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BIOLOGICAL CHARACTERISTICS OF THE EFFECT OF LEAD ON THE ORGANISM

(Possible biological indices)

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INTRODUCTION

In the last decades, industrial medicine has shifted away from a pure applied and descriptive discipline towards basic science research.

Owing to the application of biochemistry, cytology, pharmacology, histochemistry, genetics and immunology, the field of study in which industrial toxicology is engaged is considerably broadened. Less emphasis is being placed on the value of routine studies dealing with the evaluation of toxic effects of hazardous substances; on the contrary the importance is stressed of research, which aims at elucidating the toxic mode of action of these substances and in studying the pathogenesis of occupational diseases. As a result of this modern concept, great advances have been scored in the knowledge of how toxic agents, foreign to the body actually act. The new insights that have been gained are based upon systematic observations, being the results of studies in various fields. It has to be realized however, that although these studies have clarified many problems, new ones have been opened up. Moreover, a great deal of uncertainty remains in the interpretation of many findings and in the assessment of the relationship between clinical symptoms and biochemical observations. Profound studies have revealed that the clinical manifestations of toxic substances have their counterparts in injurious effects

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at subcellular or biochemical level.

Toxicants disrupt vital metabolic processes of cells or subcellular structures, consequently leading to functional impairment of organs and to clinical symptoms. The interference with sub cellular processes is commonly accompanied by the presence of biochemical disorders. It is axiomatic that biochemical pathways may be impaired and these biochemical lesions occur whenever functional dysfunction in a tissue is apparent. The application of the concept of biochemical lesions has directed the attention of toxicologists to the development of screening methods, suitable for the early diagnosis of poisoning. The aim is to control the physical and chemical factors of the environmental air that contribute to the development of chronic diseases. Since it has been recognized that toxic agents are capable of producing manifold longrange, chronic, genetic or carcinogenic effects, research is focused on the understanding of biochemistry and pharmacology of these agents.

This approach has deepened the study on possible metabolic derangements, that ultimately might lead to subclinical effects. These derangements may have their own functional significance. Apart from the existence of specific damage to some target organ, studies in this field may reveal less obvious non-specific alterations. Slight non-specific responses, as a compensatory activity of the body has been detected, following exercise of low levels of poisons. Chemical tests, based on this approach, may supplement classic methods of physical and hematological examination. They should not only be of importance in assessing and predicting the toxicity of a compound, but preferentially be capable to disclose disorders before the occurrence of pathological manifestations.

Moreover the concern is to search for indicators (enzymes, metabolites, etc.) whose alterations are in a certain relation to the exposure intensity and duration. Such alterations, frequently become apparent in the body fluids (blood, urine). It is the elaboration of tests on biological samples, which might be used to estimate the integral absorption and the proper magnitude of the exposure to which increasing importance is being attributed.

From the viewpoint of possible metabolic activities, lead has been shown to be involved in many processes. Contrary to previous

concepts the action of lead is now revealed to be far from simple. The aim of this paper is to describe the close association which exists between the multiple biochemical lesions, provoked by the metal and the clinical picture resulting from these disorders.

INFLUENCE OF LEAD ON THE HEMATOPOIESIS AND ON ERYTHROCYTES

The study of lead toxicology has been dominated by the researches on the influence of the metal on the formation and degradation of hemoglobine and erythrocytes. The hematological disturbances, with resultant anemia, manifest in a condition of increased lead absorption or poisoning, actually has been the subject of numerous investigations. The emphasis that has been placed on this aspect of lead toxicity has two compelling reasons: the attempt to elucidate the biochemical mechanism which lies at the basis of the toxic anemia produced by lead and the occurrence of pathological levels of some metabolites, related to the synthesis of hemoglobine in case of increased lead intake. Since lead is capable of attacking the hematopoietic system in an early stage of exposure, tests on these metabolites in biological samples could be developed, which determine the pretoxic stage, and even could be elaborated into reliable parameters for the degree of exposure. These tests being of great value in the medical supervision of workers may be used in the practice as integral absorption indexes and render the possibility to follow up even slight responses of the organism.

Although many papers have appeared, dealing with the genesis of lead anemia, the process whereby the metal induces this toxic phenomenon is by no means clear and there remains a great deal of uncertainty in the interpretation of the results.

The older theory, which explains the anemia almost entirely to the hemolytic action of lead is no longer accepted. This action cannot be excluded in acute poisoning, but is in case of chronic poisoning of minor importance.

Newer insights have been gained by experimental evidence, explaining the drop of hemoglobin by a profound inhibitory action of

lead on the metabolic chain of hemesynthesis (porphyrin disturbances). Biochemically, this disturbance implies that lead attacks enzymes (or systems) at various points of the porphyrin synthetic chain. The defective hemoglobiniration is fundamentally based on the profound effect on the blood-forming systems in the bone marrow cells exerted by the metal. Secondary to this effect are the changes observed in the peripheral blood, e.g. polychromasia, basophilia and reticulocytosis.

Concerning the toxic action of lead on the hematopoiesis, three different aspects can be distinguished:

a) the influence of lead on circulating erythrocytes
b) the impairment of Hb-production, as a result of the injurious effect of lead on bone marrow cells
c) the stimulation of the erythropoiesis in the bone marrow.

These features are all closely related to the manner in which lead is able to cause anemia. As yet it is impossible to give a satisfactory picture of the background of lead anemia, which takes into account the various experimental observations. In spite of the numerous investigations, it remains for more study to give an over-all embracing theory, which should cover and explain the manifold experimental findings. The matter is complicated by the fact, that some of the experimental findings are apparently contradictory. Furthermore it is generally agreed that there is no single factor which determines the genesis of the anemia. In the context of this paper, the views on the genesis of lead anemia can only be briefly outlined.

The views related to the action of lead, are based on the appearance of the following biochemical events occurring in lead poisoning in vivo:

<u>urine</u>

- porphyrinuria; increased excretory output of coproporphyrine III (CP III) and to a lesser degree of type I
- elevated excretion of 5-aminolevulinic acid (5-ALA) and of aminoacetone (AA)
- slight rise of the urinary level of porphobilinogen (PBG)
- increased excretion of iron (Fe).

blood

a) serum:
- increased contents of 5-ALA and of AA
- increased Fe concentration, together with lowered Fe-binding capacity
- increase of plasma porphyrins (protoporphyrin - PP IX and to a slight degree of CP's)

b) mature erythrocytes of the peripheral blood:
- elevated level of PP IX
- increase of non-hemoglobin Fe
- decrease of Hb
- reduction of the red bloodcell count (RBC)
- decrease of Hb-synthesizing enzymes; 5-aminolevulinic dehydratase (ALAD) and of heme synthetase
- occurrence of punctate basophilia
- siderocytes
- reticulocytosis

The erythrocytes are characterized moreover by increase of the osmotic resistance (O.R.), enhanced K^+-outflow and shortened survival time.

bonemarrow cells

The occurrence of morphological entities, like basophilia, siderocytes, increase of erythroblasts and sometimes of megalo- and macroblasts, and degenerative alterations of the mitochondria with the presence of ferritin-molecules.

tissue cells:

accumulation of porphyrines in liver and kidney cells in some animalspecies in experimental poisoning.

