Arh. hig. rada, *15* (1964) 57

PRELIMINARY INVESTIGATIONS OF PROLONGED OCCUPATIONAL EXPOSURE TO TOXIC SUBSTANCES ON THE LEVEL OF SOME SERUM ENZYMES*

J. Z. HANKE

Institute of Occupational Medicine, Lódź

Received for publication January 13, 1964

The activity of four serum enzymes – aldolase, alkaline phosphatase, glutamic-oxalate transaminase, and glutamic-pyruvic transaminase – in two groups of workers chronically exposed to benzene and its homologues, as well as to lead has been investigated. Statistical analysis of the results obtained has shown that the contact with benzene and its homologues produces a drop in serum alkaline phosphatase activity and also, in some persons, a considerable drop in aldolase and glutamic-pyruvic transaminase serum level. The group of workers exposed to lead showed lower values of aldolase activity but higher SGOT/SGPT ratio than the control group. There was no evidence that these changes were caused by some other diseases, or malnutrition, or alcoholism. They seemed to be due only to a chronic exposure to benzene and lead.

INTRODUCTION

Many authors have recently published interesting data on the influence of toxic substances upon the biocatalytic system of the organism. *Cornish* (1) found very distinct changes in the level of glutamic oxalate transaminase in the blood serum of rats exposed to the action of CCl_4 (air concentration 50–100 p. p. m.) through a period of ten days. *Ball* (2) examined the longstanding influence of this drug on the activity of blood cholinesterase in rats and observed considerable fluctuations in the level of this enzyme. *Cenacchi* (3) investigated the behaviour of both transaminases (G. O. T. and G. P. T.), aldolase and malic acid dehydrogenase in the serum of rats treated with plumbic oxalate for 30 days. The enzymatic changes appeared already at the beginning of the treatment and showed a tendency to increase. Organ injuries were found only toward the end of the exposure. *Nicola* (4) caused a chronic intoxication in rats and a subacute intoxication in rabbits using benzene in both cases. The activity of transaminases, aldolase and malic acid dehydrogenase in red

* Delivered before the First Yugoslav Congress of Occupational Medicine, Beograd, November 1963.

cells showed a drop in intoxicated animals. A similar experiment on rats was carried out by *Gabor* (5), the exposure time ranging up to 47 weeks. According to the investigator's opinion, changes in the activity of leukocytic peroxidases, blood catalases and leukocytic indophenoloxidase appear early (after 42 days of exposure to benzene) and are therefore a sensitive index, which can be more easily interpreted than blood changes (white and red blood count, haemoglobin, leukocytes differential count).

With regard to the observations concerning human beings only isolated reports are available.

Strauss (6) observed a drop of serum cholinesterase activity in persons occupationally exposed to trichlorethylene. In people continuously exposed to the action of some sulphur compounds or di-aethyl-p-diamine *J. Golubowski* (7) noticed a rise in the level of alkaline serum phosphatase correlated with the degree of liver injury.

F. Fournel (8) found pathologic values of blood cholinesterase in people exposed to the hazard of organic phosphorus compounds. According to his opinion, the enzymatic disturbances could be considered as early symptoms of intoxication. This opinion is shared by other authors.

In a study of 25 persons exposed to lead (*Waldman*, 9) it has been shown, that S. G. O. T. activity was increased when blood lead concentration were elevated in this instance without clinical symptoms of poisoning.

The purpose of this work was to collect some observations about the behaviour of four enzymes (aldolase, alkaline phosphatase, glutamic-oxalate transaminase – G. O. T. and glutamic-pyruvic transaminase – G. P. T.) in the serum of people chronically exposed to toxic substances.

METHODS

Two groups of workers were examined.

1st group of 42 persons exposed to prolonged action of benzene or its homologues.

2nd group of 20 persons exposed to longstanding action of lead.

