Erythrocyte fluorescence and lead intoxication

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Clark, K. G. A. (1976). *British Journal of Industrial Medicine*, 33, 193-195. **Erythrocyte fluorescence and lead intoxication.** Blood samples from people exposed to inorganic lead were examined by fluorescence microscopy for excess erythrocyte porphyrin. With continued lead absorption, fluorescent erythrocytes appeared in the circulation of workers handling this metal or its compounds, and they progressively increased in number and brilliance. These changes ensued if the blood lead concentration was maintained above $2.42 \mu\text{mol/l}$ ($50 \mu\text{g}/100 \text{ ml}$), and preceded any material fall in the haemoglobin value. At one factory, 62.5% of 81 symptomless workers showed erythrocyte fluorescence attributable to the toxic effects of lead. Excess fluorocytes were found in blood samples from a child with pica and three of her eight siblings. These four were subsequently shown to have slightly increased blood lead concentrations (2.03 to $2.32 \mu\text{mol/l}$). Fluorescence microscopy for excess erythrocyte porphyrin is a sensitive method for the detection of chronic lead intoxication. A relatively slight increase in the blood lead is associated with demonstrable changes in erythrocyte porphyrin content. The procedure requires little blood, and may be performed upon stored samples collected for lead estimation. The results are not readily influenced by contamination, and provide good confirmatory evidence for the absorption of biochemically active lead.

Lead intoxication causes an increase in the porphyrin content of erythrocytes which become autofluorescent. This abnormality may be detected by fluorescence microscopy. It has been claimed that more than 10% fluorescent erythrocytes (fluorocytes) is suggestive of lead intoxication (Nelson *et al.*, 1968). Although there are a few reports on the use of fluorescence microscopy for the rapid diagnosis of lead poisoning (Whitaker and Vietti, 1959; Clark, 1973), this technique has not been widely adopted for clinical or epidemiological studies. Microscopical observations on the porphyrin content of erythrocytes from individuals exposed to inorganic lead are described below.

Material and methods

Subjects

Battery pasters, type makers, the employees of a lead works, and a group of normal individuals were tested.

Men handling lead compounds in the preparation of plastic for an extrusion process were examined for periods varying from a few days to many months. Blood specimens from a child with pica and her eight siblings were also examined.

Methods

Venous blood samples were collected and mixed with dipotassium EDTA (2 mg/ml of blood). Haemoglobin levels and red cell indices were measured on a Coulter Model S counter. Thin wet preparations for fluorescence microscopy were made from strong (50%) suspensions of washed erythrocytes in isotonic saline. These were examined for porphyrins using a Zeiss photomicroscope equipped with an incident light fluorescence illuminator, HBO 200 mercury vapour lamp, exciter filters transmitting between 300 and 450 nm, and barrier filters allowing only wavelengths greater than 530 nm to reach the eye. The proportion of fluorocytes in a given field was estimated in comparison to the same area examined by phase contrast, and recorded as < 1%, 1 to 5%, 6 to 25%, 26 to 50%, or > 50%, since rapid fading of fluorescence made precise counts impractical.

Blood lead estimations were performed by the Department of Employment Medical Advisory Service Laboratory, using the punched disc method and atomic absorption spectrometry.

Results

In this paper, 'lead intoxication' is used to refer to all states characterized by demonstrable toxic effects of the metal, whether these appear to be solely biochemical or are accompanied by clinical signs of poisoning. The increase in erythrocyte porphyrin content which results from excessive lead absorption is one such effect, and may be detected by fluorescence microscopy.

Only one of 19 normal individuals showed an occasional fluorescent red cell. In contrast, the erythrocytes of eight persons suffering from biochemically confirmed clinical lead poisoning were all strongly fluorescent. The fluorescence was a bright but fugitive pink, which faded and disappeared within a few seconds. Compared with the erythrocytes in patients suffering from untreated iron deficiency anaemia, protoporphyria, or Günther's disease, the fluorescence caused by lead intoxication appeared more uniform from cell to cell. There was no localization to any recognizable intracellular organelle. Blood samples made incoagulable with K_2 EDTA and kept in the dark at a temperature of 4°C showed no appreciable change in red cell fluorescence during a period of 14 days, provided they were not contaminated by bacteria. The fluorescence of blood samples kept in the dark at room temperature did not deteriorate for at least 72 hours.

With continued absorption of lead, the erythrocytes of previously normal people gradually became fluorescent. Some men showed more than 50% fluorocytes within three months of beginning work involving lead. This change was related to the blood lead concentration, but preceded any material fall in haemoglobin level (Figure). The blood of one man employed in a battery factory showed weak uniform erythrocyte fluorescence, but his observed blood lead was only $0.63 \mu\text{mol/l}$. This value would be low for an unexposed adult living in Britain, and was probably an underestimate; unfortunately the tests could not be repeated. Newly exposed workers sometimes had raised blood lead values before their cells became fluorescent, but such levels were not always sustained. Individuals who were severely intoxicated by lead showed fluorescent erythrocytes for several months after removal from the risk of further absorption although their blood lead levels were falling.

