BIOCHEMICAL MECHANISMS OF CADMIUM-INDUCED ANEMIA

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Like so many other nonferrous metals, the production and use of cadmium has shown tremendous expansion in the last two decennials. New important fields of its utilization have been created by the development and increased use of nuclear energy devices, modern electronic equipment and last but not least--space research.

Cadmium is well known to be toxic to humans and animals. Chronic exposures cause loss of weight, anemia, dysproteinemia, proteinuria increased aminoaciduria, emphysema and bone lesions. It has been suggested more recently by American investigators that the steady accumulation of cadmium in the kidney with age may play a role in the development of hypertension and atherosclerotic heart disease.

The biochemical mechanisms underlying those phenomena are not well understood. In particular, the mechanism of cadmium-induced anemia was almost completely unclear when this investigation was started. Although since then some hitherto unknown facts have been revealed, the problem of cadmium-induced anemia is still far from being elucidated.

The whole problem is complex and may be approached from many different angles. In this investigation attention has been concentrated on the biosynthetic pathway of porphyrins in both in vitro and in vivo experiments. In addition, some aspects of protein metabolism have been added. Heavy emphasis has been put on the interdependence of cadmium with some other metals as zinc, copper, and lead.

As pure industrial cadmium exposure cases were not available, the work was limited to experiments on animals. As a matter of fact, Polish workers engaged in cadmium production are liable to a multi-metal exposure with a heavy impact on anemia causing agent such as lead.

The ultimate aim of this investigation was to find--in accordance with modern trends in industrial toxicology--one or more pretoxic test which would facilitate the early diagnosis of imminent clinical intoxication syndromes.

Cadmium is a very tricky toxic agent. It accumulates in the soft tissues with special preference for liver and kidney from where it

does not and can not be removed for many years, even after the cessation of exposure, going on to exert its harmful effects on the whole organism. In view of that, the availability of a reliable exposure test is of crucial importance for the prophylaxis of cadmium intoxications and for maintaining the health of exposed people at a level required by modern medicine.

To summarize, the objectives of the investigations were:

- to elucidate the influence of cadmium on porphyrin metabolism.
- to examine the effects of simultaneous exposure to cadmium and metals which are most frequently accompanying cadmium in industrial operations.
- to find a pretoxic test which would make it possible to work out prophylactic measures based on early biochemical derangements.

In order to answer the important question whether cadmium exerts an influence on the metabolic pathway leading from simple chain metabolites to the complex porphyrin ring, several series of experiments in vitro have been performed in which in presence of a broad range of different cadmium concentrations delta-aminolevulinic acid has been incubated with tissue homogenates and red blood cell suspensions and the rate of porphyrin formation measured.

Figure 1 presents the rate of protoporphyrin formation as plotted against increasing molar cadmium concentrations. The biosynthesis activity in the presence of cadmium is expressed as the percentage of values obtained for the control samples which have been incubated in parallel with delta-aminolevulinic acid but did not contain any added cadmium ions. Each point represents an average value of tissue samples taken from 15 different animals. To avoid contamination of tissues with the synthesizing system present in red blood cells, the livers and kidneys have been perfused in vivo with isotonic saline solution.

As can be seen, cadmium exerts a slightly stimulating effect up to the molar concentration of 10^{-5}. Its further increase is accompanied by a strong inhibition of protoporphyrin formation. The course of the curve depends on the biological material tested. Liver and kidney follow an almost identical course. The porphyrin synthesizing system present in blood is a little more resistant to cadmium ions than that of the other tested tissues. At a concentration equal to 10^{-3} when porphyrin formation comes to a stop in the liver and kidney, the erythrocytes retain most of their initial synthesizing capability.

A similar picture has been obtained for the coproporphyrin formation in presence of cadmium (Figure 2).

Similar to the protoporphyrins, the accumulation of coproporphyrins becomes strongly inhibited in molar concentrations between 10^{-5} and 10^{-3} with the red blood cells showing less sensitivity to cadmium ions than the other tissues.

Additional chromatographic studies did not disclose any of the abnormal types of porphyrins which have been reported in experiments with lead and other anemia causing agents.

The hitherto discussed experiments have been carried out in tissues of healthy, unexposed animals. An identical experimental procedure was followed in tissues of animals to whom cadmium was administered parenterally and who developed cadmium deposits in the tissues tested. A comparison of data obtained in these two types of experiments should provide a clue to the question whether cadmium deposited in liver and kidney was active metabolically or if it was inactive in respect to the pathway under investigation. In the first case, a surplus in the tissue concentration should have shifted the curve obtained in vitro towards values reflecting higher cadmium concentrations. The results are shown on Figure 3. The continuous line represents protoporphyrin formation in the liver of unexposed animals. The dotted line shows the same process in livers of animals previously subjected to parenteral administration of cadmium chloride which caused after several weeks an average final cadmium concentration of approximately 0.5 mg per gram fresh liver. This means 4×10^{-4} M in the incubation mixture. The two curves are different. It is evident that the cadmium deposited was bound in an inactive compound, most probably with liver proteins.

To examine the influence of cadmium on porphyrin formation in the presence of zinc, both metals have been incubated in different concentrations with delta-aminolevulinic acid, the formed porphyrins separated and determined quantitatively. Zinc is known to exert some antagonistic effects on cadmium and has been claimed to behave as its antimetabolite.