The first group consisted of 35 workers (1a) employed in a plant in Lódź, who for several months had a professional contact with benzene in higher concentrations than the permissible one. The people came to our Institute half a year after the discontinuation of exposure to benzene. The enzymatic examination of these persons included only the estimation of serum alkaline phosphatase. Among the first group there were classed also seven patients (1b) of our clinical department suspected to be chronically intoxicated with benzene and its homologues. The enzymatic test carried out in this group included a threefold estimation of serum alkaline phosphatase, aldolase, G. O. T. and G. P. T.

Table I.

Enzyme	Methods		
	Method of estimation according to	Physiological fluctuation range	
		Own data	Data of other authors
Alkaline Phosphatase	Bessey, Lowry, Brock (11)	mean value ± standard deviation **1.6 mMol** μ ± 0.4 fluctuation range **0.9 — 2.1 mMol** μ	fluctuation range 0.9 — 4.1 **mMol** μ Suedhof, Steindorf, Goetze, Voss, Loehr (12)
Glutamic-oxalate Transaminase (Got)	Reitman, Frankel (13)	mean value ± standard deviation **19.0** μ ± **4.7** fluctuation range **12.0 — 32.0** μ	fluctuation range 10.0 — 40.0 μ La Due, Wróblewski, Karmen (14)
Glutamic-pyruvic Transaminase (GPT)	Reitman, Frankel (13)	mean value ± standard deviation **16.0** μ ± **4.3** fluctuation range **6.0 — 24.0** μ	fluctuation range 5.0 — 35.0 μ Wróblewski, La Due (15)
Aldolase	Bruns (16)	mean value ± standard deviation **6.4** μ ± **0.7** fluctuation range **5.3 — 7.8** μ	fluctuation range 3.0 — 8.0 μ Bruns (16)

The second group consisted of men, working during the last years in the atmosphere polluted with lead. Four above mentioned serum enzymes were estimated three times on three successive days.

The members of all groups passed through the medical and haematological examination. The last one included red and white blood cells count, the examination of blood smear, the reticulocyte- and platelet-count. In people having contact with lead the number of red cells with basophilic stippling, the amount of coproporphyrin in urine and the excretion rate of lead in urine after intravenous injection of EDTA were also estimated. The EDTA-test was performed according to the method described by *Z. Byczkowska* (10).

The estimation of serum enzymes activity was carried out by colorimetric methods. The necessary substrata were supplied by the firm Boehringer, Mannheim G. F. R. All the data concerning the applied methods and range of physiological fluctuations are given in Table 1.

The physiological fluctuation range of serum enzymes was established by some authors (cited in the above mentioned table) in large populations. The values were checked by means of control groups of 16–17 healthy men and women, not exposed to chemical risk. As these findings are in complete accord with literature data, the latter were used in determining the limits of physiological activity of enzymes.

RESULTS

The exposure to benzene and its homologues

a) In 18 out of the group of 35 workers exposed to prolonged action of benzene a drop of alkaline phosphatase activity under the lowest limit of physiological fluctuation range was observed.

The rest of the examined group showed values within the lowest third of this range. The difference found between the mean value for the control group and that for the group under the test was statistically significant.

The tested group		The control group			
$\bar{x}$	n	$\bar{x}$	n	t	p
0.91 ± 0.24	35	1.60 ± 0.40	17	7	$p < 0.01$

$\bar{x}$ = mean value ± standard deviation
n = number of cases
k = number of estimations
t = value »t«
p = confid. level

b) In the group consisting of seven clinical patients the enzymatic changes concerned three of the examined enzymes: alkaline phosphatase, aldolase and glutamic pyruvic transaminase. The differences between the mean values for the control group and those for the tested group were statistically significant.

Enzyme	The tested group			The control group				
	$\bar{x}$	n	k	$\bar{x}$	n	k	t	p
Alk. phosph.	0.99 ± 0.56	7	21	1.60 ± 0.40	17	17	4.0	$p < 0.01$
Aldol.	3.60 ± 1.89	7	21	6.40 ± 0.70	17	17	6.0	$p < 0.01$
G. P. T.	8.00 ± 7.30	7	21	16.00 ± 4.30	16	16	1.7	$p = 0.01$

$\bar{x}$ = mean value ± standard deviation
n = number of cases
k = number of estimations
t = value »t«
p = confid. level

On the contrary, no important disturbances could be observed in the level of S. G. O. T. However the S. G. O. T./S. G. P. T. ratio was higher than normal in all examined persons.

