The phosphatases of peripheral white blood cells in workers exposed to trichloroethylene and perchloroethylene

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The activity of alkaline phosphatase in human neutrophilic leucocytes (L.A.P.) depends upon, among other things, heredity factors (Santachiara-Benerecetti, Cesari, and De Carli, 1967; Winkelstein, Goldberg, Tishkoff, and Sparkes, 1967; Gropp, Fischer, Niederald, Klesse, and Hensen, 1968), pathological conditions, e.g., hypophosphataemia (Beisel, Benjamin, and Austen, 1959), the excretory system (Griffin and Cox, 1966; Rosner and Lee, 1965), and the relationship between serum and leucocyte alkaline phosphatases (Lohmann and Weissel, 1964). Many papers have dealt with L.A.P. activity in various diseases, the levels found being important in diagnosis (Sigg, 1966). As phosphatases are important basic enzymes I have investigated the activity of alkaline and acid phosphatases in the white blood cells of people exposed to trichloroethylene or to perchloroethylene (tetrachloroethylene) or to both.

Materials and methods
In this work 23 people exposed to trichloroethylene, seven exposed to perchloroethylene, and four exposed to both were studied. All were exposed continuously during work. Every subject was given a clinical examination to eliminate those suffering from illnesses which could influence phosphatase levels (diabetes, liver disease, and pregnancy). A control group of healthy, unexposed volunteers was also investigated.

Exposure was estimated by determining urinary trichloroacetic acid by a modification of the Fujioke method (Soucek and Franklová, 1952) and trichloroethanol as described by Vlachová (1956), also by taking into consideration the condition of the working environment. From the concentrations of trichloroacetic acid in their urine the workers exposed to trichloroethylene were divided into three groups. Those whose urines contained at least 100 mg. trichloroacetic acid per litre were the most heavily exposed, and were graded +++; those whose urines contained 50 to 100 mg. per litre were graded ++; and those whose urines contained 0 to 50 mg. per litre were graded +.

Alkaline phosphatases were stained and scored by the method of Kaplow (1963), modified as described by Hayhoe and Quaglino (1958). The periodic acid Schiff reaction (P.A.S.) was performed with and without diastase digestion, as described by Rosenszajn, Marshak, and Efrati (1963).

Results

Trichloroethylene
In the 20 controls the L.A.P. levels ranged from 35 to 95 with a mean value of 58. These levels were similar to those found by Hausser and Stroun (1962), Kaplow (1955), Knoblauch (1962), and Merker and...
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Heilmeyer (1960), and confirmed that, in people without exposure, L.A.P. levels rarely exceed 100.

In nearly all those with medium (+++) or heavy (+++) exposure the phosphatase activity was higher than normal (Figure). Subjects were investigated several times, and when their L.A.P. scores did not differ much only the mean values are given. There was only one person with a low (+) exposure and his L.A.P. score (49) was essentially normal.

Two cases illustrate the effects of interrupted exposures:

1. A 39-year-old man had medium exposure for six months, and his L.A.P. score was 50. The exposure then increased considerably, he developed signs of poisoning, and his urine contained 383 mg. of trichloroacetic acid per litre and 493 mg. of trichloroethanol per litre, providing confirmation of a very heavy exposure, and his L.A.P. score rose to 147. He was removed from work for two weeks. After he had been back at work for two days his urine contained 42 mg. of trichloroacetic acid per litre and 60 mg. of trichloroethanol per litre, corresponding to low (+) exposure, but his L.A.P. score remained elevated at 150.

2. A 48-year-old man worked for four weeks after a three-month break. His urine contained 84 mg. of trichloroacetic acid per litre and 123 mg. of trichloroethanol per litre (+++), but his L.A.P. was essentially normal (score 64). After six weeks’ further exposure followed by a two-week holiday his urine contained little trichloroacetic acid or trichloroethanol (13 mg. per litre and 0 mg. per litre respectively) and his L.A.P. score had fallen a little to 46. After further work at heavy exposure (+++) his L.A.P. score rose to 167.

**Perchloroethylene**
The L.A.P. scores were sometimes raised (Figure) but the mean was within the normal range.

**Trichloroethylene and perchloroethylene**
The L.A.P. scores were raised as in those heavily exposed to trichloroethylene alone (Figure).

**Acid phosphatase**
The scores were raised in all subjects exposed to trichloroethylene, and in two of the subjects exposed to perchloroethylene alone. The P.A.S. reaction was positive in all cases, and after diastase digestion the magenta colour disappeared.

In the controls the scores agreed with those found by Rosenszajn and his colleagues (1963).

**Discussion**
The activity of L.A.P. is increased in many conditions and can be explained as a defence mechanism against bacterial infections, etc. The enzyme plays an active part in phagocytosis (Merker, 1963). The relationship between malignant tumours, cytostatic treatment, and the activity of L.A.P. suggests a relationship between the effectiveness of the defence mechanisms and the activity of L.A.P. Osten (1966) demonstrated a temporary decrease in the activity of L.A.P. during cytostatic treatment, which was, however, followed by an increase in L.A.P. activity in those cases with better prognosis. The opposite was true for unfavourable cases. The findings of Lorbacher, Fischer, and Schmähl (1963), who studied experimental tumours in rats, were more equivocal.

Apart from defence mechanisms local phenomena may affect the L.A.P. scores. Vanotti (1963) pointed out the significance of the pH of the blood which may be affected during the metabolism of trichloroethylene.

Melnikovych, Bishop, and Swayze (1967) described their findings on L.A.P. in cell cultures under the influence of hormones. They noted difficulty in interpreting their results 'because of the lack of information about the physiological role of alkaline phosphatase which, because of its broad substrate specificity, can break down many important metabolic intermediates including mononucleotides, esters of primary and secondary alcohols and phenols'. This suggests that L.A.P. may have some function in the detoxication of trichloroethylene.

A connexion between increased activity of L.A.P.
and exposure to trichloroethylene has been demonstrated in this paper. It is noteworthy that after a prolonged absence from work the excretion of metabolites decreased as did the activity of L.A.P. The changes in the level of trichloroacetic acid in urine and the activity of L.A.P. were not, however, concomitant. L.A.P. scores rise more slowly and remain elevated for longer than the levels of trichloroacetic acid. This phenomenon is similar to the relationship of fever and leucocytosis with L.A.P. in the pyrexia experiments described by Merker (1963; 1965).

An interpretation of the increased activity of acid phosphatase is difficult. The P.A.S. reaction (with and without diastase digestion) did not indicate a pathological condition, so presumably there was no disorder in glycogen metabolism (Gahrtion, 1966; Wulff, 1967).

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References


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