

The influence of nutritional factors on lead absorption

D. BARLTROP
M.D., F.R.C.P.

H. E. KHOO
B.A.

Paediatric Unit, St Mary's Hospital Medical School, London

Summary

The nutritional factors which affect lead absorption have been studied. Synthetic diets of known composition were compounded to contain 0.075% Pb as PbCl₂ labelled with ²⁰³Pb. Rats were exposed to lead for periods of 48 hr. The dietary intake was then measured and the absorption of lead determined by means of a whole-body counter. Lead absorption was increased by high fat, low mineral, low protein and high protein diets but was decreased by high mineral diet. Low fat, low fibre, high fibre, low vitamin and high vitamin diets had no effects on lead absorption.

Introduction

The major source of lead for the unexposed human population is its diet. It has been calculated that the average daily intake of lead of an adult is 300 µg from food and beverages (Barry and Mossman, 1970) and of this, about 10% is absorbed (Kehoe, 1961). There is evidence that various dietary factors will influence the absorption of lead from the gastrointestinal tract. Milk has been found to increase lead absorption from the gastrointestinal tract in rats (Kello and Kostial, 1973). Calcium and phosphorus added to rat diets decreased the accumulation of lead in femurs and kidneys (Sobel *et al.*, 1940; Six and Goyer, 1970). Conversely, low dietary iron has been shown to enhance lead uptake from the gastrointestinal tract in rats (Six and Goyer, 1972). Protein has also been found to affect lead absorption and toxicity (Baernstein and Grand, 1942) so that rats fed both low-protein and high-protein diets had an increased lead uptake (Milev, Satler and Minden, 1970). Vitamins C and D may each modify the absorption of lead. Thus a low intake of Vitamin C may enhance the severity of plumbism (Pillemer *et al.*, 1940). Vitamin D added to the diet will increase lead toxicity (Sobel *et al.*, 1940) although this has been denied (Tompsett, 1939). Conflicting reports exist about the effect of increased dietary fat on lead absorption. Tompsett (1939) found no relationship between dietary fat and lead absorption but Weyrauch and Necke (1932) reported an increase in lead absorption with increased dietary fat. The common food additives, 'alginates', have been

reported to diminish lead uptake from the intestine of newborn rats (Kostial, Simonović and Pisonić, 1971a) but this has not been confirmed (Harrison *et al.*, 1969; Carr, Nolan and Duraković, 1969).

The experimental designs used in previous studies have not been uniform and interpretation of their data is difficult. Many variables will influence lead absorption. Using carrier-free ²¹²Pb, Forbes and Reina (1972) showed that age and weight affect lead absorption in rats, with immature rats absorbing more lead than do mature animals. The chemical and physical form of lead has been reported to modify its absorption and toxicity (Fairhall and Sayers, 1940). Recently this has been confirmed in our laboratory (Barltrop and Meek, 1975). Carrier-free isotopes of lead have been used in some studies (Milev *et al.*, 1970; Kello and Kostial, 1973; Kostial *et al.*, 1971a, 1971b) but it is not known whether carrier-free lead behaves like stable lead in the gut under all experimental conditions. Comparison therefore requires careful control of the age and weight of the experimental animals, the chemical and physical form of the lead supplied and specific activity of any isotope used.

In this paper, a systematic study of the effect of individual dietary factors on lead absorption from the gastrointestinal tract of the rat is reported. This work was undertaken using diets compounded in this laboratory and containing 0.075% lead as PbCl₂ labelled with ²⁰³Pb. The effects of varying fibre, vitamin, protein, fat and mineral content of a synthetic diet on the absorption of lead are presented.

Materials and methods

Male Wistar albino rats of 100-115 g body weight, aged 30-32 days, were used. Each experimental group comprised six animals but each rat was housed individually. The experimental diets and de-ionized water were given *ad libitum* for 48 hr but food and water intake over the experimental period was recorded.

The control diet was compounded in the laboratory as in Table 1. Other diets were prepared by varying the proportion of different constituents. The protein and fat content was varied by adjusting

TABLE 1. Composition of control diet

		%
Protein	Casein	20.0
Fat	Corn oil	5.0
Fibre	Cellulose	3.0
Carbohydrate	Molasses	5.0
	Sucrose	28.0
	Corn starch	33.0
	Minerals*	5.35
	Vitamins†	0.62
	PbCl ₂	0.101
		100.0

* The mineral mix provided the following quantities of nutrient in g/kg of diet: NaH₂PO₄ · 2H₂O, 25.13; CaCO₃, 17.5; MgCl₂ · 6H₂O, 5.43; KCl, 3.43; NaCl, 1.27; MnSO₄ · H₂O, 0.168; FeSO₄ · 7H₂O, 0.105; ZnCO₃, 0.050; CuSO₄, 0.020; (NH₄)₆ Mo₇O₂₄ · 4H₂O, 0.005 and KIO₃, 0.0003.

