no further increase in ZPP was apparent (Table 1).

Individuals with high ZPP/Hb ratios almost invariably had a concomitant increase in the blood lead level (Fig. 2). The correlation between the two measures is highly significant ($r_s = 0.86; p < 0.001$). The data shown in Fig. 2 indicate an exponential increase in ZPP with increased lead exposure. The regression lines indicate that the upper limit of the reference interval, 110 $\mu$mol ZPP/mol Hb, would correspond to about 1.2 $\mu$mol Pb/l blood (25 $\mu$g/100 ml). Almost all subjects (98%) with a ZPP/Hb ratio within the reference interval had a blood lead level of 1.5 $\mu$mol/l or less. The recommended permissible limit for lead in blood, 2.9 $\mu$mol/l, would correspond to about 500 $\mu$mol ZPP/mol Hb(Fe). However, several individuals with a blood lead level in excess of the limit had a comparatively low ZPP/Hb ratio. The validity of different cut-off levels for ZPP/Hb ratios is shown in Table 2. It appears that very few subjects with a high blood lead levels also had a ZPP/Hb ratio below the 300 $\mu$mol/mol limit used in this screening study.

In 210 males from the exposed population, the haemoglobin concentration was determined and was found to be significantly associated with the ZPP/Hb ratio ($r_s = -0.42; p < 0.001$) (Fig. 3).

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**Table 1** ZPP/Hb ratios ($\mu$mol/mol) in relation to duration of lead exposure in 94 workers manufacturing electric storage batteries

<table>
<thead>
<tr>
<th>Duration of exposure (yr)</th>
<th>Number of workers</th>
<th>ZPP/Hb ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>95% confidence limits to median</td>
</tr>
<tr>
<td>0.5–1.0</td>
<td>18</td>
<td>616</td>
</tr>
<tr>
<td>1–2.0</td>
<td>19</td>
<td>692</td>
</tr>
<tr>
<td>2.1–5.0</td>
<td>23</td>
<td>492</td>
</tr>
<tr>
<td>5.1–10.0</td>
<td>19</td>
<td>412</td>
</tr>
<tr>
<td>&gt; 10-</td>
<td>15</td>
<td>313</td>
</tr>
</tbody>
</table>

The blood lead level, assessed in the same blood samples in 202 of these individuals, was significantly associated with haemoglobin ($r_s = -0.31; p < 0.001$) (Fig. 4). With increasing levels of ZPP and lead in the blood, the haemoglobin concentration decreases, and the proportion of haemoglobin values in the lower normal range increases. The cut-off limits for ZPP are more effective than blood lead limits in distinguishing low from high haemoglobin concentrations (Tables 3 and 4).

**ZPP Levels in different Occupations**

The ZPP/Hb ratio seems to be a convenient measure of lead exposure, and it was not possible to find any difference between different occupations in the relationship between ZPP and blood lead levels. Thus,

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**Fig. 1** Median and 95% confidence limits to median of zinc protoporphyrin/haemoglobin ratio in different age groups ($N = 1295$; Spearman rank correlation coefficient, $r_s = 0.23; p < 0.001$). The dashed line is the regression line ($y$ on $x$).

**Fig. 2** Relationship between zinc protoporphyrin/haemoglobin ratio and lead concentration in the blood of 305 men with occupational lead exposure. The two regression lines are shown.
The Relationship between ZPP/mol, umol Pb/umol Hb(Fe) and zinc protoporphyrin/haemoglobin ratio in the blood of 210 men with occupational lead exposure. The two regression lines are shown.

**Fig. 3** Relationship between haemoglobin concentration and zinc protoporphyrin/haemoglobin ratio in the blood of 210 men with occupational lead exposure. The two regression lines are shown.

**Fig. 4** Relationship between haemoglobin and lead concentration in the blood of 202 men with occupational lead exposure. The two regression lines are shown.

**Table 2** Validity of ZPP determinations with different cut-off points compared with a blood lead limit of 2.9 µmol/l

<table>
<thead>
<tr>
<th>ZPP limit (µmol/mol Hb (Fe))</th>
<th>Sensitivity (% true positives)</th>
<th>Specificity (% true negatives)</th>
<th>Validity (sensitivity + specificity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>750</td>
<td>62</td>
<td>87</td>
<td>149</td>
</tr>
<tr>
<td>500</td>
<td>56</td>
<td>92</td>
<td>148</td>
</tr>
<tr>
<td>300</td>
<td>45</td>
<td>98</td>
<td>143</td>
</tr>
</tbody>
</table>

**Table 3** Haemoglobin concentrations (mmol/l) compared with ZPP/Hb ratios (µmol/mol) in 210 lead-exposed workers

<table>
<thead>
<tr>
<th>ZPP/Hb ratio</th>
<th>Number of workers</th>
<th>Haemoglobin (µmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;110</td>
<td>76</td>
<td>9.7</td>
</tr>
<tr>
<td>110-500</td>
<td>71</td>
<td>9.2*</td>
</tr>
<tr>
<td>&gt;500</td>
<td>63</td>
<td>9.0†</td>
</tr>
</tbody>
</table>

†p < 0.05 and ‡p < 0.001 compared with the preceding group by a two-tailed Mann-Whitney U-test.
§p < 0.05 compared with the preceding group by a two-tailed x² test.