The importance of these phenomena in the clinical diagnosis of inallowable lead absorption as well as their order of appearance will be described in connection with the three features of action of lead, mentioned on pag. 4.

a. the influence of lead on circulating erythrocytes

The assumption originally held by early investigators, that the anemia should be caused mainly by a direct influence of lead on peripheral blood red cells is in disagreement with most recent

observations. The older theory stated that the anemia is predominantly hemolytic in character; the decrease of Hb should result from increased viability of the erythrocytes, terminating in elevated destruction-rate of these cells. Even, the porphyrin excretion in disease was considered to be derived from the acceleration of Hb-breakdown[1]. At present all evidence is in favor of the view that lead anemia arises mainly from enzymic blockage of the heme synthetic chain at various sites. Nevertheless, the adverse effect of lead-ions exerted on erythrocytes, both in vitro and in vivo, has been unequivocally demonstrated. Lead ions possess a great affinity to these corpuscles and especially to the cellular membrane. This interaction brings on both biochemical and physiological alterations. Morphologically, the cells, affected by lead ions undergo shrinkage; the cellular membrane is altered which involves a net loss of osmotic active substances (K^+) and water. Increase of the O.R. has been reported by several authors in experimental poisoning as well as in patients suffering from chronic disease[2-5]. The findings, regarding the mechanical fragility of the red cells do not allow a definite conclusion whether this property is changed in lead poisoning.

Leakage of K^+-ions from the red blood cells, due to permeability changes of the erythrocytary membrane, with resultant elevation of the plasmatic concentration of this cation, has been observed in vitro experiments in which lead salts were incubated with blood samples[6-8], as well as in lead workers[9]. The latter did not exhibit obvious signs of toxicity, which leads to the conclusion that estimation of the K-ion concentration might be a valuable parameter of industrial lead exposure. It appears, that the loss of the cation is an event which occurs in an earlier stage of lead absorption than the alterations in respect to the physical properties (osmotic and mechanical fragility). It is likely that the erythrocytary membrane becomes early affected. Evidence for this view is provided in addition by the observation that the activity of the cation-dependent ATP-ase is diminished not solely in lead-poisoning but also before illness. Heavily exposed lead-workers, furthermore, showed a distinct reduction in the uptake of isotope-labeled phosphorons (32_p) by the membrane[10, 11]. This finding indicates that the P-metabolism, and particularly

the incorporation rate of P^{32} into membranal phosphatidic acid is disturbed under the influence of small lead amounts.

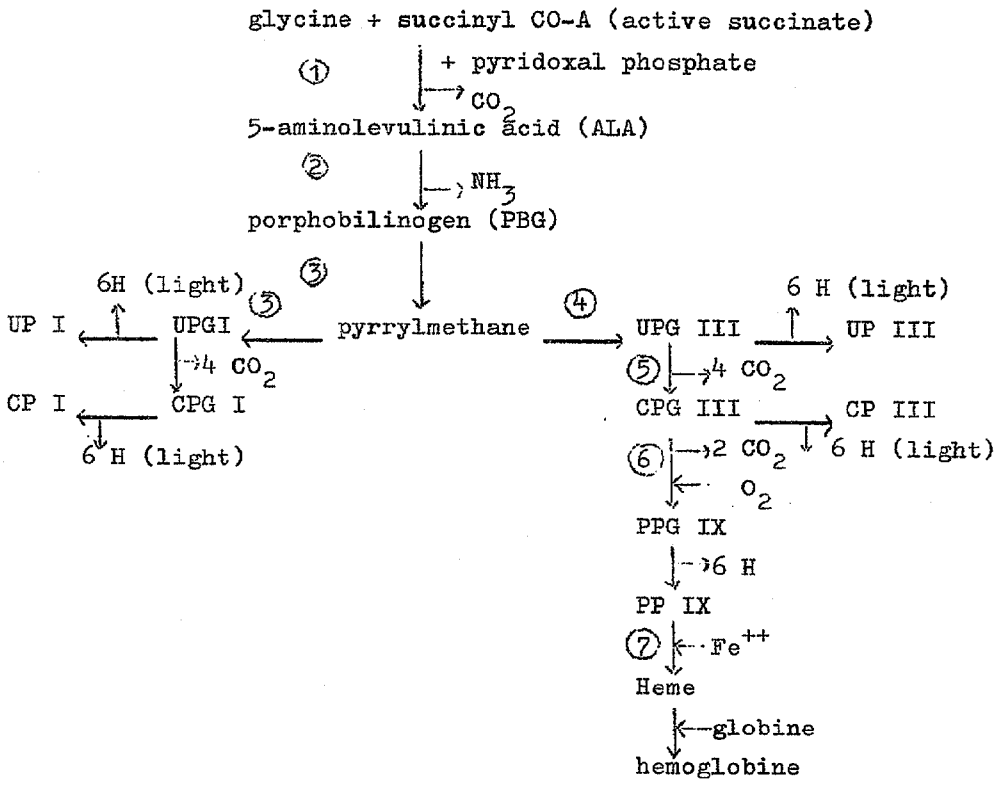
Contrarily to these membranal effects, the metabolism within the red cell corpuscles seems not to be severely affected in lead poisoning. The experiments indicate solely slight changes of the oxygen consumption and the glycolytic system. This, again suggests that the membrane has to be considered as the chief site of attack. The glucose consumption of erythrocytes, derived from lead workers is elevated in comparison with unexposed individuals, whereas the lactic acid production was insignificantly raised. This differences should be accounted to the relative abundance of young cells (reticulocytes) among the erythrocytes in the exposed group. The elevation of the glucose consumption was shown to be correlated with the excretion of coproporphyrine[12].