To evaluate the effect of multimetal exposure, we have to know the effect exerted by the single metals involved first. The results obtained with zinc alone are shown on Figure 4. It shows that molar concentrations higher than 10^{-4} cause a stepwise inhibition of porphyrin formation which ceases at 10^{-1}. The inhibition range runs through a comparatively broad concentration range.

Figure 5 shows the influence of three different concentrations of zinc on the protoporphyrin output in liver in the presence of three different

cadmium concentrations. Only the range in which rapid changes in the investigated activity occur has been plotted. The continuous curve represents the results obtained with cadmium alone. Values representing simultaneous cadmium and zinc exposure have been marked by crosses. The corresponding small circles represent the respective activities displayed in experiments with single zinc exposure and serve as reference points.

The analysis of data given on this slide points to the conclusion that at low cadmium concentrations of 10^{-7} - 10^{-5} which by themselves do not cause any derangements in porphyrin formation, the addition of zinc does not alter significantly the overall picture of the process involved. However, at critical cadmium concentrations of 10^{-3}M, zinc added in sufficiently strong concentrations (10^{-3}M) can substantially reduce the inhibition caused by cadmium.

Similar experiments have been performed on porphyrin formation in presence of both cadmium and copper. The activity of protoporphyrin biosynthesis in presence of copper alone is shown on Figure 6.

It can be seen that the curves reflecting the activity of protoporphyrin formation in presence of copper are almost identical with those which illustrate the rate of this process in presence of cadmium. A slight increase in porphyrin formation up to the molar concentration of 10^{-5} is followed by a strong and rapid inhibition which brings the process to a stop at 10^{-3} in liver and kidney and to approximately 20% of the initial activity in the erythrocytes. In view of the fact that copper has been found to play a direct role in the synthesis of haem, this seems to be a very interesting finding.

Even more interesting are the results of incubation experiments in presence of both cadmium and copper (Figure 7). It is evident that concentrations of cadmium which by themselves do not cause any significant changes in protoporphyrin formation (10^{-7} - 10^{-5} M) can reduce to a considerable extent the inhibition caused by high concentrations of copper. On the contrary, copper has no ability to alleviate the inhibition caused by cadmium.

And finally, the simultaneous effect of cadmium and lead on porphyrin formation has been studied. The effects of the presence of lead alone are shown in Figures 8 and 9.

At very low concentrations lead exerts a stimulating effect on the biosynthesis of protoporphyrin from delta-aminolevulinic acid. The inhibition begins at the molar concentrations of 10^{-3} in liver and kidney and 10^{-5} in erytrocytes. Incidentally, the strikingly stronger

resistance of the liver and kidney tissues as compared with red blood cells has been also known from experimental lead intoxication in animals.

Figure 10 illustrates the rate of protoporphyrin formation in liver in the presence of cadmium and lead. At concentrations in which cadmium by itself does not inhibit protoporphyrin synthesis, it strengthens the stimulating action of lead. On the contrary, lead at sufficiently great concentrations (10^{-4} - 10^{-3}) can reduce to a great extent the inhibition caused by cadmium.

Almost all the data presented so far were concerned with protoporphyrin formation. Corresponding data concerning coproporphyrins do not introduce any substantially new elements into the problem. They rather strengthen the data presented so far.

The last and very important step in haem synthesis, the incorporation of iron into protoporphyrin, is still under investigation and results are not available at this moment.

To summarize, the results so far obtained from experiments in vitro indicate that:

- cadmium exerts a strongly inhibiting effect on the biosynthesis of protoporphyrin in molar concentrations higher than 10^{-3}.

- zinc can reduce the inhibition caused by cadmium to a certain extent.

- copper has no effect on the inhibition of porphyrin formation caused by cadmium whereas cadmium can reduce to a considerable extent the inhibiting effect of copper.

- lead can reduce substantially the inhibiting effect of cadmium on the biosynthesis of protoporphyrin.

The problem arises whether, in which way and to what extent these results can find confirmation from experiments performed in vivo.

In the vivo experiments more than 200 rabbits have been used, divided in groups of 15 to 30 animals according to the type and duration of exposure. Cadmium was administered intravenously as a chloride solution in a daily dose of 1 mg per kg body weight. The control animals received injections of isotonic saline solution instead.

The dose of 1 mg cadmium/kg body weight proved most convenient. It caused in a reasonable time the development of a typical cadmium intoxication syndrome with marked anemia. In addition, the deposits of cadmium in tissues attained the same range of concentrations in which important derangements in porphyrin metabolism have been disclosed in experiments performed in vitro. Attempts to use doses of 2 and even 5 mg per kg body weight caused most animals to die within 24 hours. Additional metals have been given by mouth at a level of 400 ppm for zinc and 100 ppm copper in the ingested food.

It turned out quickly that a period of 6 to 10 weeks of cadmium administration was most suitable. The full intoxication syndrome developed by the fifth or sixth week. At the chosen daily dose the experiment could not be carried much longer because of heavy death toll to approximately 30% of the animals.

The development of cadmium anemia is illustrated on Figure 10. The continuous lines represent the intoxicated animals, the dotted ones the controls. The mean hemoglobin concentration in peripheral blood in a group of 15 rabbits during a 6 week period of cadmium administration at a daily dose of 1 mg per kg body weight dropped steadily to approximately 60% of the initial value.

