POTENTIAL ESSENTIALITY OF LEAD

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Toxicity cannot be used as an argument against the possibility that an element may be essential. An attempt to determine whether or not lead is essential is therefore being made in our laboratory, using a technique which has been successful in several other cases (tin, vanadium, fluorine, silicon). It consists of the application of ultraclean, more or less trace-element sterile (but not bacteriologically sterile) isolators, and highly purified amino acid diets. All compounds tested are added to the diet. Young rats maintained in the trace-element isolator system develop deficiencies when unidentified essential trace elements are missing from the diet, the most important symptom being lack of growth. Other signs are shagginess of the fur, seborrhea, lack of incisor pigmentation, etc. In order to work with lead, an air filter system has been developed which removes not only dust particles, but also aerosols and substances present as vapors.

The basal diet contains 53 individual components (21 amino acids, 13 vitamins, sucrose, 2 fats, 3 salts and 13 trace element compounds). It was difficult to obtain all of these in lead-free form and monitor their lead content. Special problems were encountered with calcium phosphate, certain amino acids and the fats. The basal diet contains approximately 0.2 ppm of the element.

In 13 successive experiments, carried out in 1972/73, growth responses were seen when 1.0—2.5 ppm of lead in form of lead subacetate was added to this ration. The growth effects were statistically highly significant, even though they amounted on the average to an increase of less than 20%. Not only lead subacetate but also lead oxide and lead nitrate produced this response. In extended trials aimed at amplifying these initial data we were at first unable to repeat the results. Screening of possible sources of lead in the system showed that extreme caution was indicated. The lead content of plastic bags, used for storing diets, varied greatly, and a labeling tape used for numbering of diets and animal cages contained 0.56% (56000 ppm) of the element. Elimination of these potential sources of lead, and continuous monitoring of all plastic components was necessary for the demonstration of the growth

promoting effect. Our data now indicate that the tentative lead requirement of the rat under these experimental conditions lies at approximately 1 ppm of the diet, an amount that is readily supplied by normal foodstuffs.

GENERAL CONSIDERATIONS

It is the purpose of this paper to present some evidence for the potential role of lead as an essential element. Over the past two decades, research on essential trace elements has entered a new era, beginning with the identification of cobalt as an integral component of vitamin B_{12}, and with the discovery of selenium as an essential trace element. (1) Selenium is required at levels which range between 7 to 50 parts per billion. It prevents severe, usually fatal, deficiency diseases, such as muscular dystrophy, heart muscle degeneration, liver necrosis, etc. It was primarily the discovery of selenium that opened up a new phase in nutrition research, i. e., in the investigation of new essential dietary agents. While attempts to identify new amino acids or new vitamins have become areas of greatly diminishing returns, the discovery of new essential trace elements is currently progressing at a rapid rate. The main *organic* components which are indispensable in the diet and are needed for the maintenance of normal functions, growth, and health are identified and available today. Indeed, it is exactly this circumstance that makes it possible to study new, hitherto unrecognized *inorganic* trace requirements. Nutrition research is inevitably driven in this direction.

Until 1957, only 7 elements were known as essential trace elements for the mammalian organism. These were iron, iodine, copper, zinc, manganese, cobalt and molybdenum. To these seven, seven more have been added over the past years as indispensable in more or less small amounts. The 7 new elements are: selenium, chromium (2), tin (3), vanadium (4, 5), fluorine (6), silicon (7, 8), and nickel (9). Six of these were discovered in my laboratory, as reviewed elsewhere (10, 11).

Some interesting observations emerge if one studies the distribution of elements which are essential for warm-blooded animals with reference to their position in the periodic system (Fig. 1). In this illustration, the essential trace elements are categorized by individual boxes. In contrast to most of those elements which are essential in trace amounts, the main components of biological systems, i. e., the 11 atoms which constitute the bulk of living matter, are all of very law atomic weight. They belong to the smallest 20 elements in the periodic system. Slightly above this range, in the first series of transition elements; we have a sequence of eight individual elements, from vanadium to zinc, which are all identified as essential. Each one has peculiar and specific functions in which it cannot be replaced by any of the others. A high degree of specificity prevails even though *in vitro*, especially with respect to bivalent cations, replacement reactions can occur and indeed are the order of the day.