† The vitamin mix provided the following per kilogram of diet: vitamin A, 19,800 i.u.; vitamin D, 2300 i.u.; vitamin E, 7 i.u.; choline chloride, 4.65 g; ascorbic acid, 991 mg; *p*-aminobenzoic acid, 110 mg; inositol, 110 mg; niacin, 99.1 mg; calcium pantothenate, 68 mg; menadione, 49 mg; thiamine hydrochloride, 22 mg; pyridoxine hydrochloride, 22 mg; riboflavin, 22 mg; folic acid, 1.98 mg; D-biotin, 0.44 mg; vitamin B₁₂, 0.029 mg.

the content of casein and corn oil in the diet. Fibre was varied using different proportions of cellulose. Corn starch and sucrose content were then adjusted to make the diets isogravic and isocaloric. With the exception of diets containing fat in the range 15–40%, feeds were prepared by moulding into sticks and heating at 100°C for 20 min. The high fat diets were fed in powder form to the rats kept in individual glass metabolic chambers. Each diet contained 0.075% Pb as PbCl₂, labelled with ²⁰³Pb to a specific activity of approximately 100 nCi/mg Pb. The experimental period of 48 hr, age and weight of experimental animals and lead concentration of 0.075% in the food were determined in preliminary studies (Bartrop and Meek, 1975).

After the end of the experimental period, the rats were killed by ether anaesthesia and counted in a Packard Armac small-animal whole-body counter. Approximately 3 ml of blood was obtained by venepuncture and collected into heparinized vials. After counting, each carcass was dissected and the gastrointestinal tract, kidneys, spleen, liver and femur were removed. The lead content of residual food, carcass without the gastrointestinal tract, carcass without any organs, whole liver, kidneys, spleen and femur were determined by measurement of the gamma-emission of ²⁰³Pb. Residual food, carcass and liver were counted in the Packard Armac whole-body counter. Specimens of blood, kidneys, spleen and femur were counted in a Hewlett-

Packard Auto-Gamma counter. The ingested dose was determined by the difference between the food given and the residual food. Lead content of the carcass and individual organs was expressed as percentage of ingested dose.

Results

The values for lead retention by animals fed on the control diet are given in Table 2. Whole-body retention was only 0.65% of the ingested dose compared with the value of 10% that has been reported for human adults (Kehoe, 1961). Of the organs, liver had the greatest retention of lead with progressively lesser values in kidney, femur and blood respectively.

TABLE 2. Controls*. Lead in organs as % ingested dose

	$\bar{x} \pm \text{s.d.}$
Blood	$(0.20 \pm 0.05) \times 10^{-2}$
Femur	$(2.08 \pm 0.51) \times 10^{-2}$
Kidneys	$(2.82 \pm 0.50) \times 10^{-2}$
Liver	$(6.62 \pm 1.20) \times 10^{-2}$
Whole body without gut	0.65 ± 0.12

* $n = 11$.

TABLE 3. Effects of different diets on lead absorption (ratio of mean retention experimental : control)

Diet	Blood	Kidneys	Femur	Liver
Low protein	5.1	2.5	2.8	2.2
High protein	1	3.7	2.6	1
Low fat	1	1	1	1
High fat	9.6	7.6	4.8	4.2
Low minerals	17.7	11.9	13.7	8.8
High minerals	0.2	0.2	0.1	0.1
Low fibre	1	1	1	1
High fibre	1	1	1	1
Low vitamins	1	1	1	1
High vitamins	1	1	1	1

The effects of various experimental diets on lead distribution between the organs are given in Table 3. All results which are not significantly different from the control are presented with the experimental : control ratio as 1. In the low factor diets, the nutritional factor concerned was omitted while in the high factor diets it was increased three- to four-fold. Low protein, high fat and low mineral diets increased the blood lead concentration and high mineral diet decreased it (Fig. 1). The lead concentration of liver showed a similar relationship. In the kidneys and femurs, however, a high protein diet was also associated with an increased lead content. Low fat, low fibre, high fibre, low vitamin and high vitamin diets had no effect on lead absorption.