The red blood cell count displays a continuous fall as well. After a six week exposure the mean group values attain approximately 70% of the initial value. The comparison with hemoglobin behaviour indicates a slightly hypochromic type anemia. The right side of the slide shows the growth of the animals. The control rabbits gained weight while those receiving cadmium displayed lost weight in parallel to the drop of hemoglobin content and red cell count.

Figure 11 illustrates the course of the Price-Jones curve in a group of cadmium intoxicated animals as compared with controls. A distinct shift to the left and a flattening of the curve indicates that cadmium-induced anemia is of the microcytic type.

Figure 12 shows the effect on hemoglobin content and red blood count of the simultaneous administration of cadmium and zinc. The separate lines represent groups of animals which received cadmium alone, zinc alone, cadmium and zinc and controls receiving none of those metals. To bring the lines to a common reference, the values are given as percents of the values obtained at the beginning of the experiment.

As can be seen, zinc has by itself caused a drop in hemoglobin content. In combination with cadmium a cumulative effect is present, producing

a very deep drop both in the hemoglobin content and the red blood count.

Figure 13 gives the results of experiments in which in a similar way the interdependence of cadmium and copper has been checked. It is evident that copper alone in the doses used has no effect on hemoglobin content or red blood count. In contrast to zinc the administration of copper alleviated the cadmium-induced anemia. This was particularly evident in the red blood count values.

In view of the fact that cadmium and lead exert some antagonistic effects on porphyrin biosynthesis, the urinary excretion of haem precursors in cadmium intoxication has drawn special attention. It is well known that lead causes a strongly increased urinary excretion of delta-aminolevulinic acid and coproporphyrins both in men and in rabbits. In addition, in rabbits an increased output of porphobilinogen is observed.

Figure 14 presents the excretion of all three haem precursors in a group of cadmium intoxicated rabbits. The black blocks represent the mean group values and the white ones the respective standard deviations. The excretion at the beginning of the experiment is compared with that found at the end of the sixth week. It is obvious from the diagram that no statistically significant changes in the excretion of haem precursors have been found in cadmium intoxication.

Despite the negative outcome of the investigations on the excretion of delta-aminolevulinic acid and porphobilinogen, the determination of the activity of the enzyme responsible for the biosynthesis of porphobilinogen from delta-aminolevulinic acid namely the delta-aminolevulinic acid dehydratase (ALAD) brought some unexpected results. The red blood cells of almost one hundred animals have been tested for the enzymes' activity. They represented the following exposures: cadmium alone, cadmium and zinc, zinc alone, cadmium and copper, copper alone and the control group receiving none of those metals.

Figure 15 illustrates a part of the results. The ALAD's activity is expressed as units per 1 milliliter of red blood cells. As can be seen, cadmium causes a rapid and strong influence on erythrocytic ALAD--increasing its activity. Already after a ten day exposure a significant change is observed. After 40 days a fourfold increase in respect to the initial value is produced.

Zinc had no direct significant influence on the enzyme but reduced the effect of cadmium--suggesting some antagonistic action. The

administration of copper did not change the activity of ALAD by itself nor did it influence the changes caused by cadmium. It is well established by now that lead exerts a strong inhibiting effect on ALAD. This effect has been used for the detection of lead exposure. It seems as if the same test with the opposite direction of changes could be used for the detection of cadmium exposure. It could not be proved on humans so far, because of lack of individuals exposed exclusively to cadmium.

The question arises what was the mechanism of the activation of ALAD by cadmium ions. Only hypotheses can be put forward at this moment. According to American investigators copper is necessary for the activity of this enzyme. A tentative explanation based on the assumption of the possibility of a cumulative effect of cadmium and copper has still to be verified.

So far, the anabolic part of haem metabolism has been discussed. A few words about its catabolic part. The only attempt to explain the mechanism of cadmium-induced anemia presented so far was made by Swedish investigators who claimed to have found increased hemoglobin breakdown and associated cadmium anemia with a hemolytic type anemia. Some years ago, when no data on the derangements of haem biosynthesis caused by lead were available, the mechanism of lead-induced anemia was explained by increased hemoglobin breakdown as well. This view could not be held in view of new experimental data.

Under this investigation the level of serum bilirubin in cadmium intoxicated animals has been checked. No increase in this parameter has been found. Increased erythrocyte destruction does not seem to play the chief role in cadmium-induced anemia.

It is well known that cadmium intoxication is accompanied by derangements in protein metabolism. Anemia may be just an expression of these derangements. While the synthesis and metabolism of proteins in the developing and maturing blood cells have not been followed in this investigation, the experimental animal material was used for a broad spectrum of analyses thought to be helpful in elucidating the investigated problem although seeming not to be directly correlated with anemia at the first glance.

The electrophoretic pattern of serum proteins in cadmium intoxication has been investigated in several countries. In this investigation the moving boundary electrophoresis technique was used and the existence of hypoalbuminemia, and hyperalpha$_2$- and gamma-globulinemia were confirmed (Figure 16).