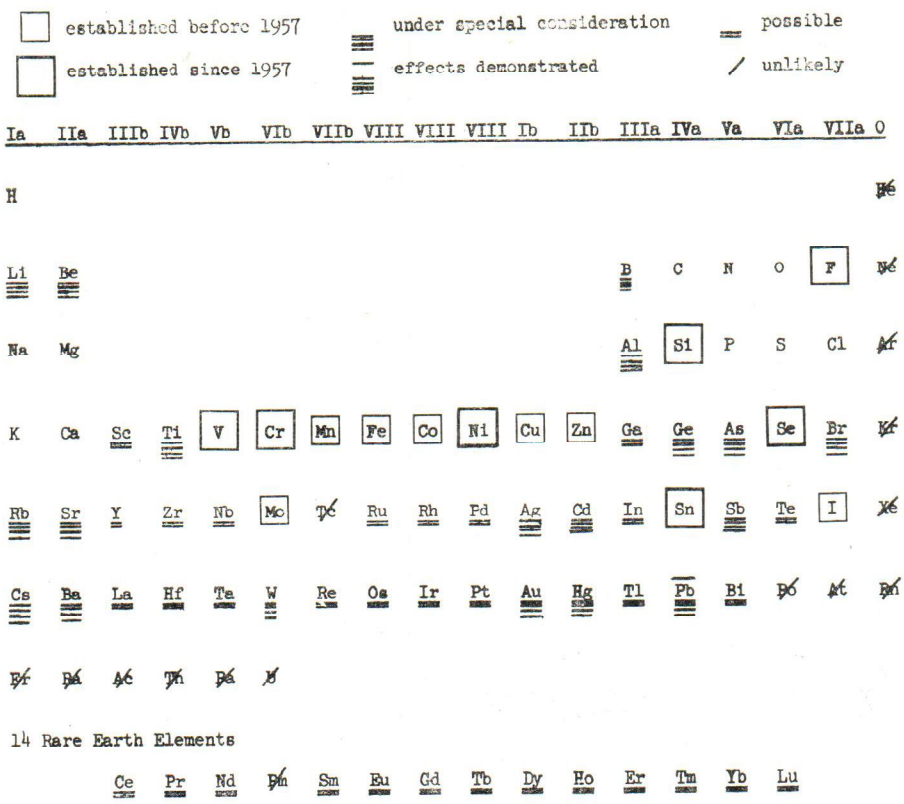

Fig. 1. *Periodic system distribution of trace elements of known and potential importance for warm-blooded animals. Status as of 1973*

If one compares the position of lead in the periodic table to that of other elements recognized as indispensable, it is seen that lead, with respect to its atomic weight, is outside of the area in which essential trace elements have been identified thus far. It is said that until now, none of the elements beyond iodine, with an atomic number of 53, has been shown to be of physiological importance to bacteria, protozoa, animals or man. Even though this statement is not entirely accurate, it would be an important development indeed if one could demonstrate that lead is needed.

By way of definition, an essential trace element is an element present in small amounts whereby the question what establishes a »trace« is a matter of convention. Not all elements which we find in tissues or living organisms are necessarily essential. They can be present as accidental contaminants. The essential trace elements are different from

those which are present by coincidence in that they seem to be present in the organism by design: they are usually incorporated in highly specific molecular structure and fulfill special, indispensable functions; they have been called the inorganic vitamins. Indeed, in this respect they are quite analogous to the vitamins with the possible exception of silicon which has been shown recently to fulfill structural tasks as a crosslinking agent in connective tissues (12).

Lead fulfills most of the postulates which can be used to characterize potentially essential trace elements.* It is present in the biosphere and is normally found in the organism and in tissues; it is present in egg, milk, and in the newborn, the latter indicating placental transfer; there may be a mechanism to maintain constant levels of lead in blood plasma, but not the erythrocytes; the element is present at »physiological levels« in normal diets; indeed it is ubiquitous in almost all natural materials of the plant and animal kingdom. The obvious question whether or not lead is essential is therefore an important one.

In discussing lead, the problem of its potential toxicity invariably arises. However, we must be aware of the fact that toxicity does not mitigate against potential essentiality. There is no functional relation whatsoever between toxicity, on the one hand, and essential biological function, on the other hand. Trace elements which are toxic at relatively small dose levels may be highly essential and indispensable for normal body function at even lower, physiological amounts. Thus, most of the essential trace elements are toxic when given in excess. Obvious example are iron, iodine, copper, and especially also selenium.

As a matter of fact, in biology and medicine selenium was *only* known for its toxicity until I discovered in 1957 that it is essential in very small amounts. Today we know fatal, selenium responsive deficiency diseases in over 20 species, including not only laboratory animals but sheep, goats cattle, horses, monkeys, chickens and turkeys. Yet, shortly before its physiological role was uncovered, *Trelease* and *Beath* stated in the introduction to an excellent book on the geological occurrence and biological effects of selenium, » . . . as far as we know, it is toxic but never beneficial to animals or man« (16). It could well be that we are in a similar quandry today with lead.

If one investigates the relation between requirements and toxicity, trace elements usually have the same »therapeutic index«, the same ratio between effective dose level and toxic dose level as other dietary ingredients, for instance table salt, magnesium, calcium, amino acids, etc. This point is illustrated in Fig 2, which shows the areas in which selenium, as well as potassium, are compatible with life of a warm-blooded animal, the rat. The bracket for iron is also shown. A logarithmic scale going from one atom to 10^{24} atoms (the number of atoms in a gram equivalent) is used. It is seen that for each of these elements there exists a rather

* Reviews are found elsewhere, for example in (13—15)