The exposure to lead

The values of serum enzymes below the lowest limit of physiological fluctuation range were found only in isolated cases. But the large fraction of aldolase and S. G. P. T. values was localised near this limit. The

Enzyme	The tested group			The control group				
	$\bar{x}$	n	k	$\bar{x}$	n	k	t	p
Aldol.	4.3 ± 1.89	20	60	6.4 ± 0.7	17	17	10.0	$p < 0.01$
G. P. T.	12.0 ± 6.30	20	60	16.0 ± 4.3	16	16	1.75	$p = 0.32$

$\bar{x}$ = mean value ± standard deviation
n = number of cases
k = number of estimations
t = value »t«
p = confid. level

difference between the serum activity of aldolase in the control and in the exposed group was not statistically significant.

Although the level of S. G. P. T. was not statistically significantly lower, in most examined persons S. G. O. T./S. G. P. T. ratio was higher than normal.

The values of S. G. O. T. in a number of cases were a little higher than normal (although still within the physiological fluctuation range) which influenced the S. G. O. T./S. G. P. T. ratio too.

In Table II data are recorded concerning the results of other tests carried out in this group of workers.

Table II.

Changes in blood, urine and Pb excretion in the group of workers exposed to lead

Red cells with basophilic stippling per million of erytrocytes	no	single	300–5000
	75%	15%	10%
	minus	intermediate	plus
Coproporphyrin in urine*	< 60 gamma/24h	60–280 gamma/24h	> 280 gamma/24h
	50%	30%	20%
Lead level in urine volumen per day after EDTA dosage (1 g intrv.)	less than 500 gamma	500–1000 gamma	more than 1000 gamma
	15%	20%	65%

* The estimation of coproporphyrin in urine was carried out by a semiquantitative method acc. *Brugsch*. The results are expressed in terms: »minus«, »intermediate«, »plus« which approximately corresponds to the values; 0–60 gamma/24 hr, 60–280 gamma/24 h and more than 280 gamma/24 hr.

DISCUSSION

The data obtained confirm the opinion that longtermed exposure to the action of toxic substances can produce changes in the activity of some serum enzymes. Examining the group of workers who had contact with benzene we observed a characteristic drop of serum alkaline phosphatase. However, no clear relationship between the activity of this enzyme and the number of morphologic blood elements (neutrophilic cells, lymphocytes, red cells, reticulocytes) per cubic mm could be proved. Nevertheless, it is impressive (see Table III) that the divergences from normal in the blood composition seem to be more frequent in persons with lower

Table III.

The percentage of persons showing pathological values concerning some of the six blood factors (neutrophilic cells, lymphocytes, platelets, reticulocytes, red cells and haemoglobin)

Persons showing	Number of persons (100%)	Serum alkaline phosphatase level	
		Normal	Lower than normal
no pathological values	3	100%	—
pathological values concerning:			
one factor*	6	66%	33%
two factors	11	39%	61%
three factors	8	50%	50%
four and more factors	5	20%	80%

* in five cases a drop of lymphocytes count, in one a drop of the number of platelets.

values of serum alkaline phosphatase than in persons showing normal activity of this enzyme. With respect to this problem the obtained results were similar to those we had found in the group of workers exposed to the prolonged action of ionizing radiation (17). Other data obtained by *Abramova* (18) in her experiment on mice confirm the parallelism between the action of benzene and that of ionizing radiation.

As to the mechanism of the influence of benzene on the enzymatic system there is no unanimity in the literature on this subject. Some of the authors underline that these changes depend on the disturbances of red-ox system. In an early stage of benzene intoxication the body loses considerable quantities of sulphur, which involves a drop of glutathion and cystein level in the blood (19).