**Discussion**

Determination of the ZPP/Hb ratio represents the easiest and quickest method available for the assessment of lead exposure. The haematofluorometer provides the result in a few seconds, and this makes immediate action possible when excessive ZPP levels are found. Some hundred blood lead determinations are performed each year by the National Institute of Occupational Hygiene, but the haematofluorometer has made it possible to examine several thousand individuals in a year. However, before the haematofluorometer is accepted as a screening method, its validity should be carefully checked.

It has been shown in this study that the ZPP/Hb ratio increases significantly with advancing age. This could be related to impaired iron absorption or reduced iron intake in the older individuals. Thus, in the elderly, an increased incidence of iron de-
Table 5  

<table>
<thead>
<tr>
<th>Industry</th>
<th>Number of firms visited</th>
<th>Number of employees examined</th>
<th>ZPP/Hb ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary lead smelter</td>
<td>1</td>
<td>67</td>
<td>479</td>
</tr>
<tr>
<td>Storage battery plant</td>
<td>4</td>
<td>264</td>
<td>302</td>
</tr>
<tr>
<td>Car radiator repair</td>
<td>15</td>
<td>45</td>
<td>208</td>
</tr>
<tr>
<td>Crystal glass manufacture</td>
<td>2</td>
<td>16</td>
<td>259</td>
</tr>
<tr>
<td>Storage battery repair</td>
<td>10</td>
<td>21†</td>
<td>116</td>
</tr>
<tr>
<td>Ship breaking</td>
<td>2</td>
<td>31</td>
<td>154</td>
</tr>
<tr>
<td>Metal foundry</td>
<td>30</td>
<td>205†</td>
<td>107</td>
</tr>
<tr>
<td>Ceramic industry</td>
<td>19</td>
<td>48†</td>
<td>77</td>
</tr>
<tr>
<td>Scrap metal handling</td>
<td>4</td>
<td>21</td>
<td>144</td>
</tr>
<tr>
<td>PVC plastic manufacture</td>
<td>6</td>
<td>32</td>
<td>102</td>
</tr>
</tbody>
</table>

†Number includes subjects examined at the Working Environment Foundation exhibitions (see text).

Table 6  

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Number of concerns visited</th>
<th>Number of employees examined</th>
<th>ZPP/Hb ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shooting range instruction</td>
<td>3</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>Leadexed pane manufacturing</td>
<td>6</td>
<td>18†</td>
<td>75</td>
</tr>
<tr>
<td>Gunsmiths</td>
<td>4</td>
<td>7</td>
<td>84</td>
</tr>
<tr>
<td>Car paint-spraying</td>
<td>5</td>
<td>26†</td>
<td>92</td>
</tr>
<tr>
<td>Type setting</td>
<td>23</td>
<td>27†</td>
<td>74</td>
</tr>
<tr>
<td>Steel rolling</td>
<td>1</td>
<td>380</td>
<td>86</td>
</tr>
<tr>
<td>Shipbuilding and welding</td>
<td>4</td>
<td>105†</td>
<td>82</td>
</tr>
<tr>
<td>Car repair</td>
<td>18</td>
<td>479†</td>
<td>82</td>
</tr>
<tr>
<td>Lead pigment handling</td>
<td>7</td>
<td>97</td>
<td>71</td>
</tr>
<tr>
<td>Soldering</td>
<td>14</td>
<td>126†</td>
<td>78</td>
</tr>
</tbody>
</table>

†Number includes subjects examined at the Working Environment Foundation exhibitions (see text).

Ficiency anaemia (Bowering et al., 1976) could lead to higher mean protoporphyrin concentrations (Lamola and Yamane, 1974; McLaren et al., 1975). On the other hand, the mean haemoglobin level is constant in adult male populations until old age, and the evidence of impaired iron absorption with advancing age is only suggestive (Bowering et al., 1976). It may therefore be of significance that the body burden of lead increases with age (World Health Organization, 1977). The increase in ZPP (Fig. 1) would correspond to an increase in blood lead of about 0.003 g mol Pb/l blood per year. Although some authors have found no increase in blood lead with age (World Health Organization, 1977), a Danish study of 63 males showed a mean annual increase of 0.005 g mol Pb/l blood (Nygaard et al., 1977). A similar increase was apparent in 136 males with low-level lead exposure in Belgium, and the age-dependent increase was even steeper in 265 males with exposure to lead-contaminated drinking water (De Graeve et al., 1975). The possible accumulation of lead in the blood and increased lead toxicity with advancing age, therefore, call for further studies.