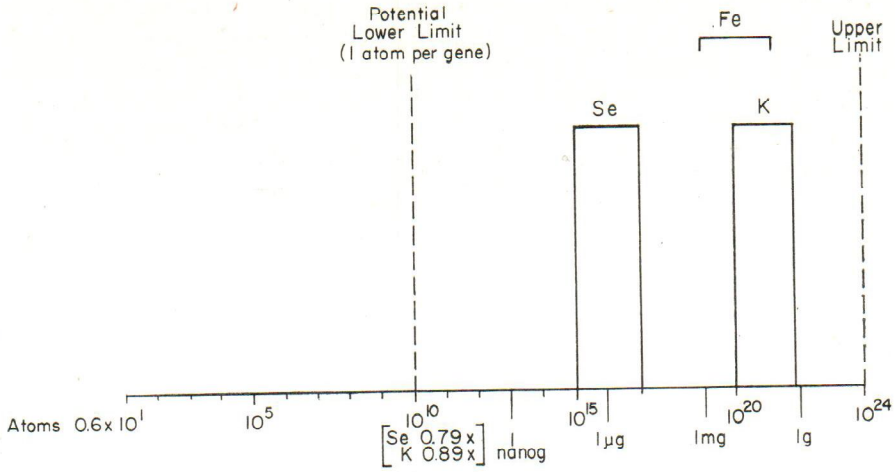

Fig. 2. *»Concentration windows« for selenium and potassium in which life is possible. Lower limit: requirement for normal functions; upper limit: toxic level. The bracket for iron is also marked*

small area, a narrow »concentration window«, in which life is possible. If the number of atoms made available to the organism lies below this bracket, a deficiency develops and life cannot proceed normally. Neither can life exist if the supply goes beyond the bracket and reaches toxic levels.

It is remarkable that we can prevail only in these rather small, confined areas of supply. This is true for all elements which are needed in our existence. It is evident from the illustration that the window for selenium is actually larger than that for potassium. Incidentally, it is also larger than the window for many of the amino acids and some of the vitamins. To illustrate the dimensions with which we are dealing, let me point out that a daily intake of 0.1 μg of selenium, consumed in 5 to 10 g of diet by a 100 g rat, is so small that we may not be able to determine it accurately even with the best available analytical techniques. We should realize, however, that 0.1 μg constitutes no less than one thousand trillion (10^{15}) atoms of selenium. If we reduce the supply to 500 trillion atoms per day per rat, half of the animals would die from liver necrosis within thirty days. The amount of selenium in liver is such that there are approximately one million atoms of selenium per liver cell. While this number may appear large to some, it is a miniscule amount considering the size of the cell relative to that of the selenium atom.

With respect to lead, we are within approximately the same area of concentration. The window — if there is a true lead requirement — would possibly be located a little to the right of that for selenium, since lead is less toxic than the latter.

If one looks at the various trace elements known to be essential, it is obvious that the problem of progressive accumulation plays a role in the physiology of some, and not in that of certain others. For some elements, the organism has elaborate mechanism preventing the accumulation of excesses in tissues. An element, for example, which does not seem to be stored is zinc. For other elements, the body has storage areas. For fluoride, the bones are storage organs. For copper and iron, liver and spleen play that role. Selenium is stored in several parenchymal organs, and iodine is stored in the thyroid. The fact, therefore, that the organism does progressively accumulate lead in bones, bone marrow and erythrocytes cannot be used as a counter argument against its potential essentiality.

EXPERIMENTAL TECHNIQUES

In our studies we applied an experimental approach which permits definite experimentation on the essentiality of specific elements. This approach was developed between 1961 and 1965 in our laboratory, following the discoveries of the roles of selenium and chromium in nutrition. Principally, the method consists of a combination of ultra-clean room and isolator techniques, on one hand, and the use of highly purified amino acid diets, on the other hand (10, 11).

The trace-element-free isolator system (Fig. 3) contains the following basic elements; isolator, airlock, blower, air-filter assembly, cage assembly, refuse trays, food cups, water bottles and scales. Plastics are used for all components; there is no metal, glass or rubber. The unit has six working sleeves and a vinyl zipper over the entire length of the top, an airlock to facilitate passage of articles in and out of the trace-element sterile environment, and air filters which remove almost all dust down to a particle size of 0.35 μm. In order to work with lead, a new filtering system has been developed which removes not only dust particles from the air but also aerosols and substances which are present as vapors (Figure 4). The unit consists of a carbon cartridge followed by a fine Millipore filter and a pump.*

For animals inside the isolator, the diet is the main source of trace-element contamination. Complete chemically-defined diets were developed; they are based on amino acids in place of protein. All ingredients are screened for trace-element contaminants. The standards of purity required far exceed those for the normal, »analytically pure« chemicals.

In speaking about trace-element deficiencies and dimensions, purity is really a relative term. One cannot categorically exclude an element from being essential except by stating that one has shown it to be not essential at a certain, accurately determined level. Thus, progress in this

* We are indebted to Mr. Manuel Brandt of the Ethyl Corporation, Detroit, for assistance in the collection of air samples and lead analyses in the development of this system.