This technique was introduced to the separation of the water soluble proteins of liver and kidney as well. There was evidence of a reverse trend in these tissues showing a positive shift in the albumin fraction and a negative one in the gamma-globulins in both liver and kidney proteins after cadmium administration. Figure 17 shows a typical electrophorogram of the water soluble proteins of liver. A typical picture seen in cadmium intoxication is shown on Figure 18.

The existence of cumulative, respectively antagonistic biological effects of cadmium with some other bivalent ions and the interference with protein metabolism directed the attention to the behaviour of some metalloproteins displaying enzymatic activity. The results obtained with a cuproprotein--delta-aminolevulinic acid dehydratase--have been already discussed.

The activity of a zincoprotein namely the alkaline phosphatase has been studied extensively. It turned out that in cadmium intoxicated animals the activity of this enzyme in liver was significantly higher than in the controls. Simultaneous administration of cadmium and zinc gave even higher results than cadmium alone. This was observed also in the pancreas. Animals fed zinc alone did not show any changes in the enzyme's activity. The administration of copper had neither effect by itself nor on cadmium-induced changes.

The acid phosphatase of animals intoxicated with cadmium was checked and a high increase of its activity in plasma found. The administration of neither zinc nor copper exerted any influence on these changes. On the other hand neither carbonic anhydrase nor carboxypeptidase A showed changes in their activity in cadmium-intoxicated animals.

To get an idea to what extent the biochemical derangements found were correlated with the quantity of free SH groups, these were determined in some of the cadmium-intoxicated animals.

Determinations in whole blood, deproteinized blood and serum have been performed. At a highly significant level of $p < 0.01$, a loss of 31% of the free SH groups in cadmium animals as compared with the control ones was found.

No significant changes in the deproteinized blood have been seen, which means that cadmium affected the protein SH groups. The lack of changes in serum as compared with full blood indicated that the derangement observed was localized in the red blood cells. It is just that part of blood where most of the cadmium present is localized.

A few lines about iron, a factor which may play an important role in the development of anemia. The level of iron in serum of cadmium-intoxicated animals was decreased as compared with control animals, whereas the iron deposits in liver were significantly higher. The answer to the question whether the utilization of iron was reduced by cadmium will be given by the investigations on the haem synthetsis.

Another metal which was found in increased amounts in livers of animals receiving cadmium was zinc. It seems as if the organism was trying to compensate the effects of the toxic cadmium by the retention of one of its antimetabolites. On the other hand, the additional administration of zinc did not influence the rate of cadmium accumulation in the tested tissues. It prevented however, the cadmium-induced sideropenia.

Contrary to zinc, the cadmium-intoxicated animals which received supplemental copper displayed decreased copper deposits in liver and an increased elimination of copper with urine as compared with animals receiving the same amounts of copper without cadmium.

All this shows that the interdependence of trace elements is a very complex one. It will need much time until all these relations and their meaning will be known.

From the data presented in this paper two facts are undisputable. First, cadmium exerts in a certain range of concentrations an inhibiting effect on the biosynthesis of porphyrins in vitro. Secondly, cadmium causes, under a sufficiently heavy exposure, a strongly expressed anemia in vivo. How can the two findings be brought to a common denominator? The clue lies in the concentrations of cadmium ions in the reacting mediums. It is a comparatively common mistake among biochemists and toxicologists to transfer conclusions drawn from experiments in vitro to life conditions in disregard of the concentrations of the active agent involved. And that is done in defiance of the commonly known fact that biologically active compounds may show in the same process reverse actions depending on their concentration. In this study that pitfall has been avoided by applying such doses in vivo which caused in a reasonable time tissue concentrations comparable to those which have been proved as causing distinctive biochemical derangements in vitro.

Having succeeded in that it seems justified to draw the conclusion that the interference of cadmium with the biosynthesis of porphyrins may play an important role in the mechanism of cadmium-induced anemia. There is lack of evidence in some points where investigations

are still going on but the data collected and presented so far give unequivocal proof of the involvement of cadmium ions in the mentioned way.

In addition, the dependence of the biological activity of one metal on the presence of other ones has found new evidence in the industrial exposures which may cause medical problems with exposed people.

And finally, a comparatively simple test has been found which might be helpful in the early diagnosis of cadmium intoxications.

As stated in the beginning, the attention in this investigation has been concentrated on the porphyrin metabolism. The probing into other metabolic pathways proved that it is worthwhile to enlarge substantially the investigations on cadmium-induced biochemical derangements as understood in a very broad meaning.

