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Study of Some Erythrocyte and Serum Enzyme Activities in Workers Exposed to Low Ozone Concentrations for a Long Time

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Summary. In 22 subjects exposed during work to ozone concentrations ranging from 0.160 to 0.290 ppm, the following enzyme activities were measured at the end of a normal work shift: AchE, G-6-PDh, PK in erythrocytes, LDH and alfa HBDH in serum.

AchE of erythrocytes and serum LDH and alfa HBDH were determined also after three weeks of nonexposure to ozone.

LDH and alfa HBDH activities decreased at the second determination; the difference between the two determinations was statistically significant ($P< 0.001$).

The results indicate that an exposure to 0.2 ppm of ozone, if prolonged in time, may affect the air-blood barrier and possibly cause alteration of some serum enzyme activities.

Concentrations of ozone around 0.2 ppm should therefore be avoided in urban and industrial areas.

Key words: Acetylcholinesterase (AchE) – Glucose-6-Phosphate Dehydrogenase (G-6-PDh) – Pyruvate kinase (PK) – Lactate Dehydrogenase (LDH) – alfa Hydroxybutyrate Dehydrogenase (alfa HBDH) measurements (activities), 0.4 mg/m^3 exposure – Ozone

In the literature several papers dealing with the action of ozone on the activities of red cells and serum enzymes can be found (Stokinger, 1965; Goldstein et al., 1968; P'An and Jegier, 1970; Goldstein and McDonaugh, 1975; Buckley et al., 1975) although little is known on the effect of prolonged exposure to ozone on enzyme activities of workers.

In 1965, Stokinger reviewed all research on pulmonary and extrapulmonary effects of ozone suggesting the possibility of an interaction of ozone with –SH groups of enzymes of the cellular membrane.

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Buckley et al. (1975), after studying the activity of several erythrocytic enzymes on volunteers exposed for 2¾ h to 0.5 ppm of ozone, found significant decreases of AchE and reduced glutathione levels.

Goldstein et al. (1968), Balchum et al. (1971) and Rohem et al. (1971) suggest that AchE inhibition is a result of the peroxidation of unsaturated fatty acids (UFA) of the erythrocyte membrane.

In the present paper we report the results of determinations of serum and erythrocyte enzyme activities in workers exposed to low concentrations of ozone for long periods.

Materials and Methods

We examined 22 subjects, 18 males and 4 females. The average age was 27.6 ± 8.1 years (range from 20 to 50). The average exposure time was 1.9 ± 0.8 years (range from 1 to 3 years).

A control group made up of 22 subjects, 20 males and 2 females, aged between 18 and 50 years (average 35.5 ± 6.3) was used. Preliminary experiments showed that there is no influence of age or sex on the examined enzyme activities. The control subjects were workers employed in an analogous type of production where no exposure to ozone existed.

The only significant exposure of the group to ozone was a professional one, since all workers lived in a scarcely urbanized area.

Exposed and control groups were given a general check-up: a blood cells analysis and X-ray of the chest. At the end of a normal 8-h work shift (first determination), the following enzyme activities were measured: AchE, G-6-PDh, PK, LDH, and alfa HBDH.

A second determination was carried out on the same subjects at the end of their holidays (three weeks) before returning to work.

Enzyme Determination

AchE was assayed in red blood cells (RBC) according to the method of De La Huerga et al. (1970) with O-acetylcholine bromide (BDH, Poole) as a substrate. The activity is expressed in mcM of hydrolyzed acetylcholine per hour per volume of RBC in ml. The normal values in our laboratory ranged between 380 and 760 mcM/ml of RBC per hour.

G-6-PDh was measured in RBC by method of Richterich (1968). This enzyme, in presence of Glucose-6-phosphate (G-6-P) and TPN, form 6-phosphogluconate and TPNH. G-6-PDh and TPN are Boehring products. The values obtained are expressed in Unit: 1 Unit is the amount of TPN that oxidate a mcmol of G-6-P in 1 min at 25° C. The normal value is 1.6 ± 0.45 U.