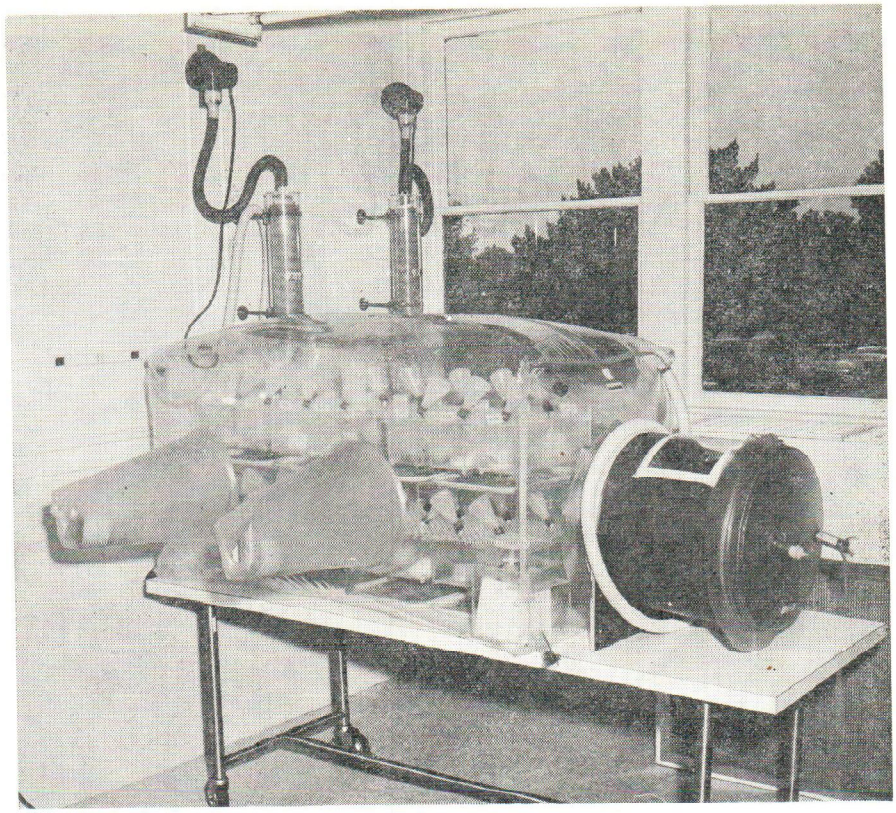

Fig. 3. *Trace-element controlled isolator*

field is singularly dependent on the sensitivity and accuracy of analytical methods which are applied to a specific element in question. Since very small, residual lead levels had to be determined in dietary ingredients with low lead contents, highly sensitive methods were needed for the current studies. In our laboratory, analyses were carried out using an anodic stripping analyzer after ashing with perchloric and nitric acid. Additional determinations were carried out elsewere by atomic absorption.

Animals used for these experiments were subjected to a carefully controlled weaning ritual which made it difficult for them to accumulate trace elements from stock diets or other sources of lead prior to the beginning of the experiment. Inbred, littermate, male, weanling Fischer