It must be added that not only benzene, but also its oxydation product phenol can influence the enzymes activity. It is well known that phenol belongs to the substances injuring the liver. The drop of some enzymes activity could be explained as an effect of diminished enzymes production by the injured liver cells (20). It is very interesting that the enzymatic disturbances produced by benzene exhibit rising of the activity of some enzymes and a drop of that of the others (5).

The second group of people exposed to the action of lead showed a drop of aldolase level in serum and a rise of S. G. O. T/S. G. P. T. ratio. No correlation between the enzymes activity in serum and the results of EDTA test could be found.

According to the opinion of *Abderhalden* (2) the low values of serum aldolase speak in favour of chronic hepatitis. The rise of S. G. O. T./ S. G. P. T. ratio seems to prove the same (22).

The liver contains lead in relatively high concentration after lead absorption from any source, particularly in acute poisoning. There are differences of opinion regarding the effect of lead upon this organ. The earlier literature tended to minimize it. The recent reports tend to emphasize the frequency and extent of hepatic damage in lead poisoning.

The next question to be discussed is whether all the enzymatic changes we met in the serum of exposed persons can be explained as due to injurious action of chemical agents only. In order to solve this problem it is necessary to exclude:

1) the influence of additional diseases,

2) the influence of other agents (e. g. malnutrition, alcoholism) that could affect the physiological status of the subject.

Table IV.

The list of diseases discovered in the examined group of workers

	Symbol of the group	Number of examined persons	Healthy	Cholelithiasis*	Adnexitis*	Neurosis univ.	Cerebrastenia
Exposure to benzene	1a	35	29	4	1	—	—
Exposure to benzene and its homologues	1b	7	4	—	—	2	1
Exposure to lead	2	20	20	—	—	—	—

* no symptoms of acerbation were found in the period of present investigation.

The medical examination discovered the existence of some diseases in a part of the workers. The full list of these diseases is given in Table IV. The available sources of information did not contain any data indicating that these diseases were caused by enzymatic changes in the blood serum. It should be stressed that the majority of the examined workers (all in the group having to deal with lead) did not exhibit any disease.

As the possible effect of malnutrition on the enzymes level, there is no apparent reason that any kind of malnutrition should be taken into account.

The problem of alcoholism needs some additional remarks. As the group of people having contact with lead consisted entirely of men it

could be taken for granted that a number of them were addicted to alcoholism. Many authors indicate that chronic alcoholism can effect serum enzymes activity. According to *Boivin* (23) a slight increase of enzymes activity in serum, particularly of S. G. P. T., is generally symptomatic for chronic alcoholism. *Amelung* (22) shares this opinion. These characteristic symptoms could be found only in one member of all three groups. They consisted of increased activity of aldolase, S. G. P. T. and S. G. O. T., the S. G. O. T./S. G. P. T. ratio being about one.

CONCLUSIONS

1) In people who had contact with benzene and its homologues a fall of serum alkaline phosphate activity was observed. Moreover, some members of this group exhibited a significant drop in aldolase and S. G. P. T. serum level.

2) The group of workers exposed to lead showed statistically significant lower values of aldolase than the control group not exposed to any chemical hazards. The S. G. O. T./S. G. P. T. ratio seemed to be higher than it was normally.

3) It is almost certain that the changes referred to were due to chronic exposure to benzene and lead. The role of additional diseases, malnutrition, and alcoholism seems to have been insignificant in the investigated cases.

