INTERACTIONS OF LEAD WITH OTHER TRACE SUBSTANCES AND DIETARY FACTORS

R. A. WILLOUGHBY,* D. G. THAWLEY** and G. BROWN***

* *Ontario Veterinary College, University of Guelph, Ontario, Canada,*
** *Department of Veterinary Medicine, Massey University, New Zealand, and* *** *Cominco Ltd., Trail, B. C., Canada*

Interactions among lead, zinc, cadmium, calcium and vitamin D have been demonstrated in the laboratory. There is evidence indicating that under certain field conditions these interactions are of more importance in terms of the level of diseases that develops than is the absolute intake of each variable.

Thirty nine parameters were measured in order to determine the extent and nature of interactions among toxic and normal levels of lead, zinc and cadmium, normal and deficient levels of calcium and normal, deficient and high levels of vitamin D.

Significant interactions affecting general metabolism, hematological tissue, blood, liver, kidney and enzyme related parameters were identified, illustrated and discussed.

The response of an animal to disease caused by elements, trace substances or other dietary factors depends upon the dosage, the species requirements and susceptibility, intercurrent diseases and interactions between and among these variables. The interactions may be additive, synergistic or antagonistic in nature and the interaction may occur during one or more phases of the absorption, distribution, metabolism and excretion of one or several systems. The large number of parameters that one must measure in studying all of these aspects of an interaction undoubtedly is the reason why comparatively little information is available on interactions among elements and trace substances that may occur in the food chain of animals (1, 2, 3).

It is the purpose of this paper to describe our studies with interactions among lead, zinc, cadmium, calcium and vitamin D in horses and rats and to discuss the application of the results.

Field observations (4) showed that of 28 horses raised within the vicinity of a lead-zinc smelter, 5 of 6 young horses and 1 of 22 horses developed illness. Lameness and unthriftiness characterized the illness affecting the young horses whereas the older horse had clinical signs of pharyngeal paralysis. All cattle and other domesticated animals raised on the same area were unaffected. These findings, not being entirely consistent with a diagnosis of lead poisoning, prompted us to undertake two studies in young horses. The first (5) demonstrated that after young horses were fed a low calcium diet for 27 weeks, the concentration of lead in the liver was higher than when adequate calcium diets were fed. These young horses did not become lame or unthrifty. Our second study (6) was conducted in order to determine the nature of zinc and lead toxicity in the young growing horse and to test for interactions between toxic amounts of lead and zinc. Young horses fed toxic amounts of zinc only or of zinc and lead together, but not lead alone, developed lameness and unthriftiness similar to the syndrome which has been observed in young horses raised in the vicinity of the lead-zinc smelters (4, 7, 8). Pharyngeal paralysis and other neurological signs were observed only in young horses fed toxic amounts of lead alone. There was evidence of an interaction between toxic amounts of lead and zinc because animals fed toxic amounts of both had higher tissue levels of lead than those that developed pharyngeal paralysis from lead poisoning but the interaction appeared to suppress the development of the nervous system signs of lead poisoning.

The experiments being reported in this paper were designed to further investigate lead interactions through the use of the factorial experimental design and the rat as a more expedient experimental animal.

MATERIAL AND METHODS

Forty-eight treatments were used in a factorial designed experiment to study the interactions among two dietary levels of lead, zinc, cadmium and calcium and three dietary levels of Vitamin D. Each treatment was added to or was present in a standard diet and was administered to two Sprague Dawley rats. Each set of 48 treatments was replicated four times. Two animals were housed in a single stainless steel cage and all received deionized distilled water free choice. The concentrations of the treatments given are listed in Table 1. The treatments were given for 42 days before the rats were killed. The 39 different parameters measured are listed at the top of Table 2. Further details of the methods used for each determination are available (9).

The data were analysed by computer using a program for analysis of variance for factorial experimentation in randomized complete block with or without subsampling.

Table 1

*Concentrations of treatments fed**

High lead	— 5000 ppm of diet fed as $PbCO_3$
Normal lead	— 7 ppm of diet (residual level)
High zinc	— 6300 ppm of diet fed $ZnCO_3$
Normal zinc	— 30 ppm of diet (residual level)
High cadmium	— 90 ppm of diet fed as $CdCO_3$
Normal cadmium	— 0.1 ppm of diet (residual level)
Normal calcium	— 0.9% of diet
Low calcium	— 0.1% of diet
Normal vitamin D	— 2000 i. u./kg of diet
Low vitamin D	— no vitamin D
High vitamin D	— 50,000 i. u./kg of diet

* The basal diet was a General Biochemicals (Cleveland, Ohio) Calcium and Vitamin D deficient purified diet.

RESULTS

The significant main effects and interactions are listed in Table 2 with the 5% and 1% levels of significance being represented by a single and double asterix, respectively. Thirty-nine tables containing the mean data and the standard deviations of the mean for each parameter of the 48 treatment combinations are available (9).

Only the major results will be discussed in this paper. All data in Figures 1 to 26 contain significant main effects and interactions. The end of the bar in each figure represents the mean value of a parameter and the line with a vertical line at either end represents one plus and minus the standard deviation of the mean.

General metabolism: The most severe growth depression occurred in animals fed high zinc low calcium diets. High cadmium diets alone or with high zinc diets also severely depressed weight gain (Figures 1 and 2). Variations in the vitamin D content of the rations did not produce dramatic effect. It was not possible with the experimental design used in this study to differentiate a direct toxic effect in appetite suppression, reduced palatability of feed, an induced metabolic effect or the three in different proportions and combinations. Other parameters measured in an attempt to demonstrate general metabolic effects parallelled the changes in weight gain to the ¾ power (Table 2).

Hematological parameters: Microcytic, hypochromic anemia was produced by the high lead, zinc and cadmium treatment diets alone. Many treatment combinations produced significant main effects and interac-

Table 2
Analysis of variance results for main effects and interactions

	GENERAL METABOLISM				HEMATOLOGICAL PARAMETERS									RENAL PARAMETERS						
	Wt. Gain	Final Wt. 34	Feed Intake	T. S. Prot.	RBC	PVC	Hb.	MCV	MCH	MCHC	[illegible]	Serum Fe	TIBC	Urine ALA	Urine Alb.	Wt.	Pb	Zn	Cd	Cu	Fe
Ca	**	**	**	**	**	**	**	**	**		**	**	**	**	**	**	**	**	**	**	**
VD	**	**	*		*	*	**		*		**					**	**	**		*	**
Pb		*	**			**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Zn	**	**	**	**	*	**	**	**	**			**	**	**	**		**	**	**	**	**
Cd	**	**	**	**		*	**	*	*	*	**					*	**	*	**	**	
Ca x VD				*	*			**	*		**					**	**	**			
Ca x Pb	**	**	**	*		*					**	*	**	**		**	**		**		
Ca x Zn	*	**		**	**		*	**	**			**		**	**		**	**	**		
Za x Cd	*	**		*	*						**						**				*
VD x Pb						**	**				*						**			*	*
VD x Zn	*	*															**				*
VD x Cd											**			*						*	
Pb x Zn			**			**	**	**	**	*	**	**		**		**	**	**	**	**	**
Pb x Cd		*	**		*		**	**	**	*							**	*	**		*
Zn x Cd	**	**	**				**		*	*		*		*			**		**		**
Ca x VD x Pb											*						**	*			
Ca x VD x Zn																	**				
Ca x Vd x Cd																					
Ca x Pb x Zn	**	**	**		*		**	**	**					**		*	**	**	**		
Ca x Pb x Cd			*		*	**	**	**	**								**		**		
Ca x Zn x Cd	**			*													**		**		**
VD x Pb x Zn																	**				
VD x Pb x Cd			**								*			*		**	**				
VD x Zn x Cd												**		*							
Pb x Zn x Cd	**	**	**		*			*	**												
Ca x VD x Pb x Zn						*											**	*	*		*
Ca x VD x Pb x Cd			*														**				
Ca x VD x Zn x Cd		*										*		*							
Ca x Pb x Zn x Cd																					
VD x Pb x Zn x Cd	*		**														**		**		
Ca x VD x Pb x Cd													**			*		*			
													**								

* significant at 5% level ** [illegible]

	LIVER						BLOOD				BONE							
	Wt.	Pb	Zn	Cd	Cu	Fe	Pb	Zn	Cd	Ga	Pb.	Zn	Cd	Ca	Mg	P	Conc.	% migrating in fast band
Ca	**	**	**	**	**	**	**	**	**		**	**	**	**	**	**	**	**
VD		*	*		*					**		**	*	*	**		**	**
Pb	**	**	*	**	**	**	*				**	**				*	**	**
Zn	**	**	**	**	**	**	**	**			**	**	**	**	**	**	**	**
Cd				**	**	**			**				**		*			
Ca x VD			**			**	**			**		*						*
Ca x Pb	*	**	**	**	*		**				**							
Ca x Zn	**	**	**	**	**	**	**	**	*		**	**	**	**	**	**	**	**
Ca x Cd				**		**			**				**					
VD x Pb		*					*		**			*						
VD x Zn			*	*		*	*					**			**			**
Vd x Cd																		
Pb x Zn		**		**	**	*	**		*		**	*						*
Pb x Cd				**	*	**			*									
Zn x Cd			**	**	**	**	**		**				**					
Ca x VD x Pb												*						
Ca x VD x Zn			**		*							*						*
Ca x VD x Cd																		
Ca x Pb x Zn		**	*	**			**				**							
Ca x Pb x Cd				**		*	*										*	
Ca x Zn x Cd			**	**	*	**												
VD x Pb x Zn		*	*		*							*						
VD x Pb x Cd									*									
VD x Zn x Cd				*														
Pb x Zn x Cd				**			**											
Ca x VD x Pb x Zn												*						
Ca x VD x Pb x Cd																		
Ca x VD x Zn x Cd																		
Ca x Pb x Zn x Cd				**		*												
VD x Pb x Zn x Cd																		
Ca x VD x Pb x Zn x Cd																		