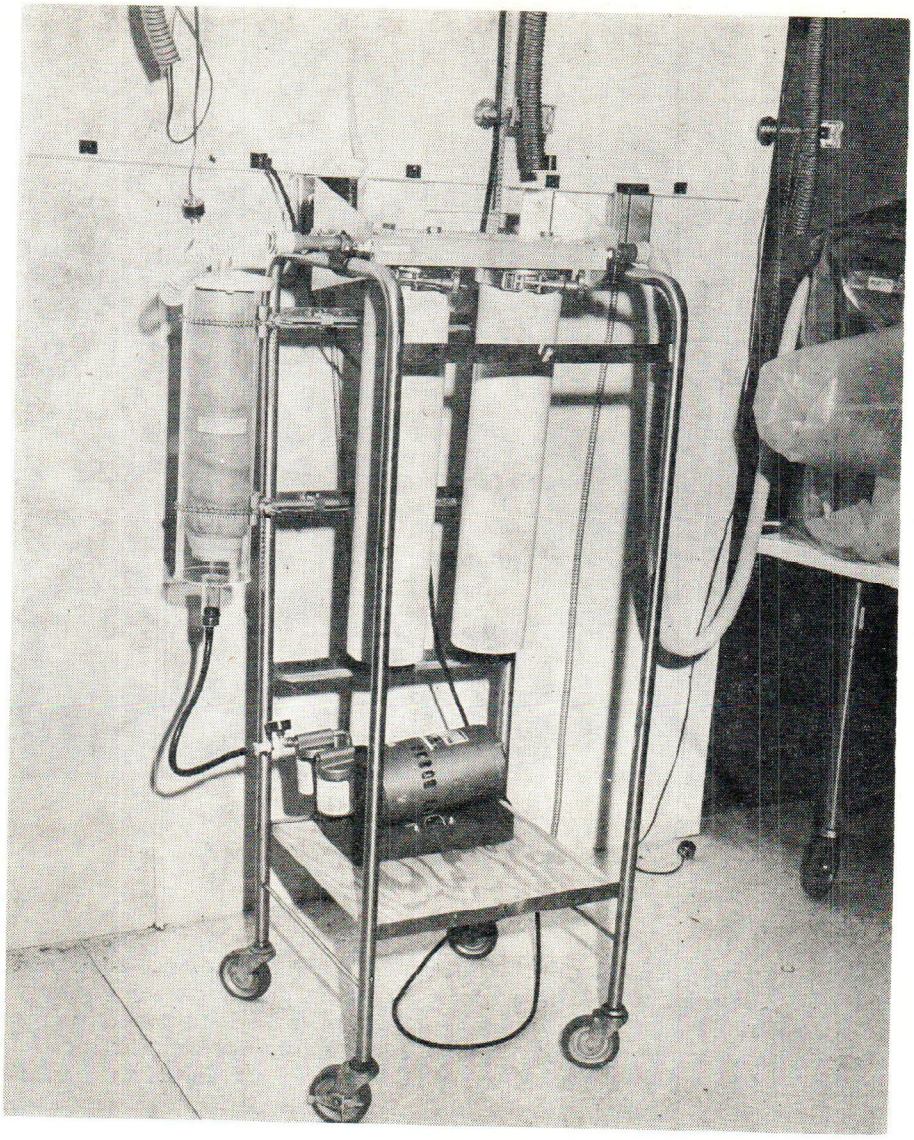

Fig. 4. *Airfilter for lead experimentation. Description: see text*

344 rats were used.* Each experiment consisted of four or five groups of six to eight animals. As an additional control to each group in the isolator, six rats were maintained in metal cages under conventional conditions, on each of the test diets. The animals were weighed and evaluated as to their appearance at 3- to 4-day intervals. Growth rates and standard errors of the means were computed by covariance analysis of the weight gains. The experiments were terminated after 28 to 32 days, at which time the rats were autopsied.

The trace-element-deficient amino acid diet which has been developed in our laboratory continuously since 1960 was changed in several important respects in order to make it possible to carry out experiments on lead. We eliminated the use of lactose as a suspending agent for vitamins and trace elements since different lots of purified lactose contained sizable levels of lead. The most important source of lead contamination in the diet was the calcium phosphate. A method was devised to produce calcium dihydrogen orthophosphate monohydrate, $CaHPO_4 . 2H_2O$ from ingredients pre-extracted with dithiodicarbamate. This procedure eliminated much of the lead contamination. Sodium and potassium were added primarily as monosodium and monopotassium glutamates. The calcium phosphate was partly supplied as part of the salt mixture and also served as the suspending agent for the vitamin supplement.

The composition of the diet is listed in Table 1. The ration contains 53 individual components (21 amino acids, 13 vitamins, sucrose, 2 fats, 3 salts and 13 trace element compounds). The main ingredients were purchased in relatively large amounts from industrial sources. The difficulties involved in obtaining all these ingredients in lead-free form and monitoring their lead content can be easily imagined (see below). Not only the salts but certain of the amino acids,** the sugar and the fats were sources of lead contamination, even though almost all of them were perfectly satisfactory with respect to lead contents if normal dietary regulations were considered. The basal diet which we could compound in this fashion contained approximately 0.2 ppm of lead.

RESULTS

In a series of successive experiments carried out in 1972 and 1973, small but consistent growth effects were observed with lead subacetate (Table 2). Dose levels ranging from 0.5 to 7.5 ppm of lead were tried. The

* Charles River Breeding Laboratories, Wilmington, Massachusetts. To prevent intake of trace elements from the mother's feed, chow pellets were removed from the nursing mother on the 17th day postpartum. The young were separated from the mother and shipped to the laboratory on the 19th day. They were kept in plastic cages on the basal amino acid diet for one day before initiation of the experiment in the isolator.

** The cooperation of Aljinomoto Company, Inc. in securing batches of amino acids with low levels of trace elements is greatly appreciated.

Table 1

Composition of basal diet for lead studies

	%
Amino Acid Mixture S-11[1]	18.76
Purified Sucrose[2]	62.75
Stripped Lard[3]	10.00
Purified Oil[4]	5.00
Salt Mixture # 10[5]	2.39
Trace Supplements # 18 and # 3Y[6, 7]	0.10
Vitamin Mixture # 228[8, 9]	1.00
	100.00

[1] Composition (g/100 g of diet; all L-amino acids except for glycine): arginine·HCl, 1.3; histidine · HCl H_2O, 0.7; isoleucine, 1.0; leucine, 1.5; lysine · HCl, 1.6; methionine, 0.8; phenylalanine, 1.0; tyrosine, 0.6; threonine, 0.8; tryptophan, 0.3; valine, 1.0; alanine, 0.4; aspartic acid, 0.3; cystine, 0.3; glutamic acid, 1.65; Na-glutamate · H_2O, 1.11; K-glutamate · H_2O, 2.3; glycine, 0.5; proline, 0.5; serine, 0.5; asparagine, 0.6.

[2] J. T. Baker lot # 408188.

[3] Canned stripped lard (Distillation Prod. Indus., Rochester, NY).

[4] Prepared by Hunt-Wesson Foods, Inc., Fullerton, CA.

[5] Composition (g/100 g of diet): $CaHPO_4 \cdot 2H_2O$, 1.875; K_2HPO_4, 0.2375; $MgSO_4$, 0.2420; $MnSO_4 \cdot H_2O$, 0.01315; $ZnSO_4 \cdot 7H_2O$, 0.01570; KI, 0.000538.

[6] Composition Trace Supplement # 18 (g/100 g of mixture): $FeSO_4 \cdot 7H_2O$, 49.80; $CuSO_4 \cdot 5H_2O$, 2.36; $MnSO_4 \cdot H_2O$, 6.16; $CrCl_3 \cdot H_2O$, 2.56; $CoSO_4 \cdot 7H_2O$, 2.385; $MoO_3 \cdot H_2O$, 0.342; $CaHPO_4 \cdot 2H_2O$, 36.393 (as a filler).