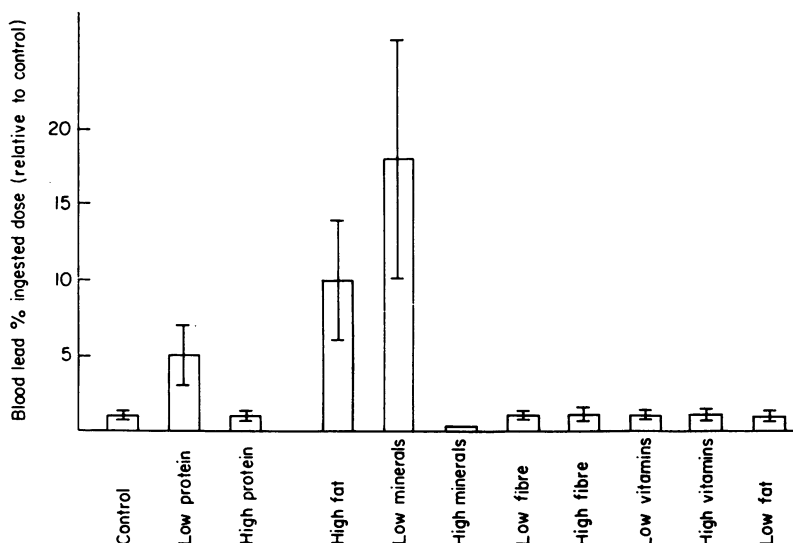


FIG. 1. Mean blood lead concentration as % ingested dose \pm s.d. for diets of varying composition relative to control diet ($n = 6$).

Protein

Detailed studies of the effects of varying dietary protein confirmed the findings in the preliminary investigations (Table 4). Diets containing 10% and 15% protein had no effect on lead absorption but diets containing 0% and 5% protein increased the lead concentration in all the organs studied. Conversely, doubling the recommended protein content to 40% by weight resulted in greater lead concentration in the kidneys (Fig. 3) but had no effect on blood, liver and femur. Further increases in dietary protein content to 80% increased the lead concentration in both kidneys and femur but did not produce any significant effects in the blood (Fig. 2) and liver.

Fat

Data showing the relationship between tissue lead and varying dietary fat are given in Table 5. De-

creasing the dietary fat content from 5% to 0 and 2.5% did not affect the lead content of the tissues studied. By contrast, increasing the fat content to 10, 15, 20 and 40% resulted in increased lead content of the tissues. There was a progressively increasing effect on lead concentration in carcass and organs from 10% to 40% dietary fat. The effects of 15% and 20% dietary fat did not differ significantly from each other. The values for blood and kidneys are given in Figs 4 and 5.

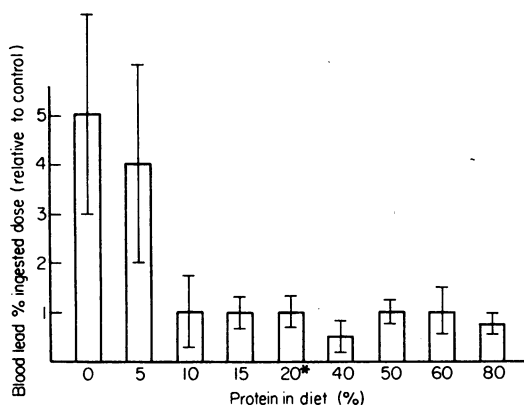


FIG. 2. Mean blood lead concentration as % ingested dose \pm s.d. for diets of varying protein content relative to control diet ($n = 6$). * Control.

TABLE 4. Effects of dietary protein on lead absorption (ratio of mean retention experimental : control)

Diet (% protein)	Blood	Kidneys	Femur	Liver
0	5.1	2.5	2.8	2.2
5	2.2	2.8	3.3	3.0
10	1	1	1	1
15	1	1	1	1
20 (control)	1	1	1	1
40	1	2.0	1	1
50	1	1.9	1.3	1
60	1	3.7	2.6	1
80	1	2.5	1.5	1

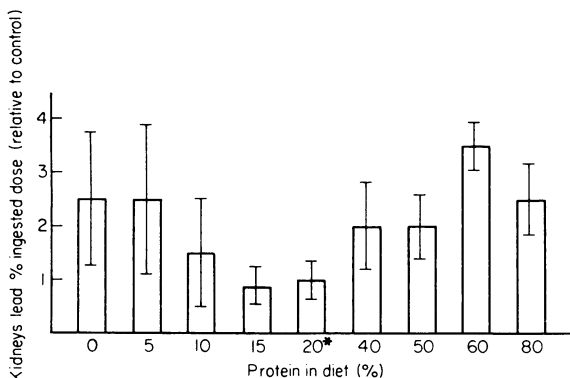


FIG. 3. Mean kidney lead concentration as % ingested dose \pm s.d. for diets of varying protein content relative to control diet ($n = 6$). * Control.