References

1. *Cornish, H. H., Block, W. D.:* A. M. A. Ind. Health *21* (1960) 549.
2. *Ball, W. L., Kay, K.:* A. M. A. Ind. Health *14*, (1596) 450.
3. *Cenacchi, G. C., Tucci, G., Lodi, A.:* Bologna Med. *5*, (1959) 519.
4. *Nicola de, P., Petronio, L., Caraccia, I. C.:* Boll. Soc. ital. Biol. Sperim. *36*, (1960) 1148.
5. *Gabor, S.:* Med. del Lavoro *50* (1959) 257.
6. *Straus, R., Wilkinson, A., Wurm, M., Harnold, B. D.:* Ind. Med. Surg. *25*, (1956) 151.
7. *Golubowski, I. J.:* Kasan. med. Z. *41*, (1960) 98.
8. *Fournel, F.:* Recueil. Trav. Chim. Pays Bas *74*, (1955) 640.
9. *Waldman, R. K., Borman, E. K., Hartford, M. S.:* A. M. A. Ind. Health *4*, (1959) 431.
10. *Buczkowska, Z., Antczak, K.:* Medycyna Pracy *3*, (1963) 211.
11. *Bessey, O. A., Lowry, O. H., Brock, M. J.:* J. Biol. Chem. *164*, (1946) 321.
12. *Suedhof, H., Steindorf, H., Goetze, Ch., Voss, I., Loehr, H.:* Dtsch. med. Wschr. *87* (1962) 249.
13. *Petzold, H.:* Dtsch. Z. Verdaungs u. Stoffwechselkh. *20*, (1960) 257.
14. *La Due, J. S., Wróblewski, F., Karmen, A.:* Science *120*, (1954) 3117.
15. *Wróblewski, F., La Due, J. S.:* Proc. Soc. exp. Biol. N. Y. *91*, (1956) 569.

16. *Bruns, F.:* Biochem. Z. *325,* (1956) 156.
17. *Hanke, J.:* Congreso Internacional del Trabajo, Madrid, 1963.
18. *Abramova, Z. J., Kuzniskaja G. J.:* Naucz. Issled. *5,* (1959) 202.
19. *Lemierre, A., Lenormant, Ch., et al.:* Traité de médecine, tome IV, Paris, 1948.
20. *Esposito, S., Petronio, L.:* Tumori *45,* (1959) 657.
21. *Abderhalden, R.:* Klinische Enzymologie, George Thieme Verlag Stuttgart, 1958.
22. *Amelung, D.:* cit. acc. *Blut 8,* (1962) 122.
23. *Boivin, P., Bessen, R., Hartmann, L., Fourent, R.:* Rev. Franc. Etudes Clin. Biol. *14,* (1959) 349.
24. *Brugsch, J.:* Porphyrine, Johann Ambrosius Barth Verlag, Leipzig, 1952.
25. *Frajola, W. T.:* Fed. Proc. *19,* (1960) 1.
26. *Gage, J. C.:* Brit. med. J. *4,* (1955) 1370.
27. *Tucci, S. G., Cenacchi, G. C., Lodi, A.:* Bologna Med. *5,* (1961) 609.
28. *Tulpe, S. G., Phansalker, S. V., Ramandhandram, M.:* Current Sci. *28,* (1959) 149.

Sadržaj

PRELIMINARNA ISPITIVANJA O UTJECAJU KRONIČNE EKSPOZICIJE TOKSIČNIM SUPSTANCIJAMA NA AKTIVNOST NEKIH ENZIMA U SERUMU

Ispitana je aktivnost četiriju enzima u serumu – aldolaze, alkalne fosfataze, glutaminsko-oksalne transaminaze i glutaminsko-piruvične transaminaze – kod dvije grupe radnika koji su bili kronično eksponirani benzenu s homolozima i olovu. Prema rezultatima koji su obrađeni statistički i uspoređeni s kontrolnom grupom, opaža se da u kontaktu s benzenom i homolozima dolazi do pada aktivnosti alkalne fosfataze, a kod nekih ispitanika i do značajnog pada aktivnosti aldolaze i gultaminsko-piruvične transaminaze u serumu. Grupa radnika koja je bila eksponirana olovu pokazuje niže vrijednosti za aktivnost aldolaze, a viši kvocijent SGOT/SGPT nego kontrolna grupa. Nije se moglo utvrditi da je do takvih promjena došlo zbog drugih bolesti, pothranjenosti ili alkoholizma već su one vjerovatno nastale samo zbog kronične ekspozicije benzenu i olovu.

Institut za medicinu rada,
Lodz

Primljeno 13. I 1964