Finally, the existence of a shortened life span of erythrocytes in lead poisoning is a well-established fact. This phenomenon may in some way be related to the enhanced K^+-loss or alternatively originate from the influence of lead on the bone marrow erythroblasts (disturbance of heme-synthesis). The experiments conducted with marked chromium (^{51}Cr)[13-16] or by using ^{3}H-DFP [17], infer to an increased turnover rate of these isotopes, indicating decreased survival time.

b. <u>inhibition of heme biosynthesis by lead (mechanism)</u>

The chief site of production of hemoglobin (Hb) is in the erythroblasts in the bone marrow. As mentioned previously, the anemia produced after the ingestion of lead is principally determined by the impairment of the biosynthesis of heme. This theory can give a satisfactory explanation for the abnormal hematological picture and the abundant excretion of substances related to heme, in lead intoxication. All of the evidence is in favor of the view that lead exerts an inhibitory action on enzymes catalysing the various steps of the heme biosynthesis. Although the sites of attack of lead on the in vitro biosynthesis of heme now is rather well documented, the exact nature of the disturbance in vivo is virtually incomplete. The lack of knowledge can be explained by the fact that heme may be synthesised also in tissues either than the bone marrow, by the hypersynthesis of porphyrin

precursors as an initial effect of lead and by the observation that the mode of action in vitro differs from that in vivo. A summary of the steps in the biosynthesis of porphyrin is given in the figure below.



Biosynthetic pathway of hemoglobine

enzymes:

① : ALA-synthetase
② : ALA-dehydratase
③ : PBG-deaminase
④ : UPG-isomerase
⑤ : UPG-decarboxylase
⑥ : CPG-decarboxylase
⑦ : heme-synthetase

abbreviations:

UP : uroporphyrine
UPG : uroporphyrinogen
CP : coproporphyrine
CPG : coproporphyrinogen
PPG IX: protoporphyrinogen IX
PP IX : protoporphyrine IX

Objection against the hemolysis-theory came first from Remington [18, 19] who presented evidence that lead anemia arises from a blockage of the incorporation of divalent iron into the protoporphyrin molecule (hemesynthetase inhibition). This theory could explain the porphyrinuria, the elevated level of PP IX in erythrocytes and the deposition of iron in serum and bone marrow. Although the inhibition of heme synthetase is a well-established fact, observed by many researchers, it is assumed that this is not the only lead-sensitive step in the porphyrinic chain. Eriksen[20, 21], Dresel and Falk[22, 23] presented evidence that lead interferes in the early stages of the pathway, e.g. before the synthesis of PBG. In vitro, the step catalysed by ALAD appeared to be extremely lead-sensitive. Similarly, strong inhibition of this enzyme was found in vivo-experiments by Goldberg et al[24]. Kreiner-Birnbaum and Grinstein[25] investigated extensively the porphyrin and heme synthesis in lead poisoned rabbits by employing isotopically-tagged substrates (precursors). They concluded from their observations on the accumulation of the various intermediates that the main blockage is at the level of ALAD. Secondly, the step catalysed by the enzyme (or enzymic system) UPG-decarboxylase most probably becomes inhibited, although to a lesser extent. Using ^{14}C-labeled CPG III as substrate, both PP IX and heme were found to be reduced in reticulocytes and hemolysates in the experimental animals. This is interpreted in a way that CPG-decarboxylase is also blocked by lead, but conclusive direct evidence for this inhibition is lacking. Blockage of this step might provide an explanation for the rise of CP III, observed in the bone marrow cells, erythrocytes and blood plasma in experimental lead poisoning (Sano[26]). In contradiction to heme-synthetase, the other enzymes of the system-ALA synthetase and PBG deaminase - appear to remain unaffected in lead poisoning. With relation to the mitochondrial enzyme ALA-synthetase, this result was confirmed previously by Pecora[27] and Gajdos[28]. It is postulated that the enzyme inhibitions might be explained wholly by the high affinity of lead towards the essential sulphydryl groups (-SH) these enzymes possess. A full explanation on this basis is however unsatisfactory, since other metals with equal or even

higher capacity to inhibit SH-groups do not disturb the in vivo synthesis. For the depression of ALAD an indirect inhibitory effect due to accumulation of intermediates (PP IX) has been proposed.

Detailed information on some of the enzymes and intermediates involved in the normal biosynthesis of heme is as yet incomplete known. This circumstance, in addition, restricts the possibility of giving a complete and comprehensive picture of the enzymic inhibitions involved in lead poisoning.

c. Stimulation of the erythropoietic activity

The precise action of lead on heme synthesis is overshadowed by the observation that in beginning exposure, the blood-forming system is stimulated recognizable from a rise of the RBC together with increase of the Hb content. This effect is primarily expressed in the bone marrow. Rabbits exposed to 50 mg Pb acetate/m^3 showed an initial rise of Hb and the RBC, followed by a depression of these values after prolonged administration[29]. The secondary changes in the peripheral blood, such as reticulocytosis and basophilia are manifestations of the increased response.

In close association several authors emphasize the promotion of the synthesis of the porphyrins. Sano[26] established that in lead poisoning the first disorder of the biosynthesis consists in hyper-synthesis of ALA, from glycine. The accumulation of PP IX is explained in a similar way. Italian authors confirmed this view, using several systems and observed an overactivity of the entire metabolic chain in vivo. According to Pecora[30, 31], the in vitro action of lead is quite different from taht in vivo: whereas in the first case the porphyrin formation is depressed, the reverse is true concerning the in vivo action at low concentrations of lead, ranging from 10^{-7} to 10^{-11}M. in the bone marrow. The sign of the effect in vivo is dose-dependent. If concentrations of lead in the bone marrow reach 10^{-4}M, there is actually a depression of the synthesis. Additional evidence for the stimulation is afforded by the fact that not only urinary CP III but also type I is elevated in lead workers in absolute magnitude[32, 33].

Other results also indicate unequivocally the capacity of lead to enhance the formation of porphyrins[34] which appears to be contradictory to the ascertained blockage of the different enzymic steps. This divergency in results, again, illustrates the difficulties encountered in establishing the real mode of action of lead in vivo.