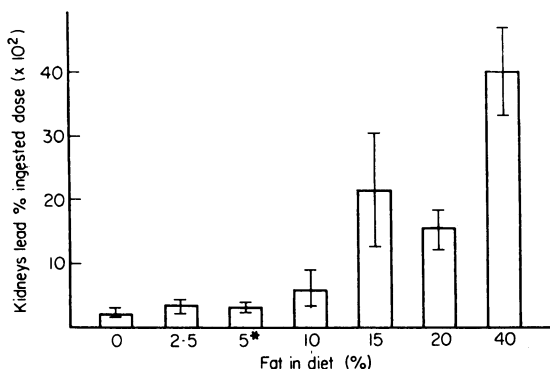


FIG. 5. Mean kidney blood lead concentration as % of ingested dose \pm s.d. ($\times 10^2$) for diets of varying fat content ($n = 6$). * Control.

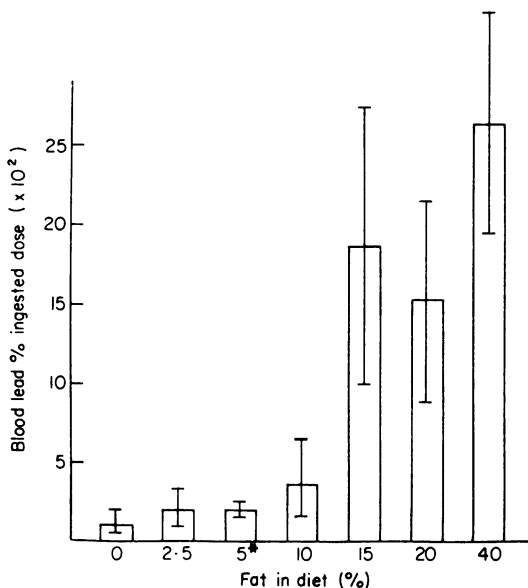


FIG. 4. Mean blood lead concentration as % ingested dose \pm s.d. ($\times 10^2$) for diets of varying fat content. ($n = 6$). * Control.

Minerals

In order to identify the individual minerals responsible for increasing lead uptake from the gut, each of the minerals selected was omitted sequentially from the diet (Table 6). Initially, the response to the omission of major and minor components was determined. Diets deficient in calcium, phosphates, magnesium, sodium, potassium and chloride resulted in a marked increase in lead absorption but exclusion of iron, manganese, copper, zinc, iodine and molybdenum from the diet had no effect. Further studies showed that sodium, potassium and chloride had no effect on lead uptake. Diets without added calcium or phosphates resulted in increased tissue lead concentrations which appear to be additive. A low dietary magnesium resulted in increased lead retention in the tissues but to a lesser extent than either calcium or phosphates alone.

Increasing the total mineral content of the diet four-fold resulted in an 80% decrease in lead retention in blood, 50% in kidneys and 90% in femur and liver. The individual minerals responsible for this effect are currently under investigation in this laboratory.

TABLE 5. Effects of dietary fat on lead absorption (ratio of mean retention experimental : control)

Diet (% fat)	Blood	Kidneys	Femur	Liver	Carcass
0	1	1	1	1	1
2.5	1	1	1	1	1
5 (control)	1	1	1	1	1
10	1.9	2	1.5	1.5	1.8
15	9.6	7.5	4.8	4.2	5.2
20	7.9	5.5	4.6	4.4	4.2
40	13.6	14.2	10.8	7.1	8.9

TABLE 6. Effects of dietary minerals on lead absorption (ratio of mean retention experimental : control)