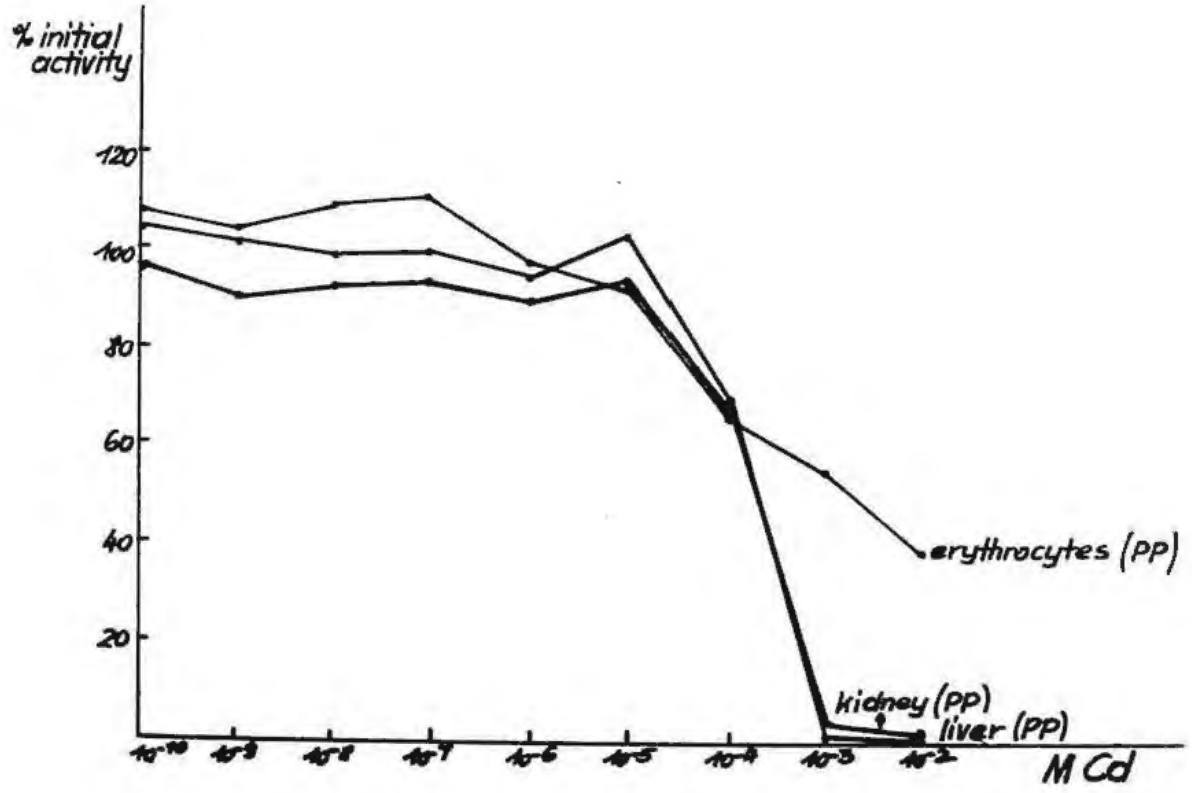

Figure 1. Protoporphyrin (PP) formation from delta-aminolevulinic acid in presence of different cadmium (Cd) concentrations.

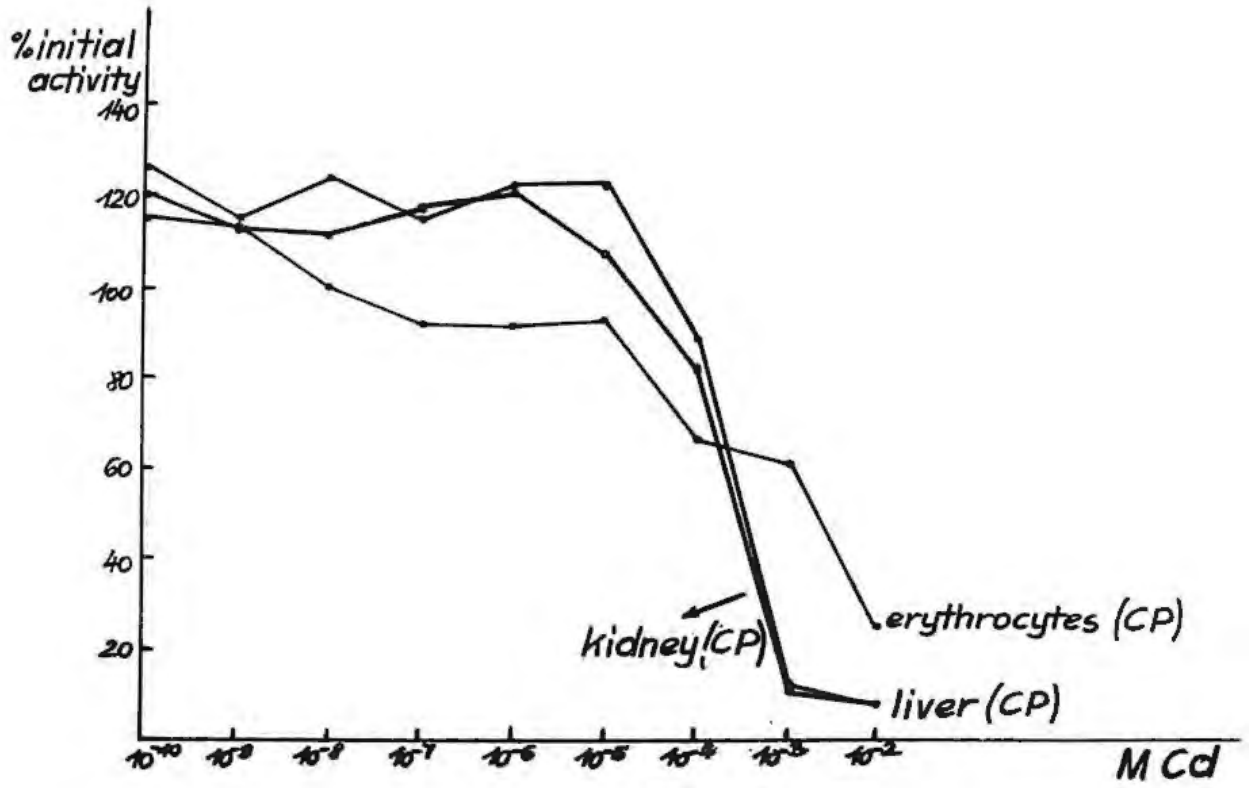

Figure 2. Coproporphyrin (CP) formation from delta-aminolevulinic acid in presence of different cadmium (Cd) concentrations.