Legend for Table 2

Wt Gain = Weight gain during 42 days
Final Wt. 3/4 = Final weight to the 3/4 power
T. S. Prot. = Serum protein, total solids method
RBC = Red blood cell count
PCV = Packed red cell volume
Hb. = Blood hemoglobin concentration
MCV = Mean corpuscular volume
MCH = Mean corpuscular hemoglobin
MCHC = Mean corpuscular hemoglobin concentration
Retic. Count = Reticulocyte count
TIBC = Total iron binding capacity
Urine ALA = Urine delta-aminolevulinic acid
Urine Alb. = Urine albumin
Wt. = Weight
Conc. = Concentration
Serum Alkaline Phos. = Total serum alkaline phosphatase
% Migrating in fast band = % alkaline phosphatase isoenzime migrating in the fast band

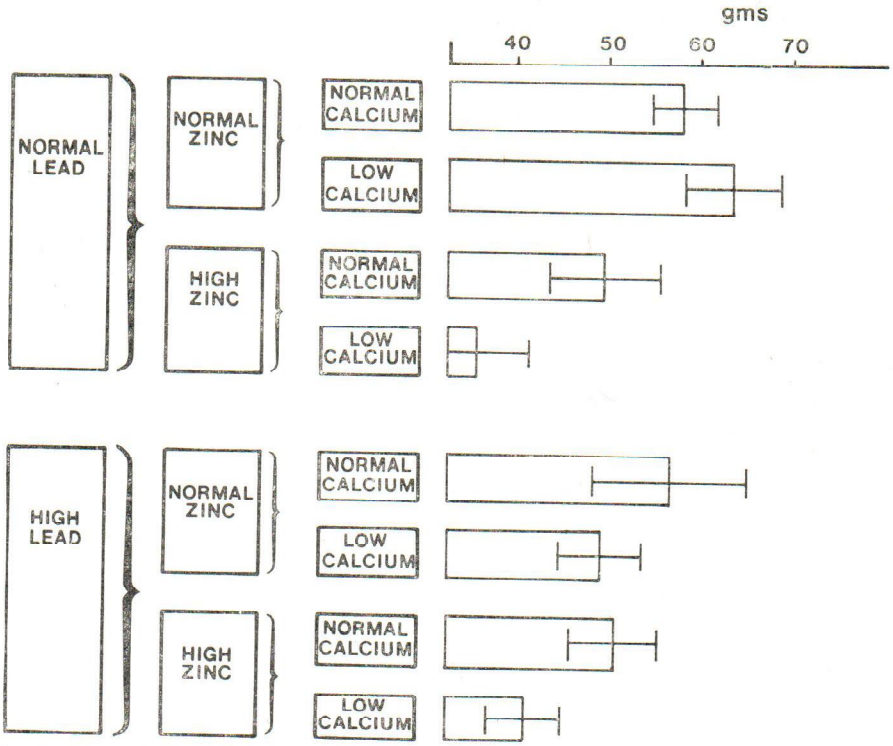

Fig. 1. *3-way interaction lead × zinc × calcium. Final weight 3/4. Normal cadmium & normal vitamin D*

tions on the hematological parameters (Table 2) but the most severe anemias occurred when high lead and high zinc or high cadmium treatments were fed together with low calcium diets (Figures 3 to 7). The high cadmium diet alone reduced the mean corpuscular hemoglobin concentrations (Figures 6 and 7) to the greatest extent. The addition of the high lead treatment to the high cadmium diet further reduced these values whereas the addition of a high zinc treatment to the high cadmium diet produced little change in either the presence or absence of a high lead treatment.

The reticulocyte counts were highest in rats fed the combined low calcium, high vitamin D and high lead treatment diet (Figures 8 and 9). The addition of high zinc, high cadmium or both treatments to the above combination of treated diets appeared to block the reticulocyte response. There tended to be a higher reticulocyte response in animals fed high

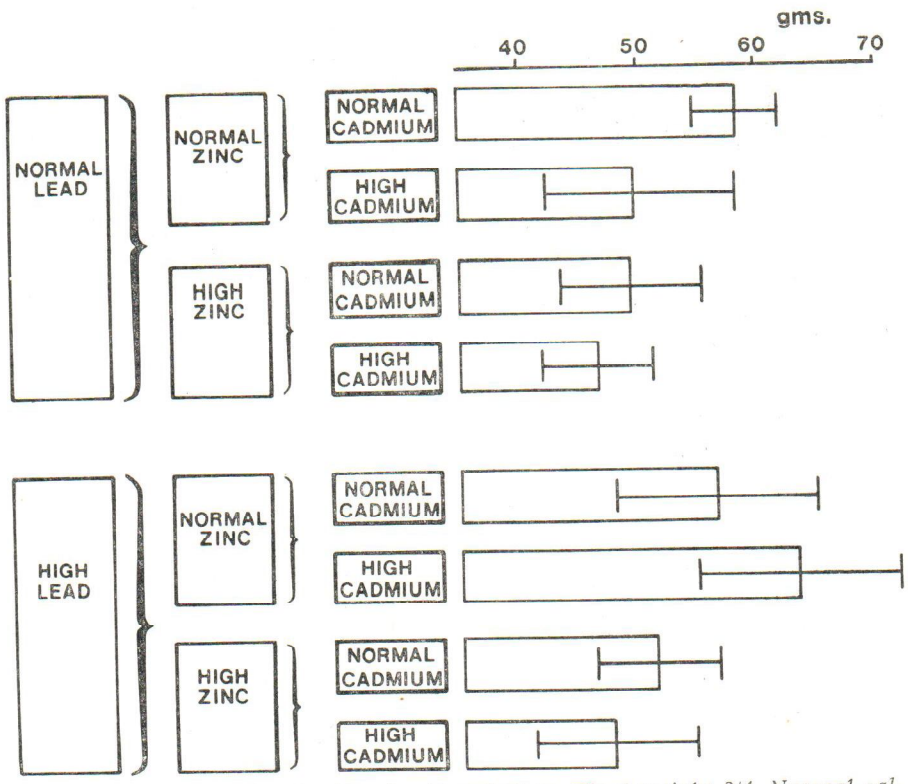

Fig. 2. *3.way interaction lead × zinc × cadmium. Final weight 3/4. Normal calcium & normal vitamin D*

vitamin D plus high lead treatments than when normal or low vitamin D plus high lead treatments were fed.

The serum iron concentrations were reduced to the greatest degree when high zinc and low calcium treatment diets were fed (Figure 10). The low serum iron values produced by these treatments were altered to a minor extent when high lead, high cadmium and different levels of vitamin D were added to the high zinc low calcium diet.

Total iron binding capacity values remained above the amount generally considered adequate for red blood cell synthesis.

Urine delta-aminolevulinic acid concentrations were increased when high lead treatments were fed. Low calcium diets (Figure 11) increased the concentration of urine ALA two-fold whereas high zinc diets when added to a high lead low calcium treatment diet appeared to reduce or block the excretion of ALA. Different vitamin D intakes (Figure 12) were

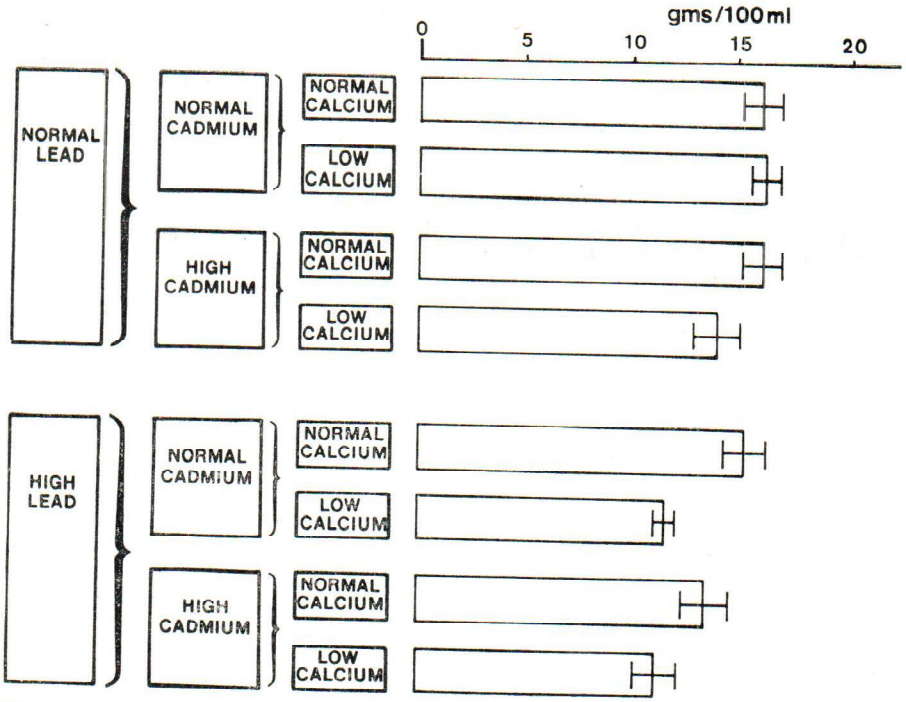

Fig. 3. *3-way interaction lead × cadmium × calcium. Blood hemoglobin. Normal zinc & normal vitamin D*

without consistent effect on urine ALA values. High cadmium diets altered urine ALA values to a minor and inconsistent extent compared to the effect of high zinc diets.

Kidney intranuclear inclusions: The low calcium and high lead treatment diet resulted in a marked increase in the number of intranuclear inclusions in renal tubule cells (Figure 13). However, high zinc diets appeared to block the appearance of lead induced intranuclear inclusions regardless of the calcium, vitamin D or cadmium intake (Figure 14).