Signilicance and reliability of various parameters as indicators of elevated lead absorption

a. basophilic stippled cells (BSC)[35, 36]

This phenomenon is manifested in the bone marrow cells and in the peripheral blood. In the marrow cells it occurs earlier and to a stronger degree than in the blood. In the latter, the enumeration of the BSC has been generally employed in workers to obtain a valid index of response, because the appearance of any increase precedes the depression of the Hb-content. There is however a wide divergency in results among the various authors, mainly due to diversity in methods. The reliability of the BSC largely depends on the counting and staining methods employed. The variability in results would b^{e} much lower using one single standardized technique. On this condition, there is a close relationship between increase of the BSC and the exposure intensity. The degree of stippling is related to the alterations of other parameters, and is paralel with the urinary levels of ALA, CP and lead. There is non-linearity between basophilia and the severity of the clinical symptoms. Owing to its high specificity, the BSC is considered as a useful aid to follow up workers and a valuable supplement of other laboratory findings.

b. reticulocytes[35, 37]

Reticulocytosis is a very early occurring symptom. Owing to the greater aspecificity of this phenomenon, estimation of the number of reticulocytes is of lesser value than determination of the BSC.

c. coproporphyrin

In the prevention of workers against inallowable lead absorption, the estimation of urinary CP has been proved to be of great value. In case of heavy exposure levels of CP may increase up to 10 to 30-fold of the normal concentrations. This phenomenon is an earlier response of the body than increase of the BSC, but has the disadvantage that it is less specific. Elevation of CP may result from a variety of other poisons and is present in certain blood diseases[38]. A high degree of parallelism has been ascertained between the intensification of the coproporphyrinuria and elevated levels of urinary and blood lead[39-42] and urinary ALA[43-46] by many authors. Coproporphyrinuria on the other hand is unrelated to the occurrence of clinical signs of toxicity.

Increased levels of CP III have also been found in mature erythrocytes[47, 48], and in the bone marrow cells and kidney tissue[49] of animals, experimentally poisoned.

d. protoporphyrin (PP IX)

Protoporphyrinemia is a phenomenon recorded by a vast number of investigators[48, 50-54]. The erythrocytary PP IX content is elevated in a very early stage of lead exposure and may reach values 10 to 50-fold higher than in inexposed subjects. In addition, it is held a more specific reaction than coproporphyrinuria. Besides in plumbism, it is encountered in several other anemias, but does not arise from intoxicants other than lead. Noteworthy is the long persistance (a few years) of the phenomenon after cessation of the exposure[53]. It is apparent that the measurement of red blood cell PP IX is one of the best indicators of disturbance of the hematopoiesis. Because of the fact that there is no simple analytical method available for determining PP IX, this parameter is less convenient for routine work. Elevation of free PP IX in the erythrocytes is related to the appearance of red fluorescence of these cells and visualized under U.V.light. According to Whitaker et al[55], the counting of the number of fluorescent cells might be helpful to diagnose lead poisoning. Additional research is needed to conclude whether this method is useful in the medical supervision of lead workers.

Increase of plasma porphyrins in lead workers has been observed by Waldron[56]; these substances consisted for the greater part of free PP IX.

c. aminoketones (blood serum andurine)

More recently, the attention is called to the value of urinary ALA determination for early detection of increased lead absorption[42-44; 57]. Intensified ALA excretion has been shown in lead workers with or without toxic manifestations as well as in animal experiments. Since Mauzerall and Granick[60] elaborated an excellent method for analyzing the concentration of ALA, determination of this parameter has been generally used in the control of occupational workers. Actually, the total amount of urinary aminoketones is determined by this method; from this ALA constitutes a part, whereas the remainder consists chiefly of aminoacetone (AA). There is however some divergency in results concerning the portion of total aminoketones which is represented by ALA[42,61-63].

Owing to the extensive studies of Haeger-Aronssen[42] on the usefullness of ALA as laboratory test, the following conclusions can be drawn:

- the increase in the urinary ALA varies directly with the degree of lead exposure; it implies a reliable indicator of the magnitude of the exposure;
- highly significant correlation coefficients have been found in lead workers between urinary ALA and lead ($r = +0.92$) (see also 64) and between ALA and CP ($r = +0.69$);
- increased urinary output of ALA is a much more specific sign of hazardous lead absorption than abnormal CP levels; raised ALA levels have been found only in a few pathological conditions, such as some types of porphyrins, in which the excretion of PBG is also strongly elevated however;
- ALA is increased above normal at exposures in which CP does not exceed the normal limits, which supports the view that it is a very sensitive and early parameter[46, 65];
- in case of heavy exposure, urinary ALA levels may reach values higher than 30-times the normal concentrations (about equally as for CP).

These features have been confirmed by many of the mentioned authors. A certain paralelism became apparent between the magnitude of ALA increase and the appearance of toxic signs. ALA determination has been proved to be suitable to define the quantity of metabolically active lead in the organism, and was highly cor-

related with blood lead values (r = +0.90)[45]. Similarly, a rather high correlation was established between the elevations of free PP IX in the red blood cells[66] or the BSC[67] and those of ALA.

The intensification of the ALA-excretion is in agreement with the proposed enhancement of porphyrin synthesis induced by lead. This results in increased production of the metabolite. Because of the blockage of ALAD, the compound cannot go through further stages of the metabolic chain. Consequently, the blood level of ALA is raised with either overflow of the metabolite into the urine or alternatively decreased reabsorption of ALA in the renal tubules. In agreement with this view, Saita et al [68], observed in persons ffected by lead an extra-increased excretion of ALA (and increased blood level) after administration of glycine per os, in comparison with unexposed individuals. Elevation of urinary aminoacetone (AA) has also been observed in lead workers: the extent at which this metabolite is increased is however less than is the case of ALA[69]. Clarification of the abnormal AA metabolism awaits further research however.

Significant elevations of ALA in serum have been shown to accompany consistently those of the urinary ALA levels[42, 66, 70-75]. A direct propostionality has been detected between both concentrations. Some evidence has been presented that serum ALA elevation even precedes the augmentation of urinary ALA. As biological exposure test, the determination in serum is less suitable, since the extent of the increase is less than for ALA in urine and the normal levels are too low to measure with great accuracy.

f. <u>iron-metabolism</u>

The disturbance of the Fe-metabolism may be explained in terms of the impairment of the heme synthesis. Blockage of heme synthetase results in a diminished utilization of iron. This is manifested in the occurrence of iron-stainable inclusions in the red cells (siderocytes[76]) and in the erythroblasts (sideroblasts) in patients and in experimental animals. These corpuscles contain molecules of ferritin inside the mitochondria and vacuoles, according to electro microscopic observations.

Hypersideremia (increase of Fe in theserum), together with a decrease of the latent binding capacity resulting in a raised saturation percentage, is a common phenomenon encountered in poisoned subjects and experimental animals by several authors[77-80]. There is no evidence that serum Fe contents are altered in exposed workers without signs of intoxication. Zegarski established a certain correlation between this parameter and urinary CP[81]. Studies on the urinary excretion of Fe revealed, that the metal may be increased in lead workers, without any signs of toxicity. The extent of the alteration was related to the duration of the exposure[82].