Diet	Blood	Kidneys	Femur	Liver	Carcass
Low total minerals	17.7	11.9	13.7	8.8	11.8
High total minerals	0.2	0.5	0.1	0.1	—
Low Ca, PO ₄ , Mg, K, Na, Cl	17.7	14.9	10.7	8.2	11.0
Low Fe, Mn, Cu, Zn, I, Mo	1	1	1	1	1
Low calcium	6.2	4.4	3.4	4.0	3.1
Low phosphate	2.7	3.0	2.1	1.9	1.8
Low calcium and phosphate	9.7	7.4	4.9	3.8	4.4
Low magnesium	2.7	1.6	1.6	1.7	1.7
Low K, Na, Cl	1	1	1	1	1

Discussion

The results show that nutritional factors have a marked effect on the absorption of lead from the gastrointestinal tract. Previous data on the apparent high absorption of lead from the gastrointestinal tract in suckling rats compared with adult rats (Pentschew and Garro, 1966; Kostial *et al.*, 1971a, b) may thus be partly explained by the nature of their diet rather than functional immaturity of the gut.

Cow's milk has been used as a prophylactic for lead poisoning but this view has been questioned by Kello and Kostial (1973) who found enhanced lead uptake in rats when milk was added to the animals' diet. The findings of this paper suggest that the high fat and protein content of milk might be expected to increase lead absorption but the effect would be counteracted by the high mineral content.

There are three possible mechanisms by which nutritional factors could affect the absorption of lead from the gut. Firstly, by the binding of lead to a poorly absorbed factor or its derivatives thus rendering the lead unavailable for absorption; secondly, by the interaction of the factor with the cellular processes regulating lead absorption; thirdly, modification of the metabolic status of tissues with an affinity for absorbed lead. At present, the data presented in this paper cannot distinguish between these possibilities. Since the experimental period used in this study was acute and the diets isocaloric, metabolic adaption is unlikely to account for the results obtained. The effects observed are more likely to be due to the nutritional factors acting directly on lead absorption from the gut.

It is not known whether lead absorption from the gut involves single or multiple pathways. The results from the protein studies seem to indicate that multiple routes might be involved since both low and high protein intake enhanced the absorption of lead. Similar results were obtained by Milev *et al.* (1970) although they found increased lead in all the organs of animals on a high protein diet, whereas in this study an increased lead content was found in

only the kidneys and femurs. Gontzea *et al.* (1970) suggested the hypothesis that a low protein diet results in impaired detoxifying mechanisms of the liver thus allowing more lead to be accumulated. This hypothesis, however, does not explain the enhanced lead uptake associated with a high protein diet.

Using kidney lead retention as an index of lead uptake, the relative importance of individual nutritional factors can be determined. A regime containing 40% fat will increase uptake fifteen-fold. A mineral-deficient diet will result in a twelve-fold increase. However, the increases in lead absorption due to the lack of the individual minerals, calcium, phosphates and magnesium do not summate to a twelve-fold increase (Table 6) so that there would appear to be a synergistic effect. Since a low calcium diet is known to modify the absorption of other minerals, for example magnesium (Morris and O'Dell, 1963) and strontium (MacDonald *et al.*, 1952, 1955), it is probable that a common pathway exists for the absorption of calcium and other minerals including lead from the gut. Both low and high protein diets will also enhance lead uptake in the kidneys 2.5 and 3.5 times respectively.

Vitamins did not have any effect on lead uptake. This is contrary to the findings in relatively long-term studies where a dietary deficiency in vitamin C (Pillemar *et al.*, 1940) enhanced lead toxicity and an increase in vitamin D (Sobel *et al.*, 1940) resulted in enhanced lead absorption. The data reported in this paper suggest that the effect of vitamins do not result from an action on the gut.

The only dietary regime that decreased lead uptake was that with added minerals. These results suggest a potential application of minerals as prophylactics for lead workers and children living in high risk situations. The marked effects of the different nutritional factors demonstrated in this report might in part explain the wide range of blood lead concentrations found in community studies.

Acknowledgments

This report is based on research being undertaken for the degree of Ph.D. in the University of London (H.E.K.) and is supported by a grant from the Buttle Trust.

²⁰³Pb was supplied by the M.R.C. Cyclotron Unit, Hammersmith, London.