Mineral concentrations in blood, liver, kidney and bone: Low calcium diets and high vitamin D diets increased lead concentrations in blood, liver and kidney. Bone lead values were increased by the low calcium diets but only by the high vitamin D diet when it was fed together with a normal calcium diet (Figures 15 to 20). When high zinc diets were fed together with high lead treatment there was a reduction in the concentration of lead in blood, liver, kidney and bone. High cadmium high lead diets when compared to high zinc high lead diets decreased the concentration of lead in blood, liver, kidney and bone. High cadmium high lead diet when compared to high zinc high lead diets decreased the concen-

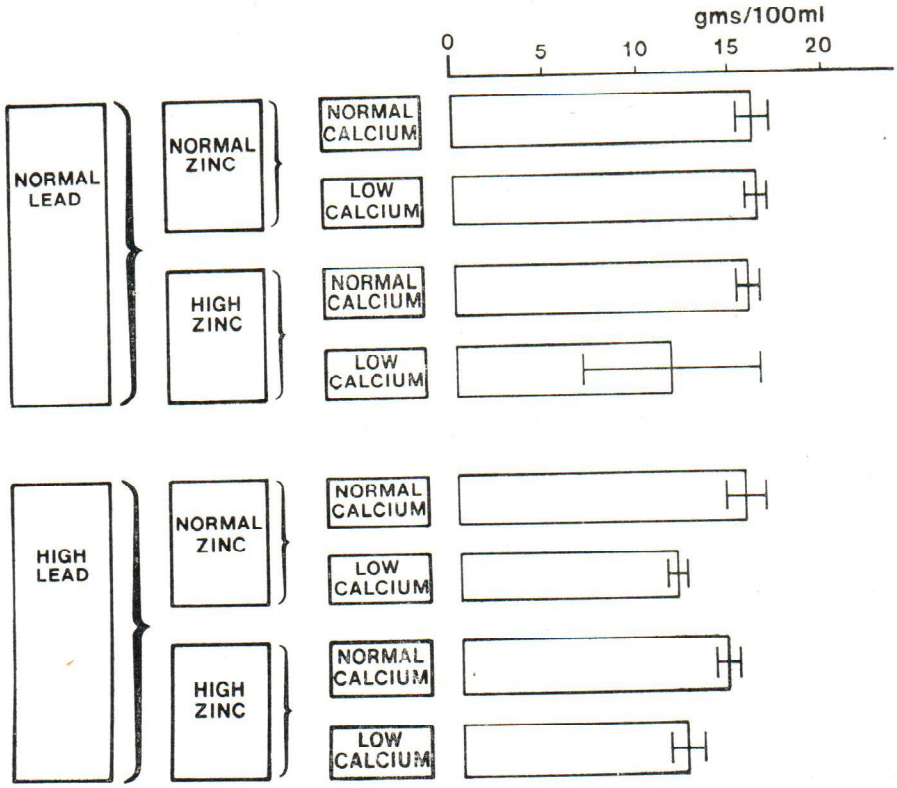

Fig. 4. *3-way interaction lead × zinc × calcium. Blood hemoglobin. Normal cadmium & vitamin D*

trations of lead in tissues and blood only to a minor extent. There was no evident combined effect on tissue or blood lead values when both high cadmium and high zinc diets were added to high lead diets.

Liver copper and iron values (Figures 21 and 22) decreased when high zinc and high cadmium diets were fed and when both were combined these values were further decreased. On low calcium diets, high lead diets caused a further decrease in liver copper values from those caused by high zinc and/or cadmium diets.

Alkaline phosphatase enzyme: There were many significant interactions based on total serum alkaline phosphatase values but no consistent pattern emerged. The trend, however, was for low calcium diets to increase the total serum alkaline phosphatase values and for high lead and high zinc diets to further increase the values. High cadmium and vitamin D diets produced a variable response (Figures 23 and 24).

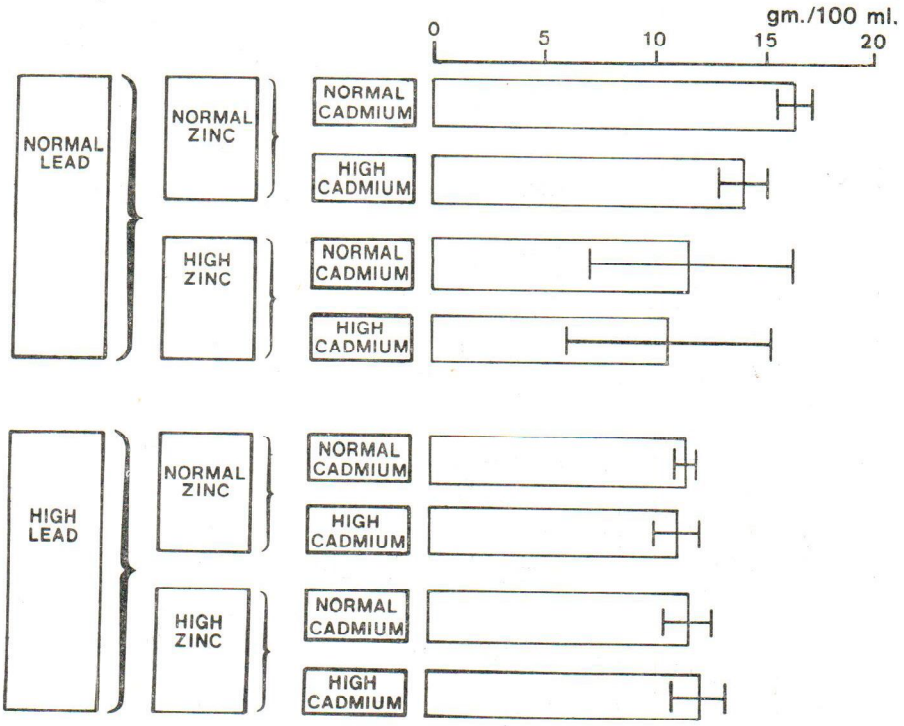

Fig. 5. *3-way interaction lead × zinc × cadmium. Blood hemoglobin. Low calcium diet & normal vitamin D*

High zinc and low calcium diets produced a marked increase in the percentage alkaline phosphatase isoenzyme that migrated in the fast moving »liver« band (Figures 25 and 26). This effect of high zinc was present to a lesser extent on normal calcium diets (Figure 26). There was no consistent effect of high lead, high cadmium or different vitamin D diets on the percent of the fast moving »liver« alkaline phosphatase isoenzyme band.

COMMENT

The main purpose of this study was to examine interactions among lead, zinc, cadmium, calcium and vitamin D in order to help predict what might occur under field conditions if animals were exposed to similar imbalances in an environment. We selected treatment concentrations that would produce a measurable effect on one or more para-

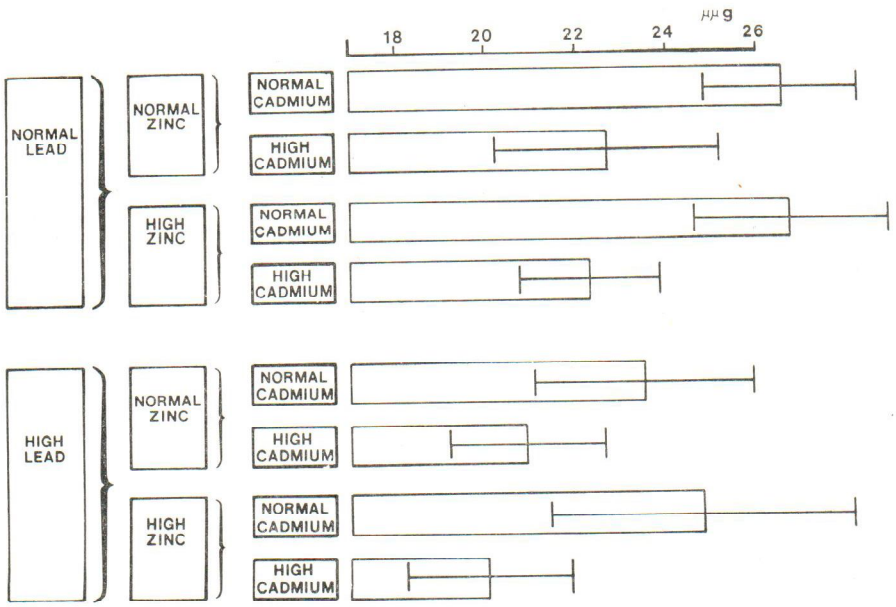

Fig. 6. *3-way interaction lead × zinc × cadmium. Mean corpuscular hemoglobin. Normal calcium & normal vitamin D*

meters, but not death, after 42 days of treatment. As will be mentioned later, different treatment concentrations and ratios of treatments could produced different results.

Low calcium diets enhanced lead, zinc and cadmium toxicity generally but in particular the induced anemias. These interactions were recognized nearly 50 years ago (10, 11). The anemia produced when low calcium high lead treatment diets were fed was decreased in severity when high levels of zinc or cadmium were fed concurrently. These results suggest a substitution or blocking effect produced by the presence of the high zinc or high cadmium diets.

The molar ratio of zinc to cadmium used in this study, greater than 90 : 1, enhanced the severity of the anemia when high levels of both were fed concurrently. Others (12, 13) have shown a protective interaction between zinc and cadmium when the molar ratio is in the order of 1 : 1. The combined high zinc high cadmium treatment also reduced the tissue copper and iron concentrations more than either treatment alone. These results indicate that further definition of the zinc and cadmium interaction over broader dosage ratios is needed, in order to determine the ratios at which addition, synergism or antagonism occurs.

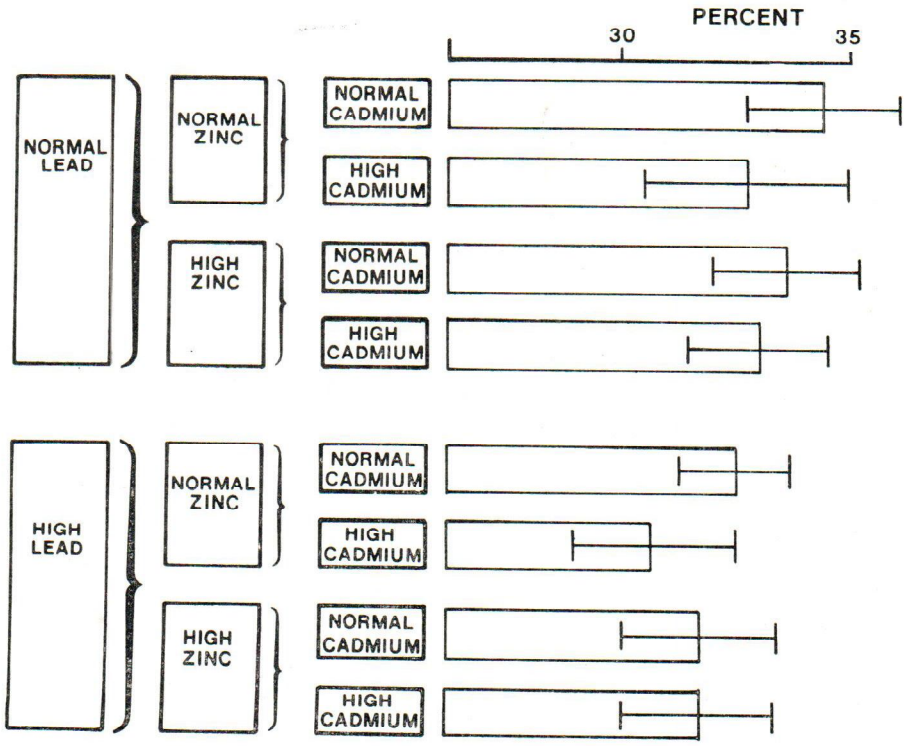

Fig. 7. *3-way interaction lead × zinc × cadmium. Mean corpuscular hemoglobin concentration. Normal calcium & normal vitamin D*

Tissue concentrations of lead, zinc and cadmium were markedly increased when low calcium and/or high vitamin D diets were fed concurrently with high concentrations of these treatments. When high lead and high zinc diets were fed concurrently, the tissue and blood lead concentrations were lower than when only the high lead treatment diets were fed. This lead zinc interaction also decreased the number of intranuclear kidney inclusion bodies as compared to those fed the high lead treatment only. This lead zinc interaction had been observed in young horses (6). The horses were fed toxic diets for a longer period and the bone lead values were lower when a high lead high zinc diet was fed. In the horses, however, the soft tissue lead concentrations were elevated when lead and zinc treatments were given together. The importance of duration of treatment, species differences and the ratio of lead to zinc in this interaction warrant further study.