Much work has been done to elucidate the complex manner in which Fe-metabolism becomes disturbed by lead. Discussion of the various findings with respect to this disorder, is however beyond the scope of this paper.

g. inhibition of ALAD

Crude preparations of the enzym, located in the erythrocytes, are inhibited by certain heavy metals and SH-group inactivating substances[83, 84]. In vivo, ALAD-activity was shown to be suppressed both in rabbits subjected to lead poisoning[85, 86] and in human plumbism[87-90]. Experiments performed on animals by means of i.v. or s.c. injections of lead salts demonstrate that the enzym inhibition occurs at extremely low levels, viz at dose levels too low to cause any change in the urinary Pb, CP or ALA levels[91]. Confirmation of this early response of the body has been obtained by assaying ALAD activity of lead workers[90]. Activities below normal were recorded in subjects with a very slight degree of exposure. In these cases the other urinary parameters remained unchanged. Hence, it is concluded that the blockage of ALAD may be considered as the most early and sensitive sign of increased lead absorption (at about 10^{-7}M). Similarly, in the rabbits experiments inhibition of ALAD preceded the appearance of coproporphyrinuria. In these studies, a marked dose-dependent relationship has been established(amount of lead injected versus degree of inhibition). In groups of occupational workers, a fairly close linear relationship existed between urinary lead, CP or ALA and the reciprocal enzyme values. This strongly strengthens the va-

lidity of ALAD activity as exposure test. However, the fact that this enzyme is influenced in such a sensitive and early way might limit its usefulness in preventing poisoning. Because of the low activities already observed in moderate exposure, difficulties would be encountered in discriminating real lead poisoning from stages with low exposure degree.

Nevertheless, the studies are valuable in demonstrating that lead can bring on an effect on the organism by intake at minimal doses.

h. heme synthetase

As pointed out already, the inhibition of the altimate step of heme synthesis has been claimed to constitute an important factor in determining impaired porphyrin metabolism.

Experiments show that lead-ions exercise a strong blocking action on the enzym at about 10^{-4}M; below this level the degree of inhibition decreases, parallelwith decreasing concentration[19,22,24]. In vitro investigations performed on red blood cells of rabbits, ducks and humans demonstrate that at 2.10^{-5} and 4.10^{-5}M the influence is neglectible[92, 93]. Heme synthetase was measured by following the rate of Fe^{59} incorporation into heme. In vivo, the inhibition of heme synthetase was shown in patients with toxic signs and in prolonged experimental poisoning[94]. According to Otrzonsek[95], heme synthetase is reduced in the bone marrow cells, but increased in hepatic tissue. Apparently, the liver is capable to take over part of the Hb synthesis from the bone marrow.

Little work is done, up to now, on the possible reliability of this enzyme as early parameter.

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MISCELLANEOUS BIOCHEMICAL ASPECTS

a. enzyme activities

The literature, dealing with studies concerning changes of enzyme activities as a result of the influence of toxic substances is steadily growing. Regarding lead two features should be distinguished from each other: elevation of certain enzyme activities in the blood plasma as an expression of some tissue damage caused by absorbed lead and secondly the inhibitory action of lead on the activity of enzymes, localized in the tissues(organs) and present in the blood serum, resulting in a lowering of these activities. Study on the enzyme changes may be helpful in clarifying the toxic mode of action of lead. It is tentatively suggested furthermore that the quantitative assay on enzymes would hold a biochemical rationale useful for the assessment of the degree of poisoning, or even might be of value for controlling the exposure of lead workers.

Several reports have been devoted to the estimation of serum enzyme activities such as the transaminases (SGOT, SGPT), aldolase (ALD), alkaline phosphatase (alk.Ph.), pseudo-cholinesterase (p-ChE) a.s.o. in case of occupational and experimental lead poisoning.

Pathological increased levels of both transaminases have been encountered by some of the investigators in lead poisoned workers[1-3]. Contradictory results, however, were obtained by several other authors[4-7]. In healthy workers, without any clinical picture, the absence of a rise of these enzymes seems well-established. Experimental poisoning resulted in an average three-fold increase of serum transaminase activity[8]. The final conclusion may be, that these serum enzymes could reach values above normal in certain circumstances of lead poisoning, most probably as resulting from hepatic disturbance.

The ALD-level is found to be increased, both in patients suspected of plumbism[5] and in healthy individuals, occupationally exposed to the metal[9]. A good correlation was found between this enzyme level and the urinary excretion of lead. Hence, estimation of ALD-activity might be considered as a valuable diagnostic procedure to control workers and to prevent them from poisoning.

The behaviour of alk.Ph has been the subject of extensive studies of Kosmider[10, 11]. He established a strong decrease of the enzyme level in animals, acutely intoxicated as well as in lead workers with signs of toxicity. In the former case, the degree of lowering of alk.Ph-activity rans paralel to the experimental dose. Moreover, a close relationship between the decrease of the enzyme activity, and changes of other parameters was established (CP, serum protein changes). The biochemical derangement, which underlies this action of lead is presumably connected with the inactivating influence of the metal on functional groups of the enzyme, essential for integral enzyme activity (-SH and/or COOH-groups), although the possibility that lead is capable to compete successfully with the activating magnesium-ions cannot be excluded. Differences in the raising or lowering of bloodenzyme activities include other enzymes than those mentioned. Thus, p-ChE activity consistently is below normal in patients suffering from chronic lead overexposure[12]; a finding which could be confirmed in experimental induced poisoning. A negative correlation was found between these decreased values and the blood lead concentrations. Enhancement of the activity of catalase[12] and depression of carbonic anhydrase[13] has been shown to occur in case of human and animal lead absorption; the decrease being in some relationship with the physical condition of the workers. The depressing enzyme activity was more pronounced with increasing disposition. Both enzymes appear to be sensitive to the action of heavy metals and oxidizing agents. Some other enzymes, involved in the carbohydrate and energetic metabolism, are affected in case of lead poisoning, but only to a slight degree. The significance of enzyme changes has been up to now incompletely established. It should be considered that the alterations might indicate not merely a toxic response of the body to the action of the metal, but apart from this may be the expression of adaptive changes of the body; in other words they could be the provoked reaction of the organism to adjust the non-specific stress exerted by the toxicant. Apparent disturbances would then be absent.

Further research is necessary to elaborate these non-specific changes to tests, potentially capable of discriminating workers showing clinical symptoms and those who do not exhibit these manifestations.