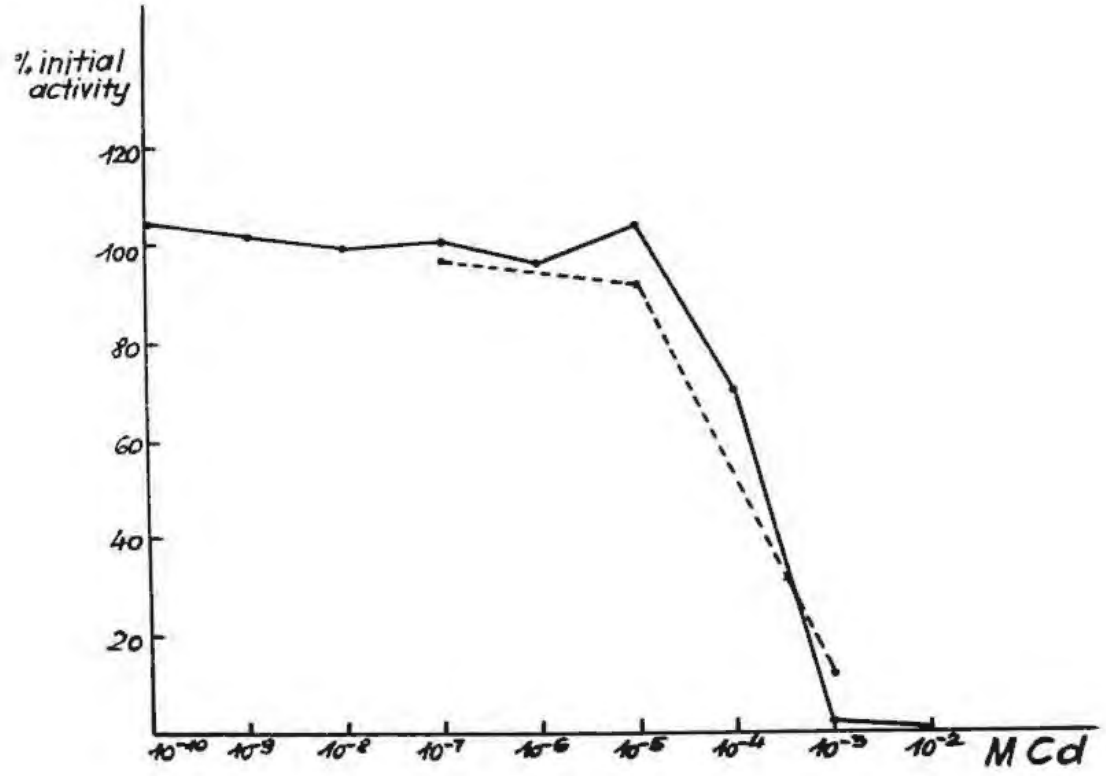

Figure 3. Protoporphyrin formation from delta-aminolevulinic acid in presence of different cadmium concentrations in livers of unexposed animals (continuous line) and animals previously administered cadmium parenterally.