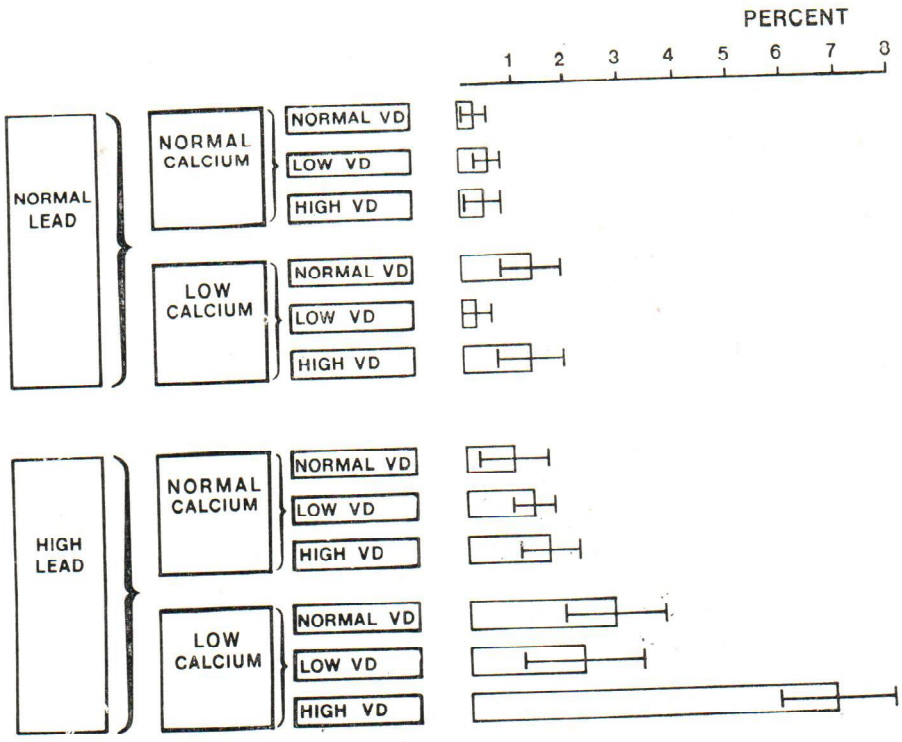

Fig. 8. *3 way interaction lead × calcium × vitamin D. Reticulocyte count. Normal zinc & normal cadmium*

We developed a method to analyse isoenzyme patterns of the metalloenzyme alkaline phosphatase in order to detect whether the different treatment combinations produced significant effects. Only the high zinc treated animals had a consistently increased amount of the isoenzyme in the faster migrating band. The control studies we conducted indicated that this fast band region corresponded with an isoenzyme of liver origin. Kinetic studies will be required to define these changes with an appropriate level of specificity to determine the nature of this altered system. High zinc diets also markedly decreased the excretion of ALA in urine. The reasons for this change also warrant more detailed study.

Our studies have indicated major interactions among lead, zinc, calcium and vitamin D with a possible additive toxic effect when the zinc and cadmium treatments were given together. More detailed studies will be required to explain why the soft tissues of the horses fed toxic levels of lead and zinc together contained higher lead levels than did compa-

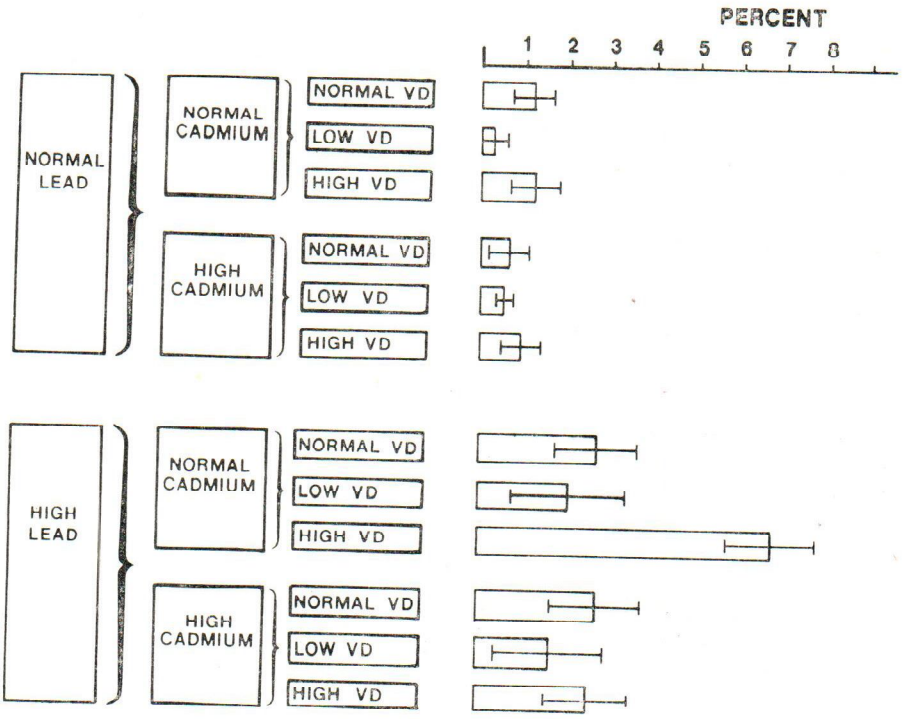

Fig. 9. *3-way interaction lead × cadmium × vitamin D. Reticulocyte count. Low calcium diet & normal zinc*

rable tissues from horses fed lead alone. The lead zinc interaction in rats appeared to lower the lead concentration in soft tissues and bone. A longer treatment period would help define the lead-zinc interaction in the rat and explain the apparent species differences in tissue lead concentrations.

There was evidence of impaired enzyme function when toxic amounts of zinc were fed. Methods similar to those used by *Vallee* (14) will be needed to further define these changes.

ACKNOWLEDGEMENTS

The authors appreciate support from The International Lead-Zinc Research Organization, the Ontario Ministry of Agriculture and Food, and Cominco Limited for this study. We give special thanks to Mr. Lauri Nicholson of Cominco Ltd., Trail, B. C. for his continued encouragement, interest and support of these studies.

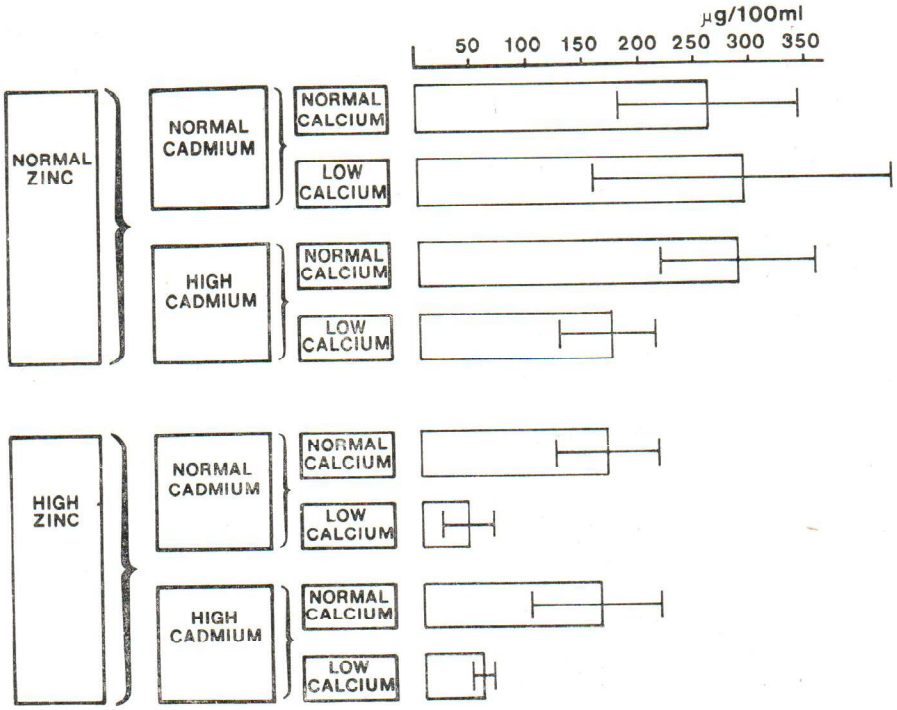

Fig. 10. *3-way interaction zinc × cadmium × calcium. Serum iron. Normal lead & normal vitamin D*