PK. Two fundamental steps are present in this enzymatic reaction: (1) Phosphoenolpyruvate (PEP) is converted to pyruvic acid by PK with ADP; (2) Pyruvic acid reacts, then, with DPNH, forming lactate and DPN (the enzyme that catalyzes the reaction is LDH). PEP, ADP, LDH, and DPNH are Boehring products. The values are expressed in Unit: 1 Unit is the moles of DPNH oxidate in 1 min at 25° C by 10^{10} erythrocytes. The normal value is 2.12 ± 0.8 U.

LDH in serum was measured according to Wroblewski and La Due method (1955) with a commercial kit (Boehringer-Biochemie, Milan). The values indicated as normal in our laboratory range between 72 and 192 U (mean 130.5 ± 33.6 U).

Alfa HBDH in serum was measured by Rosalki and Wilkinson method (1960) with a commercial kit (Boehringer-Biochemie, Milan). In our laboratory, normal range is between 9 and 110 U (mean 69.5 ± 21.8 U).

Table 1. Environmental concentrations of ozone. The air concentration of ozone was determined by the Katz method (1968)

Position in the working cycle	ppm	mg/m^3
1. Seaming process	0.160	0.320
2. Seaming process	0.215	0.430
3. Stamping	0.295	0.590

Working Place

The 22 subjects examined worked in a factory which produces polyethylene plastic bags and the working cycle included the following phases:

1. Extrusion
2. Stamping
3. Seaming process.

Extrusion consists of the production of sheets of plastic starting from the granulated polyethylene; the sheet then goes to the stamping and, before printing the wording, the top part of the sheet is treated with ozone, produced after electrostatic discharge. With this procedure the color fixation is possible.

The last phase of the process is the closing of the bottom of the bag. The machinery for the whole process consists of pieces placed in series in the same area, sufficiently close to each other so as to allow the ozone, which is produced by the stamping machine, to diffuse all over the place.

Table 1 shows the values of ozone measured at the level of the three phases in three different spots of the working place. They reflect real exposure because the same concentrations of ozone were found in different periods and because the working cycle is nonstop and the O_3-concentration remains practically constant within the factory.

The method is based on the use of a neutral, buffered potassium iodine solution as an adsorbent for the air sample. Each sampling was conducted in two midget impingers placed in series containing 1% potassium iodine in a neutral (pH = 6.8) buffer. The flow rate was 2 l/min and 30-min sampling periods were used.

Iodine is liberated in the absorbing reagent and the yellow color which occurs is measured by spectrophotometer during 60 min after sampling.

No other product can be considered as toxic agent during exposure because a ready-made polymer is the starting material.

Results

Exposed and control subjects were clinically and hematologically healthy.

Throughout all figures the first and second determination are indicated as E_1 and E_2 and the data of the control group are indicated with C_1 and C_2. The figures report all geometric individual values and geometric average values to compensate for the skewedness of the distribution. While no difference exists between the two determinations on the control group, a significant difference was found between the values of the two determinations carried out on the exposed group.

AchE. The mean value for 22 subjects was 501.6 ± 115 mcM/ml per hour of RBC. In three subjects we found a modest enzyme inhibition at the first determination; in particular, one subject showed a 33% reduction and another subject a 27% reduction.