References

- BAERNSTEIN, H.D. & GRAND, J.A. (1942) The relation of protein intake to lead poisoning in rats. *Journal of Pharmacology and Experimental Therapeutics*, **74**, 18.
- BARLTROP, D. & MEEK, F. (1975) Absorption of different lead compounds. *Postgraduate Medical Journal*, **51**, 805.
- BARRY, P.S.I. & MOSSMAN, D.B. (1970) Lead concentrations in human tissues. *British Journal of Industrial Medicine*, **27**, 339.
- CARR, T.E.F., NOLAN, J. & DURAKOVIĆ, A. (1969) Effect of alginate on the absorption and excretion of ²⁰³Pb in rats fed milk and normal diets. *Nature*, London, **224**, 1115.
- FAIRHALL, L.T. & SAYERS, R.R. (1940) *Public Health Bulletin (US)*, No. 253.
- FORBES, G.B. & REINA, J.C. (1972) Effect of age on gastrointestinal absorption (Fe, Sr, Pb) in the rat. *Journal of Nutrition*, **102**, 647.
- GONTZEA, I., SUTEZESCO, P., DUMITRACHE, S. & BISTRICEANU, E. (1970) Recherches sur le rôle de l'apport proteique sur les moyens de défense de l'organisme envers quelques toxiques chimiques. *Archives des Maladies Professionnelles, de Médecine du Travail et de Sécurité Sociale*, **31**, 471.
- HARRISON, G.E., CARR, T.E.F., SUTTON, A. & HUMPHREYS, E.R. (1969) Effect of alginate on the absorption of lead in man. *Nature*, London, **224**, 1115.
- KEHOE, R.A. (1961) The metabolism of lead in man in health and disease. The Harben Lectures, 1960. *Journal of the Royal Institute of Public Health and Hygiene*, **24**, 81; 101.
- KELLO, D. & KOSTIAL, K. (1973) The effect of milk diet on lead metabolism in rats. *Environmental Research*, **6**, 355.
- KOSTIAL, K., SIMONOVIĆ, I. & PISONIĆ, M. (1971a) Reduction of lead absorption from the intestine in newborn rats. *Environmental Research*, **4**, 360.
- KOSTIAL, K., SIMONOVIĆ, I. & PISONIĆ, M. (1971b) Lead absorption from the intestine in newborn rats. *Nature*, London, **233**, 564.
- MACDONALD, N.S., NUSBAUM, R.E., EZMIRLIAN, F., BARBERA, R.C., ALEXANDER, G.V., SPAIN, P. & ROUNDS, D.E. (1952) Gastro-intestinal absorption of ions. I. Agents diminishing absorption of strontium. *Journal of Pharmacology and Experimental Therapeutics*, **104**, 348.
- MACDONALD, N.S., SPAIN, P.C., EZMIRLIAN, F. & ROUNDS, D.E. (1955) The effects of calcium and phosphate in foods on radio-strontium accumulation. *Journal of Nutrition*, **57**, 555.
- MILEV, N., SATLER, E.L. & MENDEN, N. (1970) Aufnahme und Einagerung von Blei im Körper unter verschiedenen Ernährungsbedingungen. *Medizin und Ernährung*, **11**, 29.
- MORRIS, E.R. & O'DELL, B.L. (1963) Relationship of excess calcium and phosphorus to magnesium requirement and toxicity in guinea-pigs. *Journal of Nutrition*, **81**, 175.
- PENTSCHEW, A. & GARRO, F. (1966) Lead encephalomyelopathy of the suckling rat and its implications in the porphyrinopathic nervous diseases. *Acta neuropathologica*, **6**, 266.
- PILLEMER, L., SCIFTER, J., KUEHN, A.O. & ECKER, E.E. (1940) Vitamin C in chronic lead poisoning. *American Journal of Medical Science*, **200**, 322.
- SIX, K.M. & GOYER, R.A. (1970) Experimental enhancement of lead toxicity by low dietary calcium. *Journal of Laboratory and Clinical Medicine*, **76**, 933.
- SIX, K.M. & GOYER, R.A. (1972) The influence of iron deficiency on tissue content and toxicity of ingested lead in the rat. *Journal of Laboratory and Clinical Medicine*, **79**, 128.
- SOBEL, A.E., YUSKA, H., PETERS, D.D. & KRAMER, B. (1940) The biochemical behavior of lead. I. Influence of calcium, phosphorus and vitamin D on lead in blood and bone. *Journal of Biological Chemistry*, **132**, 239.
- TOMPSETT, S.L. (1939) The influence of certain dietary constituents of the diet upon the absorption of lead from the alimentary tract. *Biochemistry Journal*, **33**, 1237.
- WEYRAUCH, F. & NECKE, A. (1932) Zur Frage der Milch-Scheimsupper- und Fett-prophylaric bei der Bleivergiftung. *Zeitschrift für Hygiene und Infektionskrankheiten*, **114**, 629.