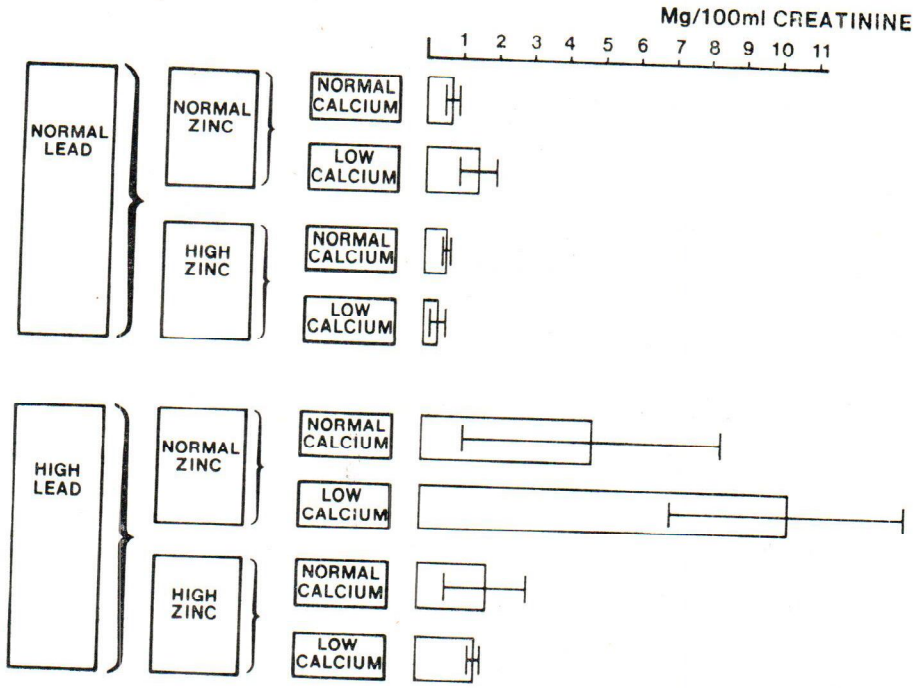

Fig. 11. *3-way interaction lead × zinc × calcium. Urine delta-aminolevulinic acid. Normal cadmium & normal vitamin D*

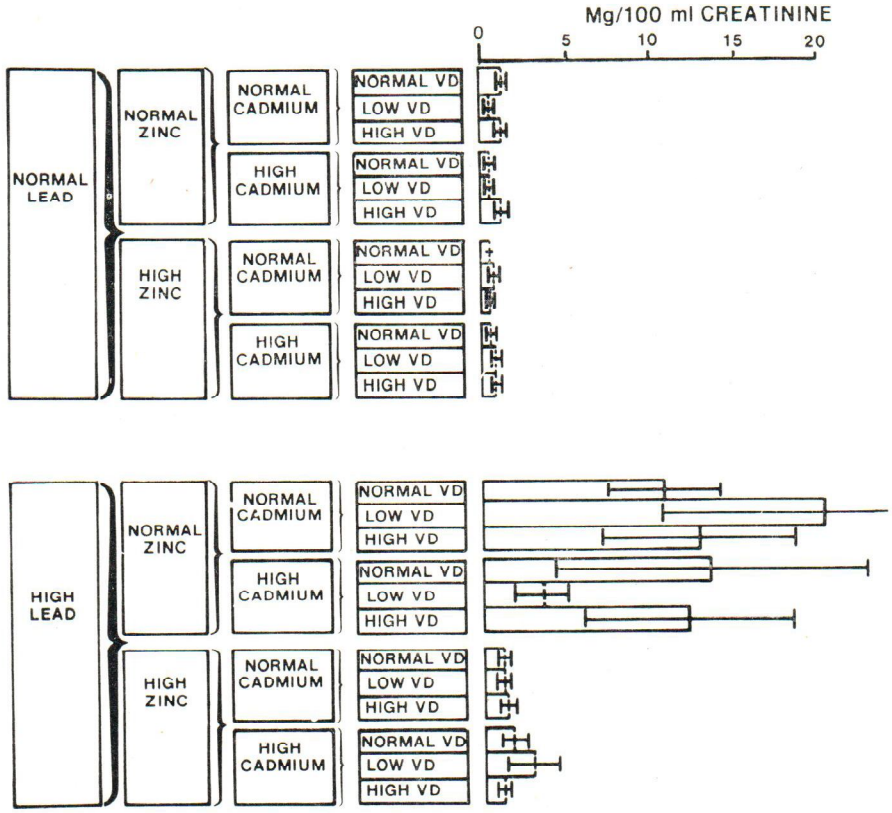

Fig. 12. *4-way interaction lead × zinc × cadmium × vitamin D. Urine delta--aminolevulinic acid. Low calcium diet*

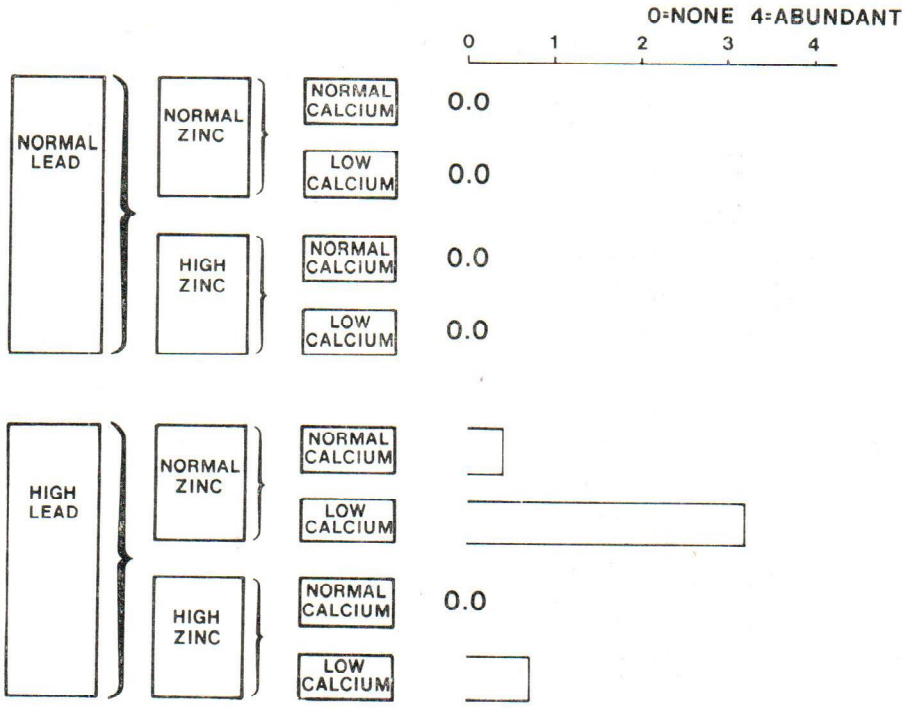

Fig. 13. *3-way interaction lead × zinc × calcium. Intranuclear inclusions kidney. Normal cadmium & normal vitamin D*

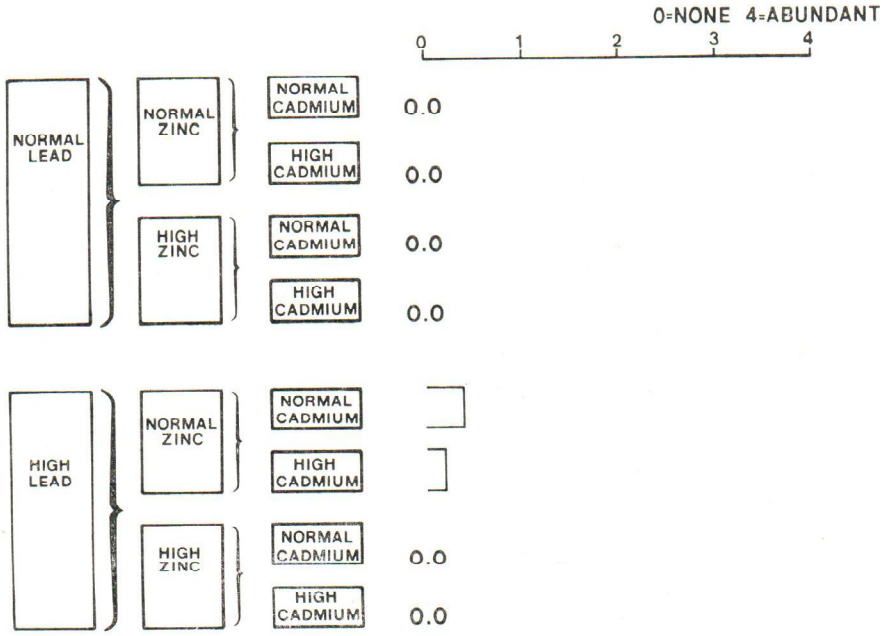

Fig. 14. *3-way interaction lead × zinc × cadmium. Intranuclear inclusions kidney. Normal calcium & normal vitamin D*

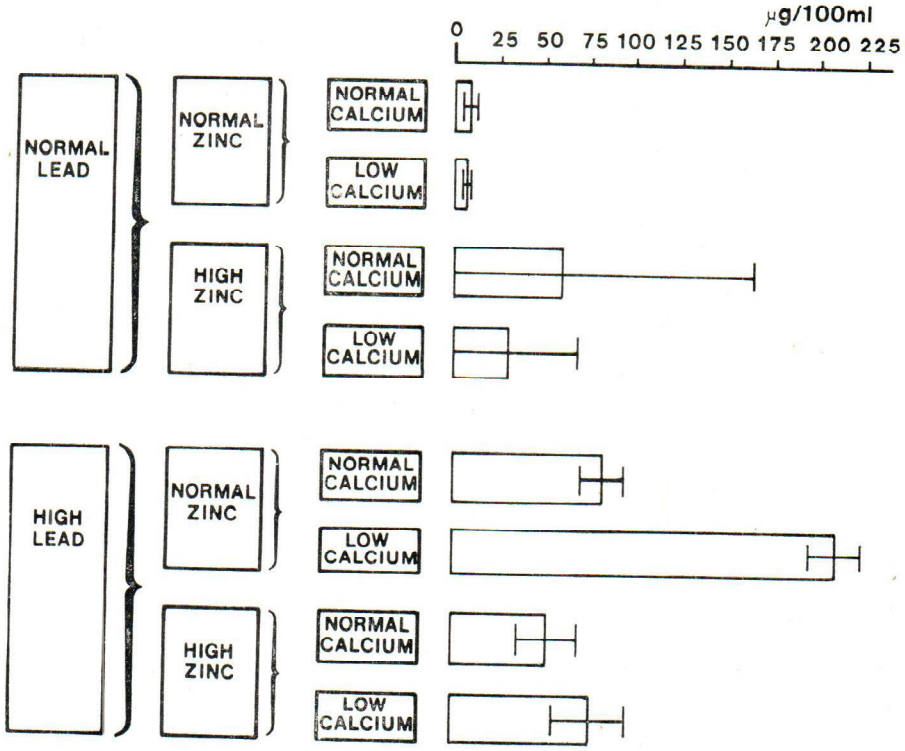

Fig. 15. *3-way interaction lead × zinc × calcium. Blood lead. Normal cadmium & vitamin D*