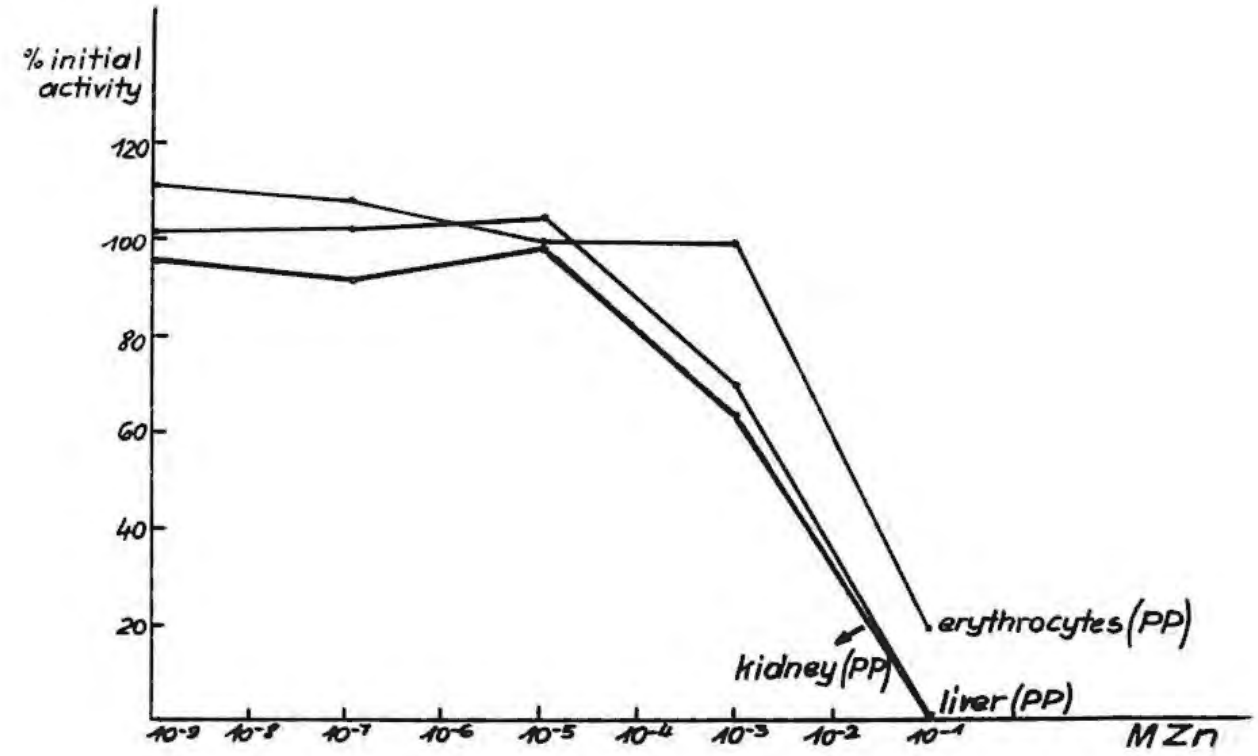

Figure 4. Protoporphyrin formation from delta-aminolevulinic acid in presence of zinc.

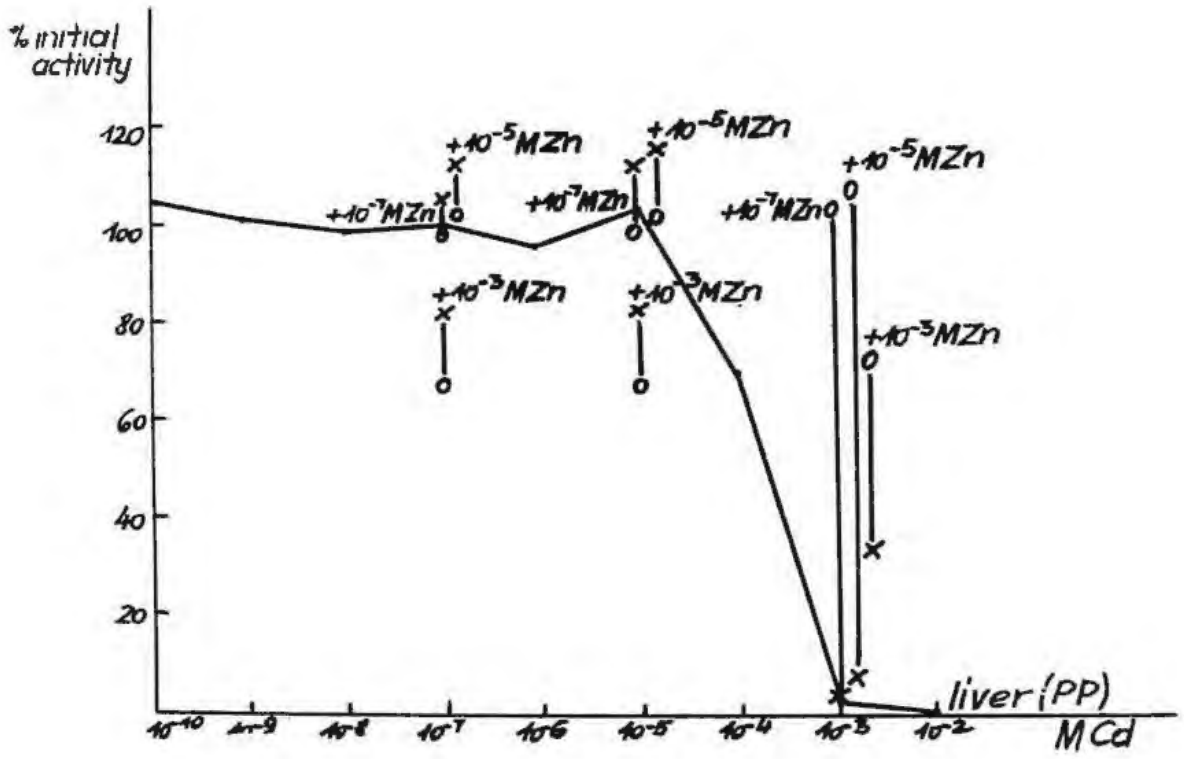

Figure 5. Protoporphyrin formation from delta-aminolevulinic acid in presence of cadmium and zinc.