Of 81 workers employed in the recovery of lead from scrap, 55 (62.5%) showed erythrocyte fluorescence attributed to lead intoxication, the others

FIGURE. Blood lead (48 observations), haemoglobin level (46 observations) and erythrocyte fluorescence in battery makers, plastics mixers, and type makers. Open circles < 5%, closed circles > 50%, triangles intermediate numbers of fluorocytes.

appeared normal. The difference in the mean haemoglobin levels of these two groups was not significant (mean $\pm$ SD, fluorescent 14.89 ± 0.36 g/dl, non-fluorescent 15.10 ± 0.98 g/dl; $t = 0.339$, $P > 0.70$).

The child with pica and three of her siblings showed abnormal erythrocyte fluorescence; these four were subsequently found to have blood lead values only slightly higher than normal. Four of the other siblings were normal by both tests, while one had borderline results (Table).

Discussion

The mean blood lead concentration in samples from

TABLE

ERYTHROCYTE FLUORESCENCE IN A CHILD (1) WITH PICA AND HER EIGHT SIBLINGS; RESULTS OBTAINED INDEPENDENTLY, PRESENTED IN ORDER OF BLOOD LEAD CONCENTRATION

Children	Fluorocytes %	Blood lead $\mu\text{mol/l}$ ($\mu\text{g/dl}$)
1	> 50	2.32 (48)
2	> 50	2.22 (46)
3	> 50	2.03 (42)
4	> 25, < 50	2.03 (42)
5	< 5	1.64 (34)
6	nil	1.16 (24)
7	nil	1.06 (22)
8	nil	1.02 (21)
9	< 1	0.82 (17)

apparently healthy populations has been shown to vary widely from place to place (Goldwater and Hoover, 1967). In Britain, values in excess of $1.74 \mu\text{mol/l}$ raise the suspicion of untoward exposure. The size of the marrow lead compartment is not accurately reflected by the quantity in the blood, which is small by comparison (Westerman *et al.*, 1965). Accumulation of lead in the haemopoietic tissues causes the formation of protoporphyrin rich fluorocytes which enter the general circulation and eventually replace the normal non-fluorescent erythrocytes. Recent evidence suggests that the excess porphyrin is present in the form of a zinc-protoporphyrin complex (Lamolo, Joselow, and Yamane, 1975). Generalized erythrocyte fluorescence occurs comparatively early in lead intoxication, well before the development of anaemia, and persists for a considerable time after absorption ceases.

It is sometimes difficult to draw firm conclusions from the results of blood lead estimations, not least because of the possibility of errors due to sample contamination or experimental technique. This is particularly so when the results fall in the range 1.69 to $2.17 \mu\text{mol/l}$. In such circumstances the presence of fluorocytes in the blood provides valuable supporting evidence of potentially harmful absorption. In Britain, a blood lead of $3.86 \mu\text{mol/l}$ is arbitrarily regarded as the danger level for exposed workers. Although obvious clinical symptoms and signs of poisoning do not necessarily develop if this concentration is exceeded, it is more likely that they might. Low levels of blood lead which are not normally regarded as dangerous may also be associated with toxic effects upon the erythrocyte precursors, leading to the production of fluorocytes. In adults, a blood lead level above $2.42 \mu\text{mol/l}$ is likely to be accompanied by biochemical changes, and the appearance of excess fluorocytes in the peripheral blood (Figure). The observations presented in the Table accord with the view that a sustained blood lead concentration of $1.93 \mu\text{mol/l}$ or more in a child suggests excessive exposure to this metal (Steinfeld, 1971). The erythrocytes are sensitive biological indicators of lead absorption, and their examination by fluorescence microscopy is a valuable technique for the diagnosis of unacceptable domestic or occupational exposure to this metal. The results of blood lead estimation and fluorescence microscopy of the erythrocytes are complementary.

Unlike the blood lead, erythrocyte porphyrin content is not readily influenced by sample contamination. It does not vary widely from hour to hour, and is comparatively stable *in vitro*. In contrast, the excretion of coproporphyrin and δ -aminolevulinic acid is very variable, and in solution in urine these compounds are comparatively unstable.

Iron deficiency anaemia, severe haemolytic anaemia, sideroblastic anaemia, and rare inherited abnormalities of erythroid haem synthesis may be associated with the production of fluorocytes. These disorders are easily distinguished from lead intoxication, but those who suffer from them should avoid occupational exposure to lead. In practice, iron deficiency is much the commonest, and may be suspected from the erythrocyte MCV and MCH, or from the appearance of the stained blood film. As a result of successful treatment fluorocytes gradually disappear from the blood.

I am grateful to Dr M. H. P. Sayers, Employment Medical Advisory Service Laboratory, for blood lead estimations.

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Received for publication 15 July 1975

Accepted for publication 9 March 1976