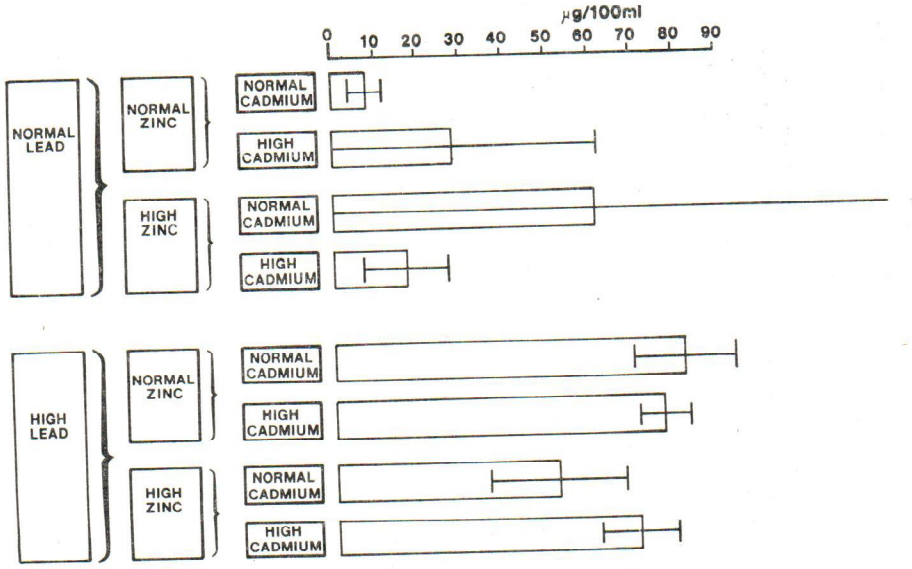

Fig. 16. *3-way interaction lead × zinc × cadmium. Blood lead. Normal calcium & normal vitamin D*

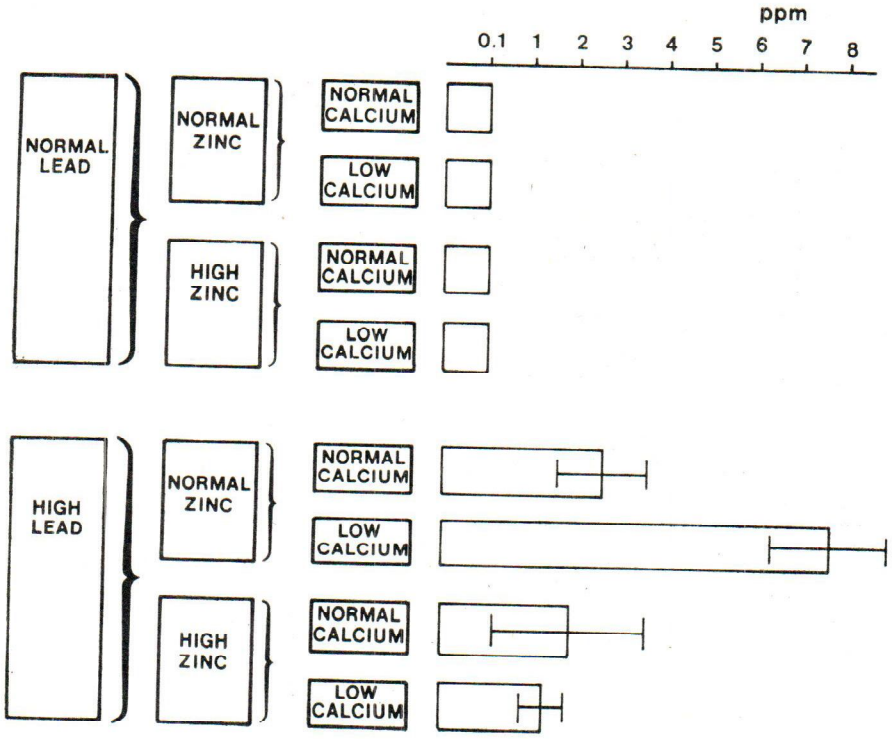

Fig. 17. *3-way interaction lead × zinc × calcium. Liver lead. Normal cadmium & vitamin D*

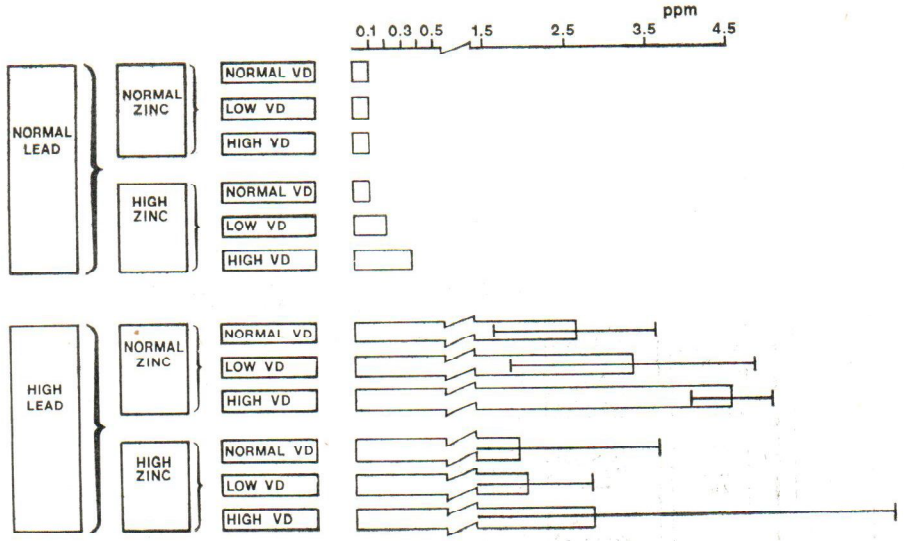

Fig. 18. *3-way interaction lead × zinc × vitamin D. Liver lead. Normal cadmium & normal calcium*

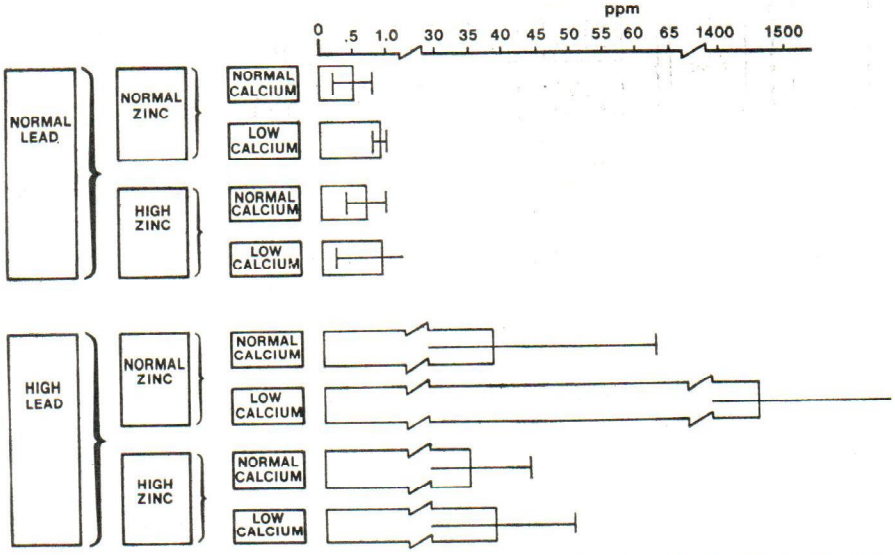

Fig. 19. *3-way interaction lead × zinc × calcium. Kidney lead. Normal cadmium & normal vitamin D*

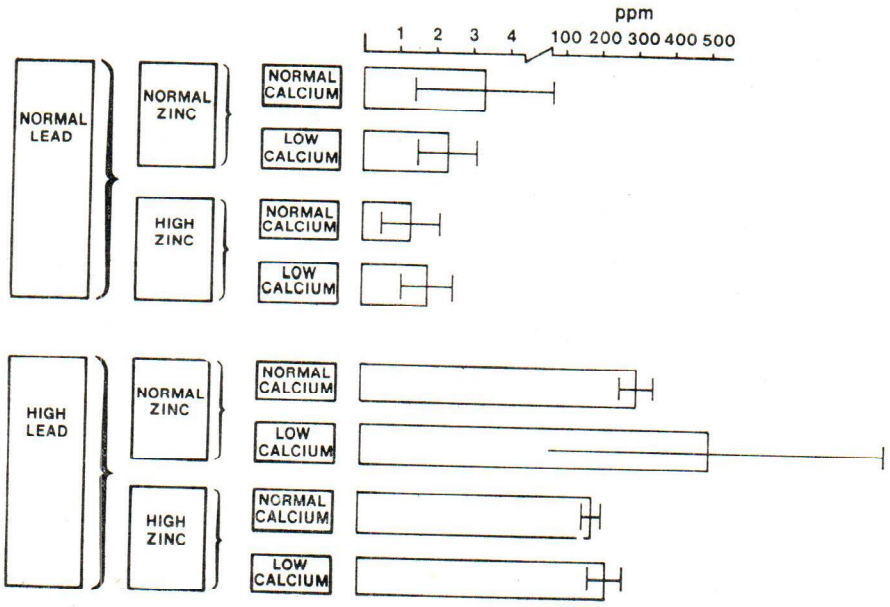

Fig. 20. *3-way interaction lead × zinc × calcium. Bone lead. Normal cadmium & normal vitamin D*