Composition Trace Supplement # 3Y (mg/100 g of diet): $K_2SnO_3 \cdot 3H_2O$, 0.5004; Na_3VO_4, 0.1805; $KF_2 \cdot H_2O$, 1.240.

[7] Selenium was added as sodium selenite (15 μg Se/100 g of diet) dissolved in 1 ml water. Silicon was added as sodium metasilicate (0.05 g Si/100 g of diet) suspended in 0.5 ml water.

[8] Composition (mg/100 g of diet): $CaHPO_4 \cdot 2H_2O$, 797.0; choline bitartarate, 179.0; thiamine · HCl, 0.40; riboflavin, 0.25; pyxidoxine · HCl, 0.20; Ca D-pantothenate, 2.0; niacin, 10.0; menadione (2-methyl-1,4-naphthoquinone), 0.10; folic acid, 0.20, biotin, 0.10; vitamin B_{12} (0.1% in mannitol), 10.0.

[9] The following vitamins were added in 1 ml absolute ethanol, in mg/100 g of diet: vitamin A acetate 2.00, vitamin D, 0.002; d-α-tocopheryl acetate, 10.0.

results obtained with 1 and 2.5 ppm of lead were statistically highly significant ($p < 0.005$), while 0.5 ppm of lead did not seem to affect the growth rate. Supplements of 5.0 and 7.5 ppm, tested in only one case each, produced small increases, but more extensive tests would have been required to ascertain their statistical validity. Even though the average growth response amounted to less than 20%, increase in the growth of up to 34% were seen in individual experiments. Lead nitrate (Table 3) and lead oxide were also effective. A preliminary report on these findings was presented in 1973 at the International Symposium on Trace Element Metabolism in Animals (TEMA 2 *(11)*).

Table 2

Growth effect of lead subacetate in ultraclean environment system (Preliminary summary, June 1973)

Dose level (ppm)	No. of expts.	No. of animals	Growth (g/day)		Growth effect (% increase)	Significance (p value)
			Control	Supplemented		
0.5	2	12	1.72 ± 0.09	1.72 ± 0.10	0	
1.0	3	23	1.79 ± 0.05	2.08 ± 0.05	16.2	<.005
2.5	8	49	1.85 ± 0.04	2.04 ± 0.04	10.3	<.005
5.0	1	7	1.53 ± 0.08	1.61 ± 0.10	5.2	n. s.
7.5	1	8	2.11 ± 0.12	2.22 ± 0.09	5.2	n. s.

Table 3

Growth effect of dietary lead on rats maintained in a trace element controlled environment

	Lead level (ppm)	Av. daily weight gain	% Increase over controls	P value
Controls		1.69 ± 0.13		
Lead subacetate	0.5	1.69 ± 0.14	0	—
	2.5	2.20 ± 0.14	30.2	<.025
Lead nitrate	0.5	1.99 ± 0.09	17.7	<.05
	2.5	1.99 ± 0.08	17.7	<.05

Following these initial, positive results, we have gone through a period during which we were unable to repeat our own experimentation. This was obviously related to changes in the lead contents of individual dietary components, new supplies of which had to be acquired from time to time. It also seemed to be related to possible lead contamination arising from the plastic components used in the isolator system. The quality of certain amino acids, fats, and also salts varied greatly from lot to lot. Examples are presented in Table 4. Lactose is included since it was a component of earlier diets used in preliminary trials. The increase in lead levels of amino acids between 1971 and 1973 appeared to be related to a sizable increase in production, necessitated by ever increasing demands for crystalline amino acids. To my knowledge, these substances are produced almost exclusively in Japan, with the exception

of glutamic acid, alanine and glycine. We have initiated the development of techniques to purify the amino acid mixture further by extraction with complexing agents.

Table 4

Lead contents of dietary components

	ppm
Alanine — 1971	n. d.
Alanine — 1973	4.6
Serine — 1971	1.1
Serine — 1973	8.8
Tryptophane — 1971	0.1
Tryptophane — 1973	8.6
Arginine HCl — 1974, Lot 59320	14.0
Arginine HCl — 1974, Lot 59330	13.0
Arginine HCl — 1975, Lot 59182	2.0
Arginine HCl — 1975, Lot 59202	0.2
Isoleucine — 1974, Lot 19790	<0.05
Isoleucine — 1974, Lot 19800	<0.03
Lysine — 1974, Lot 29674	4.6
Lysine — 1974, Lot 29664	10.0
Serine — 1974, Lot 19120	0.29
Serine — 1974, Lot 19150	1.0
$CaHPO_4$, source B, »pure, analyzed«	0.76
$Ca(H_2PO_4)\,H_2O$, source M, »analytical«	0.19
$CaHPO_4 \cdot 2H_2O$ prepared in laboratory	0.03—0.08
Lactose — Lot YDV	1.3
Lactose — Lot W2×6	19.0
Laboratory chow	1.0

From the data presented here, it is quite obvious that a systems approach is required, i.e., that dietary ingredients and all other aspects of the experimental procedure need to be continuously monitored for lead contamination. This includes the plastic items used in the isolator system. Table 5 presents values for lead, and also cadmium and titanium, obtained by semiquantitative emission spectrographic analysis of plastic components which were used in the earlier phase of our experimentation. We are under the impression that trace elements, especially metals which are present in plastic materials, are bound so tightly to the carbon matrix of the plastic that they may not be biologically available. This does not include, of course, layers of metal ions which may be attached to the surface of the plastic. We apply a very rigorous cleaning