In views of the action of heavy metals on the erythrocytes, the attention has been focused on the study of erythrocytary enzymes following in vivo lead salt intake. Extensive reference has been made on the enzyme ALA-dehydratase. Except for this enzyme, more significant enzyme deviations from normal within the red cells could be observed in lead poisoning. The studies comprise mainly the enzymes, involved in the glycolytic breakdown of substrates. The slight elevations found in certain circumstances should be ascribed to the acceleration of the hematopoiesis observed initially in lead poisoning. It seems that the penetrative capacity of the metalions is very small. It has to be considered, however, that lead-ions are accumulated in the surface of the erythrocytary membrane[14], thus interacting with biochemical processes, which regulate the transport of metabolic ions across the cellular membrane (K^+, Na^+). Functionally coupled with this process is the activity of the enzyme ($Na^+ + K^+$)ATPase, localized in the red cell membrane. The enzyme, which catalyses the hydrolysis of ATP supplies the energy, necessary to keep functioning the so-called cation pump. The pump exchanges ions (K^+) against the concentration gradient, thus maintaining a high concentration of K^+-ions within the red cells.

Hasan et al[15, 16] studied the behaviour of the enzyme in the membranal fragments of 40 occupationally exposed lead workers, free from clinical symptoms and demonstrated that the enzyme was diminished in activity (average: 30%) in comparison with a normal unexposed group. Although any correlationship between the dimination of enzyme activity and the alterations of other blood parameters, seems to be absent, the result indicates that estimation of the cation-depended enzyme may be used as a predictive test of excess lead absorption.

Histochemistry

Histochemical methods have recently opened new approaches to the study of the nature of the toxic effects of poisons exerted on tissues and particular organs. New staining methods have been successfully employed to investigate subtle alterations regarding chemical constituents and enzyme activities at cellular level.

Experimental studies of this kind in lead toxicology have revealed the multiple picture of toxic manifestations, produced by the metal. Summing up briefly the results: histoenzymatical alterations were detected in both the heart and skeletal muscle, small blood vessels, liver, kidney, intestines, and the salivary gland[17-21]. The studies include enzymes such as NADH-diaphorase cytochrome oxidase, succinate dehydrogenase, ATPase, alk.Ph., and carbohydrate splitting enzymes. The data obtained indicate that lead is enable to suppress cellular oxidation-reduction processes and to inhibit the active transport of energy-supplying organic phosphates (ATP) in the muscles. These changes have been discussed by the authors in relation to clinical signs of toxicity. Although these techniques hold very practical tools to design the functional impairment provoked by the toxicant, they do not serve as a guide for assessment of the exposure degree in occupational conditions.

b. modification of the blood serum protein pattern

As is evident from electrophoretic studies, excessive lead-absorption may result in changes in the composition of the serum proteins. The data obtained concern almost exclusively lead workers, with a certain clinical picture of poisoning, in which the observed deviations from the normal composition are the largest[22-29]. People, with long contact to lead, but without signs of toxicity only show minor changes of the serumprotein composition.

In chronic poisoning, the alterations are more pronounced according as the severity of the clinical symptoms aggravates.

The studies reveal, generally, a decrease of the A/G ratio of the serumproteins, as the most common deviation from normal. The albumin% is reduced and the globulin fractions are correspondingly increased. Especially, the α_2 and β-globulin contents are most markedly influenced, whereas the γ-globulin fraction fluctuates in a variable manner.

Similar alterations were observed by inducing lead intoxication into animals, both acutely and chronically[30, 31]. Several

investigations assume that serum electropheris might be a valuable technique in the detection of early stages of lead intoxication.

Shifts in the serum protein composition have been manifested only seldom in subjects, without clinical symptoms. Hence, it may be concluded that the method is not useful as a pretoxic test of increased lead absorption.

The basis for the interpretation of the observed dysproteinemia, provoked by the metal and its salts may be the affection of the liver. Lead, like other heavy metals is capable of disturbing the function of the liver and to bring on parenchymal damage. The involvement of the reticulo-endothelial system of the bone marrow may be regarded as an additional causative factor, however.

c. the urinary aminoacid pattern

Apart from the abundant excretion of ALA in the urine, lead may provoke an hyperexcretion of some aminoacids. Some authors reported the existence of an abnormal urinary aminoacid profile in case of accidental lead poisoning[32-34] - particularly in childhood - as well as in experimental poisoning. In these chromatographic studies, the strongly increased excretion of alanine and of glycine was predominant. It is generally accepted that this phenomenon is closely associated with the renal disturbances observed in lead poisoning. There is up to now not a consensus of opinion regarding the exact type of nephropathy produced by lead and its significance in chronic occupational exposure. As is evident from clearance-values the type of renal dysfunction depends more or less on the exposure duration and intensity. The injury is found frequently to be transitory and functional in character. Non-reversible kidney changes have only been observed in a few cases with prolonged and intensified contact with the metal.

Whenever the kidney function is impaired and particularly if the reabsorption of the proxional tubular cells is disturbed, the abnormal excretion of aminoacids may be a reflectance of this phenomenon. In view of the above findings, determination of

the urinary aminoacids may however not regarded as to be of diagnostic value in lead poisoning.

d. metal shifts in lead poisoning

Displacement of essential metals is considered as a general biological indicator of toxic response. Industrial exposure to various toxicants may result in characteristic shifts in mineral content of animal organs and blood, as has been evidenced for carbon disulfide, vanadium and beryllium. Similar phenomena have been encountered in subjects affected by lead poisoning or in workers being in long-term contact with the metal but without toxic manifestations otherwise. Besides the profound effect of lead on the iron metabolism, the observations include abnormally high serum zinc and copper quantities[37, 38]. The significance of these findings in relation to the development of clinical symptoms is still unexplained. Likewise, the role these changes play as possible prognostic indicators of immanent intoxication needs further research. The derangements regarding metal metabolism might be connected with the competitive action of lead in metalloenzymes, in which these cations are of importance. The strong elevation of copper in the red cells of lead patients may be furthermore brought into relation with the deficient hemoglobinization[39].