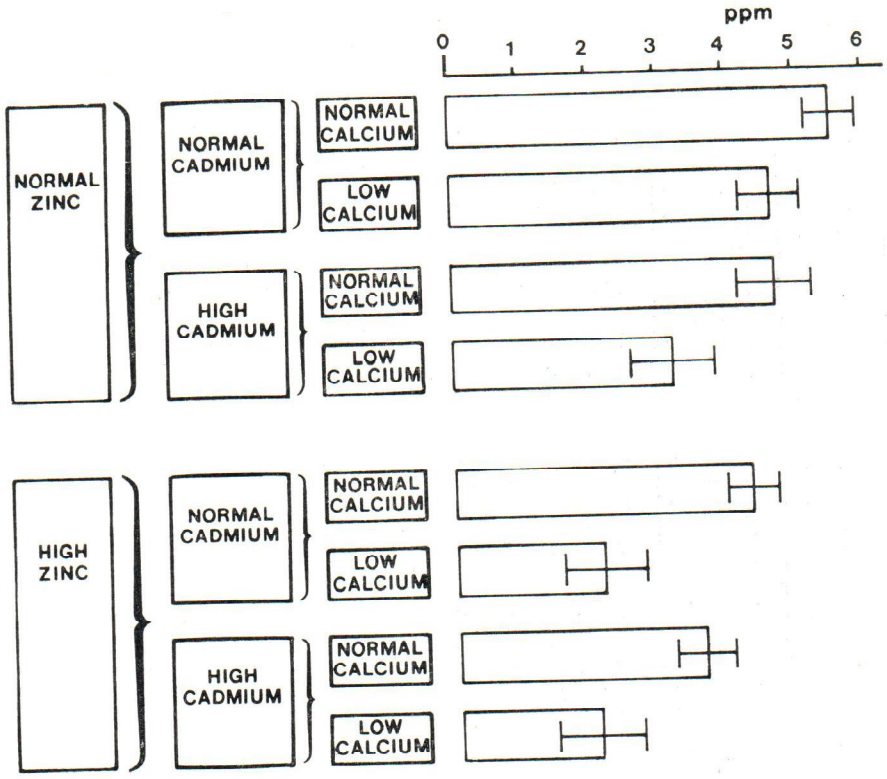

Fig. 21. *3-way interaction zinc × cadmium × calcium. Liver copper. Normal lead & normal vitamin D*

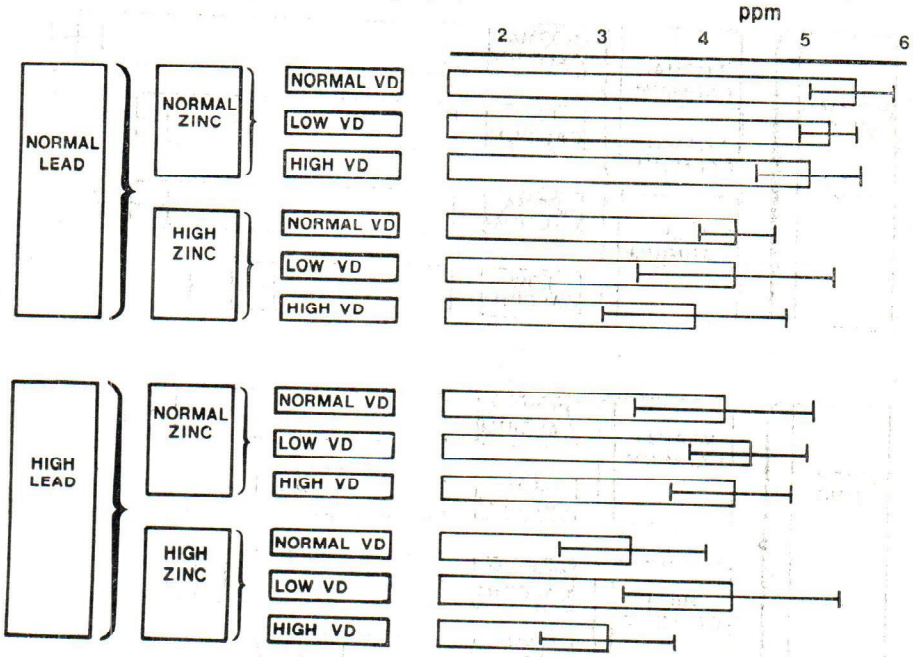

Fig. 22. *3-way interaction lead × zinc × vitamin D. Liver copper. Normal cadmium & normal calcium*

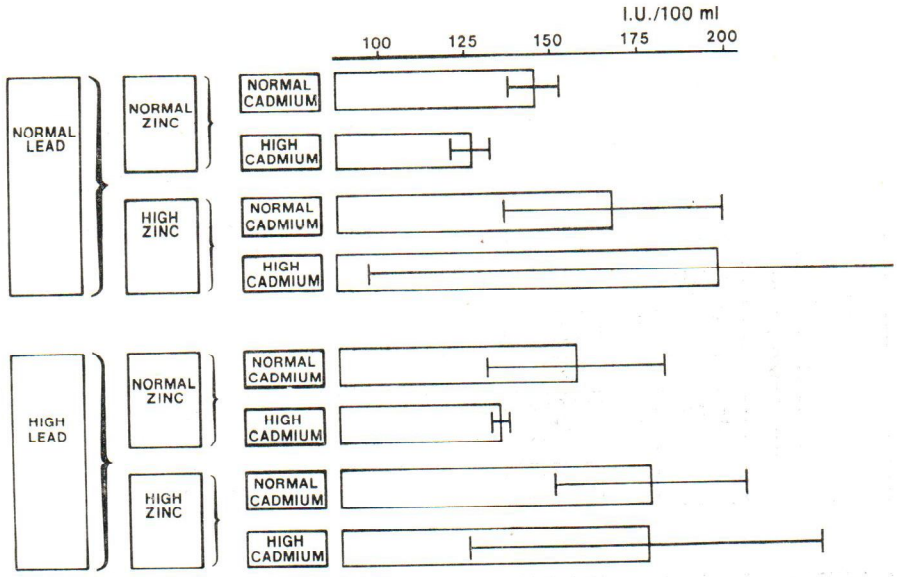

Fig. 23. *3-way interaction lead × zinc × cadmium. Total serum alkaline phosphatase. Low calcium diet & normal vitamin D*

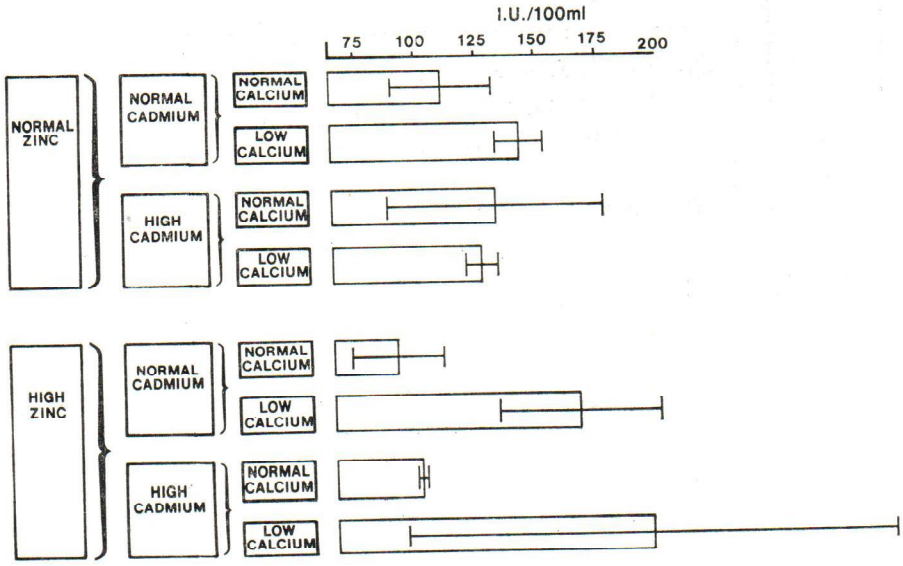

Fig. 24. *3-way interaction zinc × cadmium × calcium. Total serum alkaline phosphatase. Normal lead & normal vitamin D*

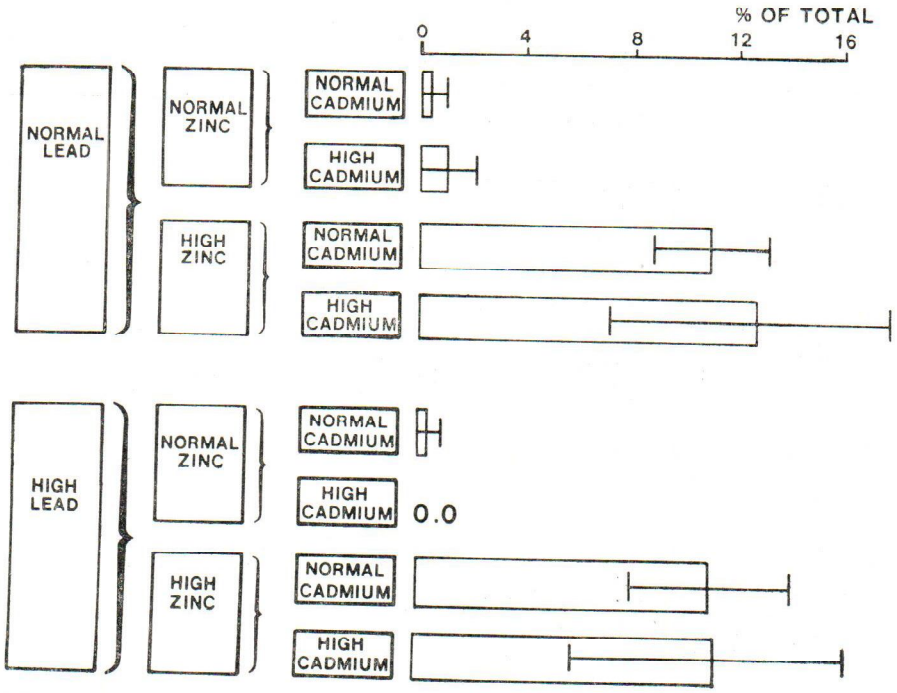

Fig. 25. *3-way interaction lead × zinc × cadmium. % alkaline phosphatase isoenzyme in »liver band«. Low calcium diet & normal vitamin D*