Table 5

Approximate amounts of lead, cadmium and titanium in plastic ware

	Pb (ppm)	Cd (ppm)	Ti (ppm)
Plexiglass cages	3.3	0.3	0.2
Nalgene food cups	2.8	1.5	15.0
Polypropylene water bottles	14.0	0.6	0.6
Nalgene water spouts	7.1	6.8	2.5
Plastic stoppers	29.0	0	0
Tygon tubing	2.8	2.2	2.5
Yellow vinyl labeling tape	5,400	0	17,000
White labeling tape	16.0	0	10,000

ritual in our experimentation. In order to work with lead, components which contain higher amounts of the element have, of course, been replaced by other cleaner plastic materials. The water bottle, for example, including the stopper and the spout, is now made of nalgene which is much lower in lead content than the previously used materials. The pitfalls which exist in this kind of experiment are exemplified by the very high lead content of the labeling tape which was used in our experiments to put numbers on diets and cages and also to tape gloves and other attachments to the isolator. The yellow tape obviously contains lead chromate (chrome yellow) as a dye. The high amount of lead in some of the plastics may arise from lead phosphate which is applied as a stabilizer.

Rigid elimination of the sundry potential sources of lead contamination has now brought us to the point where we again can produce positive growth responses by addition of lead to the diet (Table 6). It is hoped that this situation will allow us to determine the exact level of

Table 6

Effects of lead on growth
Experiment 311, March 1975
(6 rats per group)

	Average daily weight gain	Difference
	g	%
Controls	1.31 ± 0.12	
Lead supplemented (lead subacetate, 1 ppm Pb)	1.65 ± 0.12	+26

the lead requirement. Rather extensive studies, however, with series of dose levels of a number of lead compounds need to be carried out before we will have reached that goal.

It is often overlooked that a high degree of chemical specificity prevails in biological effects of any trace element. Not only in organic biochemistry but also in bioinorganic chemistry are some compounds much more effective than others. Not all selenium compounds, for instance, are protective against selenium responsive diseases; not all chromium compounds can be utilized as glucose tolerance factor; not all fluoride compounds are effective in the growth response test under our conditions, or in the prevention of caries. We are making the observation over and over again that different derivatives of an element differ greatly in their biological utilizability. The same must be expected for lead, and it will take some time and a sizable number of experiments before the most potent lead compound has been found.

The principle of chemical specificity is also valid for toxic effects. Most elements, per se, are not toxic at all. Only certain compounds of certain elements are toxic. Strictly speaking, it is nonsensical, therefore, to talk about a »toxic element«. There is no doubt that carbon, nitrogen, and oxygen, three of the main ingredients of the living matter, belong to the most toxic elements we can imagine. They are fatal if they are presented to the organism in simple structures such as cyanide, or in complex organic derivatives such as strychnine. Chemical specificity certainly is also prevailing in the toxic effects of lead compounds. One-hundred fold differences in the toxic levels of different lead derivatives can easily be demonstrated in the same species.

For a better understanding of the possible mode of action of lead in promoting growth, on one hand, and of its toxic effects if it is applied at higher dose levels, on the other hand, it is mandatory that we clarify the biochemical mechanisms of lead metabolism and the structures of lead compounds which occur in the organism.

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References

1. *Schwarz, K., Foltz, C. M.:* J. Am. Chem. Soc., *79* (1957) 3292.
2. *Schwarz, K., Mertz W.:* Arch. Biochem. Biophys., *85* (1959) 292.
3. *Schwarz, K., Milne, D. B., Vinyard, F.:* Biochem Biophys. Research Commun., *40* (1970) 22.

4. *Schwarz, K., Milne, D. B.:* Science, *174* (1971) 426.
5. *Hopkins, L. L. Jr., Mohn, H. E.:* Fed. Proc., *30* (1971) 462.
6. *Schwarz, K., Milne, D. B.:* Bioinorg. Chem., *1* (1972) 331.
7. *Schwarz, K., Milne, D. B.:* Nature (London), 239 (1972) 333.
8. *Carlisle, E. M.:* Fed. Proc., *31* (1972) 700.
9. *Nielsen, F. H., Ollerich, D. A.:* Fed. Proc., *33* (1974) 1767.
10. *Schwarz, K.:* Fed. Proc., *33* (1974) 1748.
11. *Schwarz, K.:* in Trace Element Metabolism in Animals (TEMA II), W. G. Hoekstra, et al, Eds., University Park Press, Baltimore, Md., (1974) 355.
12. *Schwarz, K.:* Proc. Natl. Acad. Sci., *70* (1973) 1608.
13. *Kehoe, R. A.:* The Metabolism of Lead in Man in Health and Disease, The Harben Lectures, 1960 London, McCorquodale (1961).
14. *Schroeder, H. A., Balassa, J. J.:* J. Chron. Dis., *14*(4) (1961) 408.
15. *Underwood, E. T.:* Trace Elements in Human and Animal Nutrition, 3rd ed., Academic Press, New York (1971).
16. *Trelease, S. F., Beath, O. A.:* Selenium, published by the Authors, New York (1949) page V.