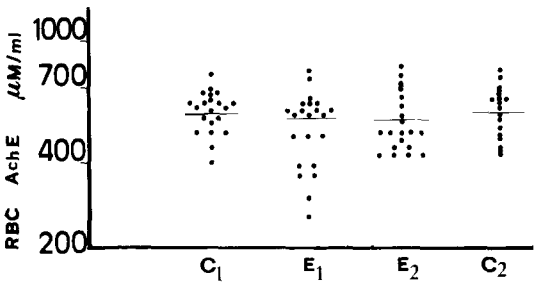

Fig. 1. AchE activity of RBC. The control group C_1 had a mean ± SD value of 539.3 ± 98.6 mcM/ml of RBC and C_2 540 ± 70.3 mcM/ml of RBC. The exposed group E_1 had a mean ± SD value of 501 ± 115 mcM/ml of RBC and E_2 500 ± 122.1 mcM/ml of RBC. The paired *t*-test value for the difference between C_1 and C_2 was 0.04 (P = N.S.), between E_1 and E_2 was 0.006 (P = N.S.). The Student's *t*-test for the difference between C_1 and E_1 was 1.4 (P = N.S.), between C_2 and E_2 was 1.6 (P = N.S.)

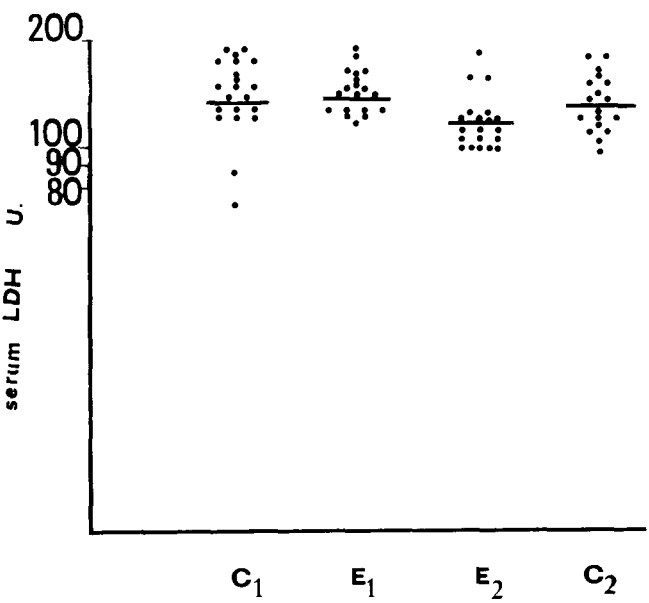

Fig. 2. Serum LDH activity. The control group C_1 had a mean ± SD value of 130.5 ± 33.6 U and C_2 123.2 ± 27.7 U. The exposed group had a mean ± SD value of 137.9 ± 24.8 U and E_2 111.4 ± 24.3 U. The paired *t*-test value for the difference between C_1 and C_2 was 0.7 (P = N.S.), between E_1 and E_2 was 4.3 ($P < 0.001$). The Student's *t*-test for the difference between C_1 and E_1 was 1.15 (P = N.S.), between C_2 and E_2 was 1.5 (P = N.S.)

At the second determination, the average value found for the whole group was 500 ± 122.1 mcM/ml of RBC per hour and none of the 22 subjects re-examined showed values lower than the normal ones.

The enzyme activity of the 3 subjects, which was reduced at the first determination, returned to normal at the second determination (Fig. 1). The difference between E_1 and E_2 average studied with paired *t*-test was not significant.