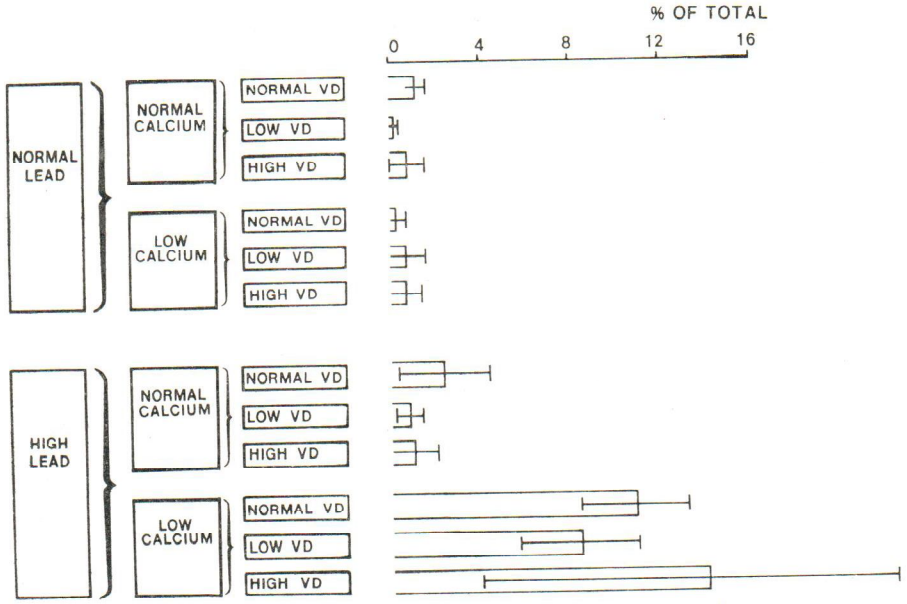

Fig. 26. *3-way interaction zinc × calcium × vitamin D. % alkaline phosphatase isoenzyme in »liver band«. Normal lead & normal cadmium*

References

1. *Mertz, W.:* Problems in Trace Element Research in Trace Substances in Environmental Health — II, University of Missouri, 1968. (Editor D. D. Hemphill).
2. *Davis S. K.:* Interaction of Trace Elements in Trace Substances in Environmental Health — III, University of Misoury, 1969 (Editor D. D. Hemphill).
3. *Mills C. F.:* Trace Element Interactions in Trace Element Metabolism in Animals — 2, University Park Press, Baltimore, Maryland, 1974.
4. *Schmitt, N., Brown, G., Devlin, E. L., Larsen, A. A., McCausland E. D., Saville, J. M.:* Lead poisoning in horses. An environmental health hazard, Arch. Environ. Health, 23 (1971) 185.
5. *Willoughby, R. A., Thirapatsukun, T. McSherry, B.:* Am. J. Vet. Res., 33 (1972) 1165.
6. *Willoughby, R. A., MacDonald, E., McSherry, B. J., Brown, G.:* Can. J. Comp. Med., 36 (1972) 348.
7. *Hupka, E.:* Wien. tieraerztl. Mschr., 42 (1955) 763.
8. *Graham, R., Sampson, J., Hester H. R.:* J. Am. Vet. Med. Ass., 97 (1940) 41.
9. *Thawley, D. G.:* Ph. D. Thesis, University of Guelph, Guelph, Ontario, Canada, 1975.
10. *Aub, J. C.* et al.: Lead Poisoning in Medicine Monographs, Vol. 7, Williams and Wilkins, Baltimore, Maryland, 1926.
11. *Cantrow, A., Trumper, M.:* Lead Poisoning, The Williams and Wilkins Co., Baltimore, 1944.

12. *Parizek, J.:* J. Endocr., *15* (1957) 56.
13. *Petering, H. G.:* The Effect of Cadmium and Lead on Cooper and Zinc Metabolism in Trace Element Metabolism in Animals — 2, University Park Press, Baltimore, Maryland, 1974.
14. *Vallee, B. L., Wacker, W. E. C.:* Metalloproteins in The Proteins, Vol. 5, Academic Press, New York, 1970.

Sažetak

INTERAKCIJA OLOVA S ELEMENTIMA U TRAGOVIMA I PREHRAMBENIM FAKTORIMA

U prijašnjim terenskim istraživanjima utvrđeno je da interakcija između kalcija, olova i cinka ima za posljedicu pojačanje kliničke slike otrovanja u konja u usporedbi s onom što bi je izazvao svaki posebno. Ta su opažanja bila povodom da su započeta opsežna istraživanja interakcija između olova, cinka, kadmija, kalcija i vitamina D. Ova su istraživanja rađena na mladim štakorima a pokusi su trajali po 42 dana. U tom su razdoblju po dva štakora soja Sprague Dawley hranjena s po jednom od 48 kombinacija i ovaj je prehrambeni postupak ponavljan 4 puta. Prije spomenute tvari odabrane su u koncentracijama koje bi kada bi se davale pojedinačno mogle izazvati uočljive toksične učinke, ali bez smrtnog ishoda u toku 42 dana. Koncentracije, izražene u ppm ili u postocima sadržaja hrane bile su slijedeće: olovo: niska koncentracija — 7 ppm, visoka — 5 000 ppm; cink: niska koncentracija — 30 ppm, visoka — 6 300 ppm; kadmij: niska koncentracija — 0,1 ppm, visoka — 90 ppm; kalcij: niska koncentracija — 0,1%, normalna — 0,9%; vitamin D: niska koncentracija — bez vitamina D, normalna — 2 000 IU/kg hrane i visoka — 50 000 IU/kg hrane.

Nakon 42 dana štakori su usmrćeni i mjereno je 39 različitih parametara: 4 vrste mjerenja općeg metabolizma, 10 hematoloških parametara, serumska alkalna fosfataza te određivanje olova, cinka, kadmija, bakra i željeza u jetri, bubrezima i kostima te naposljetku koncentracija olova, cinka, kadmija i kalcija u krvi. Rezultati su jasno prikazani grafički u 26 slika.

Valja istaknuti pojačane toksične učinke olova, cinka i kadmija u štakora hranjenih niskim koncentracijama kalcija. U istih je životinja koncentracija ovih metala u krvi, jetri i bubrezima bila znatno povećana. Slično tome toksičnost je ovih metala rasla s porastom količine vitamina D u hrani. Koncentracija olova u krvi, bubrezima, jetri i kostima bila je manja u onih štakora koji su uz olovo u hrani dobivali cink ili kadmij. Štakori koji su u hrani imali visoku koncentraciju cinka i kadmija rasli su brže od onih koji su u hrani dobili samo cink, ali su imali niži hemoglobin i manje vrijednosti bakra u bubrezima i jetri, kada se te vrijednosti usporede s onima u štakora koji su dobivali sam cink ili sam kadmij. Smanjenje koncentracije bakra u jetri i bubrezima uz povećanje koncentracije žcljeza u jetri utvrđeno je u onih štakora koji su hranjeni niskim sadržajem kalcija. Visok sadržaj cinka u hrani imao je za posljedicu značajno povećanje aktivnosti serumske alkalne fosfataze.

DISCUSSION FOLLOWING THE PAPER

TEPPER: Some interactions (chemical or physical) may occur in the gastrointestinal tract, rather than after absorption of dietary factors has occurred. For example calcium may influence phosphate, and phosphate may influence the absorption of metals. These strictly intra-lumenal interactions may lead to confusion in interpretation if the investigator regards all phenomena as related to organ or cellular biochemistry. How do you consider these possibilities in your research? Or how would you do so as your work progresses?

WILLOUGHBY: With the experimental methods used, we were unable to determine with precision whether a specific interaction on one parameter was occurring during absorption, distribution, metabolism or excretion. However, when the overall picture of statistically significant main effects and interactions is examined, it is possible to see that in some cases the elements are in fact being absorbed whereas in others it seems that the element may not have been absorbed. Kinetic techniques will be needed to specifically determine where and how these interactions are occurring.

ROSMANITH: Zu den Interaktionen zwischen Blei, Cadmium und Zink: Wir haben eine epidemiologische Unternehmung am Kindern aus einem mit Blei, Zink und Cadmium belasteten Industriegebiet durchgeführt. In dem erwähnten Gebiet wohnen etwa 9 000 Kinder im alter von 2—13 Jahren. Durch zufällige Auslese wurden aus dieser Zahl über 600 Kinder ausgewählt und zur Untersuchung eingeladen. Zur Untersuchung kamen etwa 400 Kinder (66%). Bei ihnen wurden Blei, Cadmium und Zink im Vollblut, im Urin und den Haaren atomabsorptionsspektrophotometrisch bestimmt. Im Urin wurde zugleich auch δ-ALA untersucht und auf Kreatinin bezogen. Die Ergebnisse der Analyzen wurden auf Gegensetige Abhängigkeit untersucht (t-Test Chi_2-Test, Spearman-Rang-korrelations-koefficienten Test).

Je höher die Blutbleikonzentration ist, desto höher sind auch die Haarblei-, Haarzink- sowie Urinzinkkonzentrationen und desto miedriger sind zugleich die Blut und Urincadmiumkonzentrationen sowie die Ausscheidung von δ-ALA durch Urin.

Bei gleichzeitiger kontamination mit Blei und Cadmium änderte die Intenzität der Bleiaufnahme das Verhalten des Cadmiums, indem eine erhöhte Bleiaufnahme die Cadmiumretention zu erhöhen schien.

BERLIN: From the field studies on horses exposed to high environmental levels of lead and zinc, could you indicate what lead levels in the animal diet are necessary to produce clinically observable effects?

WILLOUGHBY: There is a large number of variables which exert profound influences on the development of lead poisoning in horses. These variables include: calcium, phosphorus and vitamin D nutrition; age; concurrent exposure to zinc and possibly cadmium; and intercurrent viral, bacterial and parasitic diseases. Thus it is not possible to cite one level of exposure which will produce subclinical and clinical changes or death. If the associated variables collectively enhance the toxicity of lead, naturally the concentration of lead required to enduce toxicity will be lower than when the converse is true. For these reasons, one must always assess the casually associated factors in addition to lead, while attempting to prove a cause-effect relationship and there is no standard of lead exposure which will cause lead poisoning in horses.

THRON: a) During the first part of this very interesting report, a different behaviour of young cattle with respect to lead poisoning etc., as compared to young horses, has been mentioned. Is this a regular finding in the countryside investigated by Dr. Willoughby, and how would he explain this difference?

WILLOUHBY: a) We think that cattle are more tolerant to high concentrations of zinc than are young growing horses. This difference in tolerance probably explained why young growing horses did not survive in the vicinity of a lead-zinc smelter while cattle raised in the same area did survive.

b) No, we have not studied the influence of different concentrations of magnesium in the diet on the uptake or toxicity of Pb, Zn or Cd. However, the well known interactions of Ca and Mg within the animal and of Ca and Pb within the animal would indicate that there will be significant interactions among Pb and Mg.