Dysregularities have been reported, in addition, concerning the metabolism of the alkalimetals calcium and phosphate[40-43]. It has been established that the serum levels of calcium and of potassium are elevated in animal poisoning, whereas that of sodium is depressed. These elevations paraleled the increase of the lead concentration in the blood, and might therefore be of value in estimating the degree of exposure. Further emphasis has to be placed upon the study of the cations in the blood of lead workers to investigate whether there exists some relationship between the blood-concentration of lead and the other metals. It is suggested that the elevation of calcium in the serum has to be attributed to the mobilizing action of lead on the calcium of the skeleton. The increase of plasmatic potassium is undoubtedly by the reflectance of the potassium-loss through the erythrocytary membrane, induced by excessive lead absorption.

e. urinary excretion of steroids

The functional condition of the adrenal cortex is known to be altered under the influence of small amounts of toxic chemicals, viz. lead. Measurement of the urinary values for 17-hydroxycorticoids and 17-ketosteroids is regarded a diagnostic tool in detecting adrenal dysfunction. Based upon this fact it may be the speculated, whether analysis of the urinary steroids in lead workers might provide reliable indication of the exposure degree[44]. So far, the data obtained refer solely to animal experiments. The influence on the adrenals appear to be dependent on stage and intensity of exposure. In the early stages decreased levels of urinary steroids were noted, whereas in case of heavy exposure and in advanced poisoning the excretion was augmented[45].

f. action on the iodine metabolism

A few reports on animals indicate that lead exerts a depressant effect on the thyroid gland, as measured by the rate of uptake of marked iodine (^{131}I) by the gland[46].

The lowered level for protein (hormonal) bound iodine (PBI) in blood, found in sheep following chronic lead poisoning is in agreement with the hypoactive state of the gland mentioned above[47]. Up to now, no data have been gathered on the iodine metabolism in subjects from lead-processing industries; in view of the early response toxic substances on the thyroid and other glands on toxic substances, evaluation of the iodine metabolism might constitute reliable means for the estimation of the early diagnosis of lead poisoning.

g. blood coagulation

Altered blood coagulation has been observed in a variety of industrial intoxications. A decreased clotting tendency results from the protracted influence of lead[48], which according to Saita[49] has to be attributed to deficiency of one of the thromboplastic factors (factor VII) as well as to a slowing down of the prothrombin synthesis in the liver itself. These phenomena are

likely to be explained by assuming an impairment of the synthetic potency of the liver. The thromboelastographic technique allows the possibility of differentiating between the various modes of action, according to which toxicants influences the clotting mechanism, and affords a diagnostic means to detect slight changes of the coagulation.

h. action of lead on active chemical groups

It has been stressed upon that lead acts on several functional groups. The affinity of lead for the sulfhydrylgroup (-SH) constitutes without doubt an essential feature of certain enzyme inhibitions produced by the metal. Consequently, various metabolic processes can be disturbed in lead intoxication. The body, on the other hand, possess a natural defence mechanism to overcome these biochemical lesions. This is provided by the presence of free thiol groups in the blood and tissues (glutathione). Both in vitro and in vivo experiments, it has been ascertained that lead provokes a lowering of the glutathione content of erythrocytes[50,51].

The total amount of free thiol groups, appeared to be reduced in the sera of group of workers, even without clinical symptoms[52]. It is suggested therefore that determination of glutathione and/or the content of free-SH-groups could have some value as a diagnostic pretoxic tests in the medical prevention of lead poisoning.

Likewise, recourse can be made to the estimation of the concentration of the blood α-aminonitrogen content, which may be indicative of the affinity of lead to vulnerable chemical groups[53].

Related to the action of lead on thiol groups, is the appearance of Heine bodies, whose formation is due to the oxidation of thiol groups, which are bound to the globin moiety of the hemoglobin molecule. According to Ghelberg et al[54], children living in the neighbourhood of lead-processing industries presented a higher proportion of Heinz bodies in their blood than children, inhabiting rural environments. It is of importance to investigate, whether this is also the case in industrial lead workers.

i. immunologic response

There is much evidence that toxic substances have adverse influences on the available immunity mechanisms of the body. Agents appear to act by rendering the organism more susceptible to infections. The resistance power will be diminished. The speculation therefore arises whether occupationally exposed workers are more vulnerable to common infections than subjects not exposed to poisonous substances, but clear evidence to prove this suggestion is still absent. The influence on the immunitary defence may be an important aspect of the assessment of toxicity.

In a series of papers Fonzi et al reported the noxious effect of lead upon the defensive power of the animal body[55-58]. These studies revealed that lead-treated animals, subsequently subjected to active immunization, showed lower antibody globulin contents (γ-fraction) than unexposed animals. The normal "complement" of the blood serum, residing in the globulin fractions appeared to be progressively reduced, whereas the amount of anti-typhoid antibodies in the serum of lead-affected animals showed a relative deficiency after specific vaccination, as compared with normal animals (rabbits). There is every likelihood that lead interferes with either the synthesis of some antibody proteins or alternatively impairs the functioning of these proteins by formation of inactive metallo-protein complexes, thereby affecting the antibody protein balance in the blood. The problem arises whether this may precede the development of toxic manifestations or coincides with a later stage of the poisoning. One of the elements in the defense mechanism is lysozyme. This is a polypeptide, capable to bring on lyses of certain bacterial strains and present in body fluids and organs. Lysozyme activity appeared to be progressively reduced in the organs (spleen) and serum of dogs, chronically poisoned with lead salts[59]. The mean serum lysozyme activity was below normal in a group of workers, showing obvious signs of lead poisoning; the lowest values were obtained in the severest affected cases.

So far, none data on the altered immunologic response has been obtained in people working in non-hazardous circumstances of industrial work.

The phenomena above outlined may be brought into relation with the behaviour of the vitamin C level of the adrenal glands in case of human lead absorption. It is known that the metal exerts a depriving effect on the vitamin C content of this organ[60]. Furthermore loss of vitamin C from the cortex is associated with increased viability of the body against infection.

j. blood mucopolysaccharides

These substances occur in the tissues as prosthetic groups of conjugated proteins (muco- or glycoproteins). In the plasma they form a part of the α_1 and α_2-globulin fractions. The sialic acids, compounds derived from neuraminic acid, are also important constituents of the polysaccharide moiety of these mucoproteins (serum mucoids). In clinical medicine, some value has been ascribed to the determination of the sialic acids in respect to the diagnosis and early detection of several diseases (acute rheumatic fever). As constitutive part of the glycoproteins, it is increased in various inflammatory processes and also in silicosis. Serum mucoid and sialic acid determinations extended to the field of lead toxicology gave as result that these contents were below normal in 124 patients with slight to moderate poisonous symptoms[61]. Sialic acid in blood serum was also lowered in dogs with experimental poisoning. Serum neuraminic acid was shown to be consistently decreased both in lead-treated rats and in workers, even in the absence of apparent toxic signs. From the latter observation, it is suggested that the assay of the acid may be a poisoning index of exposure degree[62]. Further research is necessary to reveal possible correlations with other blood parameters, indicative for exposure.

k. abnormal hemoglobins

The presence of abnormal fetal type of hemoglobin, denoted as HbF, could be demonstrated in advanced stages of lead poisoning. Non-elevated levels or contents slightly above those of unexposed individuals were encountered in cases of mild poisoning or heavy exposure[63, 64]. From the data, it is concluded that HbF production is a biochemical event, which does not precede pathologic

signs of lead toxicity. In experiments on animals, it was found that there exists a distinct correlation between the HbF level and both the degree of basophilia or the erythrocyte count[63]. This suggests that the HbF content, as determined by electrophoresis, might have some significance in evaluating the degree of poisoning.