Sažetak

MOGUĆNOST DA JE OLOVO ESENCIJALNI ELEMENT

Svrha je ovoga rada da se prikažu rezultati istraživanja koji upućuju na mogućnost da je olovo esencijalni element. Do 1957. samo je sedam elemenata bilo smatrano esencijalnim: željezo, jod, bakar, cink, mangan, kobalt i molibden. Od tada je k ovima pridodano još sedam nužno potrebnih elemenata. Prema definiciji esencijalni je element onaj koji prisutan u tragovima. Što su tragovi u današnjim analitičkim mogućnostima, pitanje je konvencije. Olovo ispunjava mnoge od kriterija koji su postavljeni za esencijalne elemente. Činjenica je da se toksičnost ne može uzeti kao kriterij da li je neki elemenat esencijalan ili ne jer nema nikakve funkcionalne povezanosti između toksičnosti i esencijalnih bioloških funkcija, kao što to pokazuje slučaj selenija i drugih dokazano esencijalnih elemenata.

U istraživanjima eventualne esencijalne uloge olova u organizmu sisavaca primijenjen je postupak kojim je utvrđena esencijalnost selenija. U principu postoji ultračista soba i izolacijski postupak s jedne strane i upotreba ekstremno pročišćenih aminokiselina u ishrani životinja s druge strane. Sva se oprema sastoji od plastike i nigdje nije upotrijebljeno staklo, metal ili guma. Uveden je specijalni filterski sistem kojim se odstranjuju i najsitnije čestice iz atmosfere. U ovakvom sistemu, praktički jedini izvor kontaminacije životinja elementima u tragovima jest hrana. Hrana je sadržavala 53 pojedinačna sastojka (21 aminokiselinu, 13 vitamina, šećer, 2 vrste masti, 3 soli i 13 spojeva u tragovima). Bazična hrana sadržavala je otprilike 0,2 ppm olova.

Za pokus su uzeti štakori čija su legla bila pažljivo kontrolirana, tako da se izbjegne mogućnost akumuliranja esencijalnog elementa, u ovom slučaju olova. U svakom je pokusu bilo 4 do 5 skupina po 6 do 8 životinja. Kontrolne su životinje držane u kavezima uz uobičajene uvjete, i bile su hranjene posebnim hranama s niskim, poznatim sadržajem olova. U toku 28 do 32 dana životinje su vagane i praćeno je njihovo stanje.

U 13 uzastopnih pokusa što su provedeni tijekom 1972/73. utvrđen je utjecaj na rast već pri dodavanju hrani 1,0 do 2,5 ppm olova u obliku subacetata. Ovaj učinak na rast bio je statistički značajan, premda porast težine nije bio veći od 20%. Osim olovnog acetata i olovni je oksid pokazao jednak pozitivan učinak na tjelesni prirast. Pri ponavljanim pokusima ovaj je učinak izostao, ali je naknadno utvrđeno da je tome bila uzrok kontaminacija temeljne hrane olovom iz plastičnih vreća u kojima je hrana držana. Rezultati pokazuju da je vjerojatna potreba štakora za olovom u opisanim uvjetima otprilike 1 ppm u hrani, a to je ona količina koju organizam prima u normalnim uvjetima prehrane.

DISCUSSION FOLLOWING THE PAPER

ALEXANDER: It was a fascinating paper. Surely the final proof of the essentiality of lead can come with the identification of its presence in a metalloenzyme. How far has your team gone in the identification of such an enzyme?

SCHWARZ: We use growth as the main criterion, and no enzyme studies have been carried out. While it is true in some cases that identification of a metalloenzyme was proof of essentiality (the typical example being molybdenum), in most instances trace elements were identified as essential by means of their biological effects on other more general functions such as blood formation, growth, etc. Not all essential elements are necessarily parts of enzyme molecules.

TSUCHIYA: I wonder if you have measured lead concentrations in blood and some other organs of experimental animals of both control and lead supplemented group.

SCHWARZ: In our lead-deficient animals liver lead levels were found to be 60 ppb, i. e., 0.06 ppm of dry weight, or below. The kidney levels were similar, but a little higher. Supplements of 1 or 2.5 ppm of lead in the diet raised the levels in liver much less than those in kidney. The latter easily attained several ppm after 3—4 weeks of experimentation. Blood and bone analysis are not available.

KOSTIAL: I understand from your answer to Dr Tsuchiya that you are having analytical difficulties in determining very low amounts of lead in blood or tissues. I believe that this could be solved by using a carrier free radioisotope of lead to determine various parameters of lead metabolism in your animals raised in a lead free environment. Would not an evidence of a higher lead retention in these animals indicate the essentiality of lead — since present data do not indicate a relationship between the lead dose level and lead metabolism.

SCHWARZ: Such trials would certainly contribute valuable information on lead assimilation and metabolism. By itself, however, it would not establish essentiality; for that, the biological response is indispensable.

MOORE: Since the rat is rather tolerant to Pb what would be the requirements for another species such as the hamster which is more sensitive to Pb toxicity?

SCHWARZ: There are differences in the requirements of different species for a specific trace element, but these differences are usually not very great. I would estimate that they would be within one order of magnitude.