The upper limit of the reference interval is higher than that established by other authors (Piomelli et al., 1973; Roels et al., 1975; Sassa et al., 1975). This is explained by the fact that the haematofluorometer gives higher readings than the extraction methods, especially in the low concentration range (Blumberg et al., 1977; Grandjean and Lintrup, 1978).

The ZPP level in the erythrocytes is not determined by lead alone (Lamola and Yamane, 1974), and it is therefore not surprising that the relationship between ZPP and lead in blood is scattered (Fig. 2). However, it is probable that a closer relationship can be obtained in subjects with constant lead exposure (Sassa et al., 1975). The linear relationship between log ZPP/Hb and blood lead is a reasonable approximation (Piomelli et al., 1973; Sassa et al., 1975; Grandjean and Lintrup, 1978). A no-detectable-effect level in adult males was identified at about 1.2-1.7 g mol Pb/l blood (Roels et al., 1975), and this is in accordance with Fig. 2 in this study.

Anaemia is a well-known sign of lead poisoning, but it is not normally found until the blood lead level exceeds 5 g mol/l (Williams, 1966; Cooper et al.,...
Occupational lead exposure in Denmark: screening with the haematofluorometer

1973). Two cross-sectional studies, similar to the study described here, have shown a significantly negative association of blood haemoglobin levels with blood lead concentrations in workers exposed to lead (Wada, 1976; Lilis et al., 1977), but such a relationship could not be found in a large-scale study in Finland (Tola, 1973). A prospective study of new male lead workers showed, however, that the mean haemoglobin decreased in 100 days from 8.9 mmol/l to 8.3 mmol/l, while the blood lead level increased to a mean of about 2.4 μmol/l (Tola et al., 1973). The accumulation of ZPP in the erythrocytes is several times lower than the associated decrease in Hb (Fig. 3). Thus, interference with other steps of haem metabolism must be partly responsible for this decrease. Almost all haemoglobin values found in this study were within the normal range (above 8.0 mmol/l), and there is little evidence of any harmful effect of a low haemoglobin level as such (Elwood, 1973). However, a concomitant lead-induced decrease in other haem proteins has been demonstrated (Alvares et al., 1976; Goldberg et al., 1977). Thus, the decreased synthesis of haem should not be evaluated on the basis of haemoglobin levels only.

Hernberg (1973) reviewed the literature on the incidence of occupational lead poisoning, and distinguished between occupations with high risk and those with moderate or slight risk. These two groups are very similar to the groups listed in Tables 5 and 6, respectively. A large-scale Finnish screening study for blood lead levels concluded that the highest lead exposures occurred in the same industries as those mentioned in Table 5 (Tola et al., 1976). Shooting instructors and fingerprint experts handling lead pigment were not mentioned in the Finnish reports, but cases of poisoning have occurred in these occupations as well (Winge, 1931; Nyfeldt, 1937). By far the most extensive lead exposure is found in secondary lead smelters and in electric storage battery manufacturers (Williams et al., 1969; Lilis et al., 1977; World Health Organization, 1977). Thus the results of the present survey are in accordance with most other reports. However, one recent study of 216 workers from 10 garages in Denmark showed that 9% of the workers had a blood lead level in excess of 3.9 μmol/l, and that motor mechanics had higher levels than the platesmiths and the paint sprayers (Clausen and Rastogi, 1977). Our study, carried out two years later, included two of the garages studied by Clausen and Rastogi (1977) but it was not possible to confirm their findings. Our results are in accordance with findings in Finland (Tola et al., 1972); it appears, therefore, that in most instances car repair results in only a limited lead exposure.

A significant proportion of the work-force is exposed to lead, so that extensive monitoring is necessary. The haematofluorometer has proved very efficient for screening purposes, but an increased ZPP level is not specific for lead (Lamola and Yamane, 1974; Grandjean and Lintrup, 1978). An increased ZPP/Hb ratio should therefore be controlled by blood lead determination. A screening limit of 300 μmol ZPP/mol Hb(Fe) is convenient, as almost all subjects with a blood lead level in excess of 2.9 μmol/l (60 μg/100 ml) also exceed this limit for the ZPP/Hb ratio.

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Occupational lead exposure in Denmark: screening with the haematofluorometer.

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