1. 5-Hydroxyindole acetic acid (5-HIAA)

The level of this compound is shown to be in elevated concentrations in the urine in 227 children, inhabiting an air-polluted area in the vicinity of lead-processing industries[65]. Ghelberg et al ascertained that there exists a well-defined relationship between the proportion of cases with increased 5-HIAA-excretion and the mean concentration of lead in the ambient air. So far, no reports have appeared on the excretory output of the compound in lead workers. The finding above mentioned, infers that the assay of 5-HIAA might be a useful index of exposure degree and intensity . In view of the fact that 5-HIAA is the final metabolite of a minor pathway of the tryptophan breakdown, it is suggested that lead interferes in some manner with the enzymatic degradation of tryptophan. It has been put forward that the association between vitamin B_6 (pyridoxal phosphate) deficiency and lead poisoning lies at the basis of the impaired tryptophan metabolism. Pyridoxal phosphate functions as a coenzyme for enzymatic conversions in the major pathway of the catabolism of tryptophan. The state of vitamine B_6-deficiency, evoked by lead, should lead to a suppression of this main pathway - due to enzymatic inhibition - which in turn favors the conversion of tryptophan along the minor path leading to 5-HIAA. Some evidence is presented that lead actually operates in this way as is evident from the increased excretion of xanthurenic acid[66]. Vitamin B_6 administration resulted in a drop of the urinary output of this metabolite. A systematic analysis of the various intermediates and excretory products involved in the tryptophan metabolism (5-HIAA, kynurenic acid, xanthurenic acid, nicotinic acid) following lead intake, however, seems to be necessary to investigate this action more profoundly.

m. effect of lead on nicotinic acid

Caccuri[67] drew attention to a distinct relationship between lead poisoning and the metabolism of nicotinic acid. Lead poisoned animals exhibit a pronounced decrease of nicotinic acid in blood and urine. This fact, together with the observations made after the administration of the vitamin following lead salt intake, has forced these authors to assume that in metal poisoning utilization of nicotinic acid is greatly increased with consequently a reduction of coenzymes I and II (NAD, NADP)[68]. It is suggested that lead severely affects the pyridine nucleotides, either by blocking the synthesis of these substances or by enhancing the degradation of nicotinic acid. Another manifestation of this action is the altered nucleic acid content (RNA, DNA) of leucocytes, neutrophils and monocytes detected in patients with plumbism. Nunziante Cesaro and Granata[69] evaluated the concentration of these substances quantitatively by means of an ingenous cytophotometrical method in blood smears, and claimed that the method should be practically applied as a test to assess the severity of lead poisoning.

The interference of lead in vivo with the nucleotide metabolism and the disorders on porphyrin level evoked by the metal, seem to be related in some way. Actually, blood and urine nicotinic acid decrease, at the same time as CP III is steadily increasing. Prophylactic administration of nicotinic acid or the amide prevent the development of porphyrinuria, induced by lead intake. The anemic signs of lead intoxication could be partly reversed by administration of inosine, AMP, constitutive parts of the nucleotides[70, 71, 72]. These substances also maintain the urinary output of lead, 5-ALA and CP within the normal range. Apart from the interesting objective to elucidate more extensively the toxic mode of action on necleotide-level, studies should be focused on the possible interrelationship between nicotinic acid levels in blood and urine and the other body fluid indices, like CP, 5-ALA a.s.o., in case of non-hazardous lead absorption.

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SUMMARY

Observations of the effects of lead in living matter have effectively manifested a variety of hematological and biochemical parameters. A summary of these effects is presented in the table below.

Observed indices	Response to lead
URINE	
lead	+ +
coproporphyrine (type I and III)	+ +
5-aminolevulinic acid	+ +
aminoacetone	+ +
alanine	+
glycine	+
steroids	- -, and + in advanced poisoning
5-hydroxyindoleacetic acid	+ +
tryptophan metabolites	(?)
nicotinic acid	-
iron	+ +
BLOOD (whole blood or erythrocytes)	
lead	+ +
hemoglobine	-
hemoglobine F	+
red blood cell count	-
Heinz bodies	+ +
free protoporphyrine IX (ery's)	+ +
number reticulocytes	+ +
basophilic count	+ +
siderocytes	+ +
osmotic resistance (red cells)	+
survival time (red cells)	-
uptake of radiophosphorous (red cell membrane)	-
glucose consumption	+ +
$(Na^+ + K^+)$ ATP-ase	- -
catalase	-
carbonic enhydrase	-

Observed indices	Response to lead
5-aminolevulinic dehydratase	- -
hemesynthetase	-
UPG-decarboxylase	(-)
nucleic acids (DNA, RNA) in leucocytes, neutrophils and monocytes	+ and - alterations
pyridine-nucleitides (NAD, NADP) (erythrocytes)	-
free thiol groups	- -
glutathione	(- -)
nicotinic acid	-
K^+-loss (ery's)	+ +
copper (ery's)	+
SERUM or PLASMA	
5-aminolevulinic acid	+ +
aminoacetone	(+) or (+ +)
zinc	+
copper	+
calcium	+
potassium	+ +
iron	+
iron-binding capacity	-
serum proteins (Alb/Glob ratio)	-
α_2 and β globuline	+
γ-globuline	$\pm$
α-aminonitrogen	- or - -
prothrombine	-
thromboplastic factors (VII)	-
complement	-
antibody globuline (γ) after immunization	-
antityphoid antibodies	-
lysozyme	-
neuraminic acid	- -
seromucoid (mucopoly-saccharides)	(-)
sialic acid	-
protein bound iodine (PB I)	-

Observed indices	Response to lead
transaminases (SGOT, SGPT)	+
aldolase	+ +
alkaline phosphotase	-
pseudo cholinesterase	-

+	increase or intensification
-	decrease in or retardation
(+) or (-)	response not quite certain
+ + or - -	early response, preceding the appearance of clinical signs of toxicity