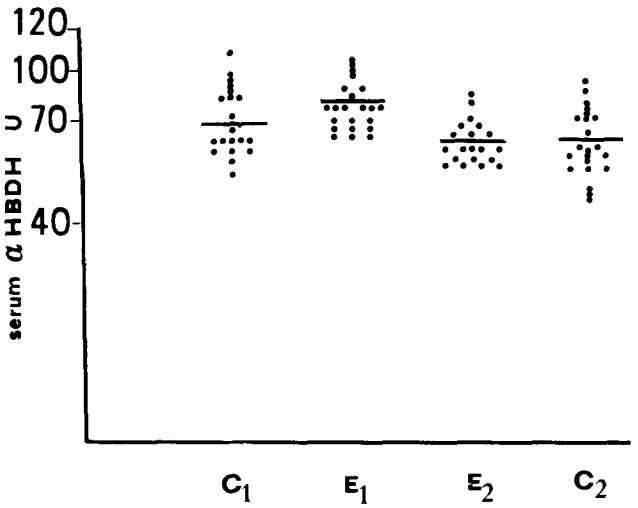

Fig. 3. Serum alfa HBDH activity. The control group C_1 had a mean ± SD value of 69.5 ± 21.8 U and C_2 59.5 ± 15.5 U. The exposed group E_1 has a mean ± SD value of 77.9 ± 17 U and E_2 59.8 ± 11.1 U. The paired *t*-test value for the difference between C_1 and C_2 was 0.73 (P = N.S.), between E_1 and E_2 was 5.4 ($P < 0.001$). The Student's *t*-test for the difference between C_1 and E_1 was 1.5 (P = N.S.), between C_2 and E_2 was 0.3 (P = N.S.)

G-6-PDh. The enzyme activity, evaluated only at the first determination, gave an average value of 1.7 ± 0.47 U. Five subjects (i.e., 23% of the total) showed values higher than the normal ones, with a value of 3.75 U in one subject.

PK. This enzyme was also assayed only in the first determination and average enzyme activity was 1.9 ± 0.31 U. Only in one subject there was an increased enzyme activity: it was the same subject that showed altered G-6-PDh and AchE activity.

LDH. The enzymatic values were all within normal limits, both in the first and second determination; the average value in the first determination was 137.9 ± 24.8 U and in the second 111.4 ± 24.3 U. The difference between E_1 and E_2 averages studied with the paired *t*-test was statistically significant ($P < 0.001$) (Fig. 2).

Alfa HBDH. This enzyme also showed normal values in both the first and second determination (77.9 ± 17 and 59.8 ± 11.1 U).

The average values between E_1 and E_2 differed significantly ($P < 0.001$) (Fig. 3).

Discussion and Conclusion

The ozone concentrations in the three spots of the working place where a measurement was carried out (0.160 ppm, 0.215 ppm, and 0.295 ppm) were higher than the TLV (0.1 ppm). For this concentration, i.e., around 0.2 ppm of ozone, in literature we did not find reported significant enzymatic alterations in the exposed subjects.

To answer the question if ozone, at relatively low concentrations, as in urban areas of big cities, is capable of passing the air-blood barrier, Buckley et al. (1975) submitted volunteers to an atmosphere with a 0.5 ppm concentration of ozone. They reported that this limit is still too high since it causes a significant alteration in the enzyme activities.

It seems necessary to us to point out that the RBC AchE values are reduced in three subjects at the first determination and return to normal values after 21 days of nonexposure, even if no conclusion can be drawn.

More interesting seems the statistically significant difference between the average values of LDH and alfa HBDH in the first and second determination in the exposed group and not in the control group, even if the individual variations were within a range of normality.

From the results obtained we suggest that an exposure to ozone concentrations of 0.2 ppm, if prolonged in time, can affect the air-blood barrier. According to the hypothesis of the experimental study by Werthamer et al. (1974), the elevation of serum LDH and alfa HBDH activities might be an adjustment to fulfill the energy requirement dictated by the increased lung tissue metabolism for repair protein synthesis.

Fundamental to us is the type of exposure of the subjects who worked in an atmosphere with ozone concentrations higher than the TLV for 8 h a day, five days a week, 11 months a year.

Obviously further and more extensive research is needed to be able to assess the lower limit of ozone concentration which is compatible with no detectable alterations in man.

From the present data we may infer that a value of 0.2 ppm is a critical one.

Abbreviations

AchE = Acetylcholinesterase in erythrocytes, G-6-PDh = Glucose-6-phosphate dehydrogenase in erythrocytes, PK = Pyruvate kinase in erythrocytes, LDH = Lactate dehydrogenase in serum. alfa HBDH = alfa Hydroxybutyrate dehydrogenase in serum

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