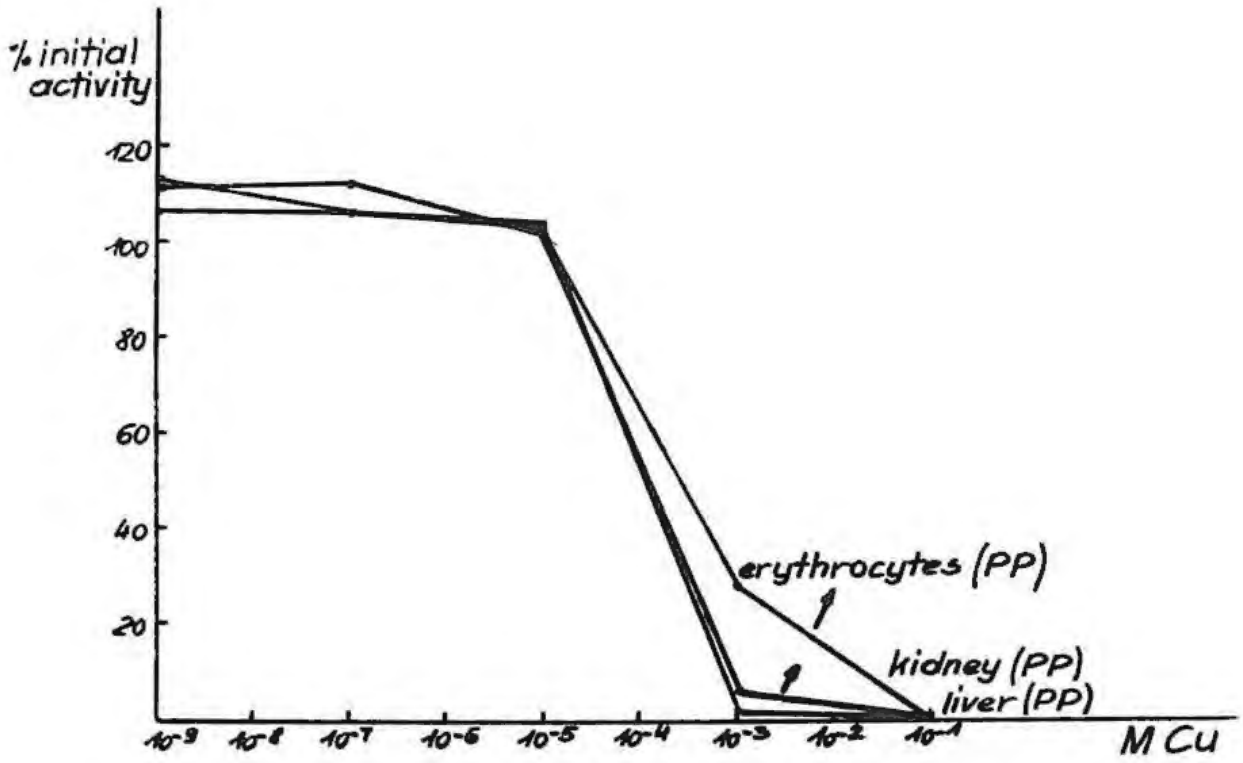

Figure 6. Protoporphyrin formation from delta-aminolevulinic acid in presence of copper.

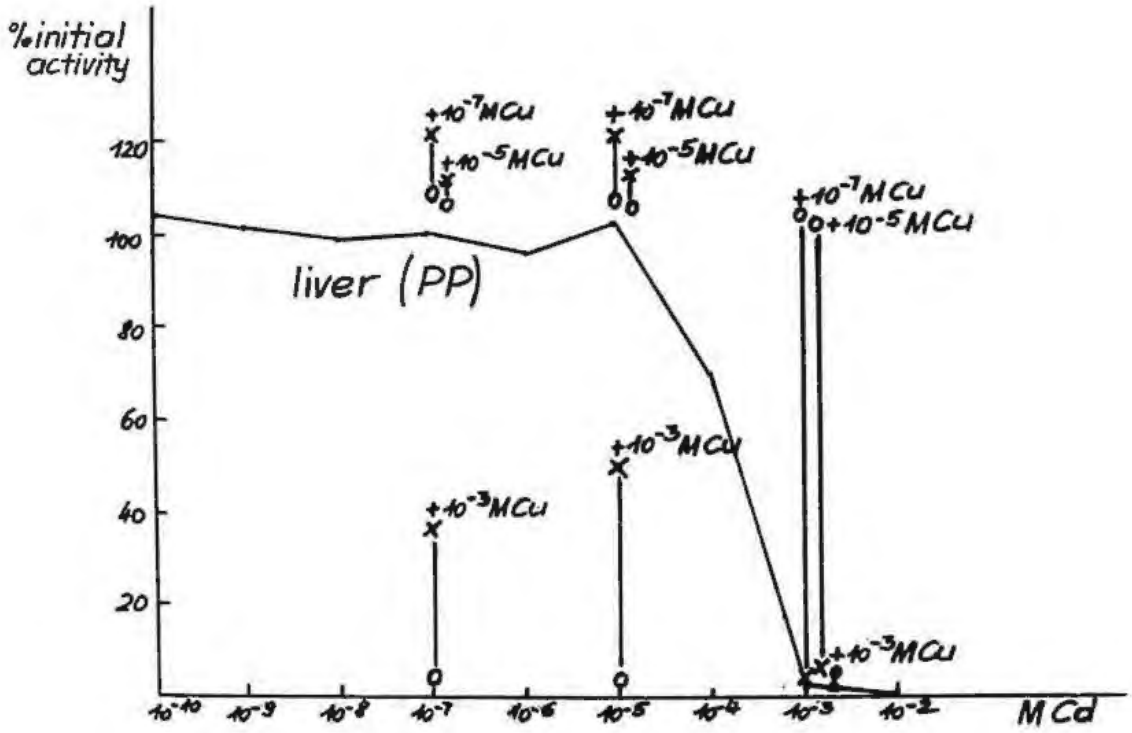

Figure 7. Protoporphyrin formation from delta-aminolevulinic acid in presence of cadmium and copper.

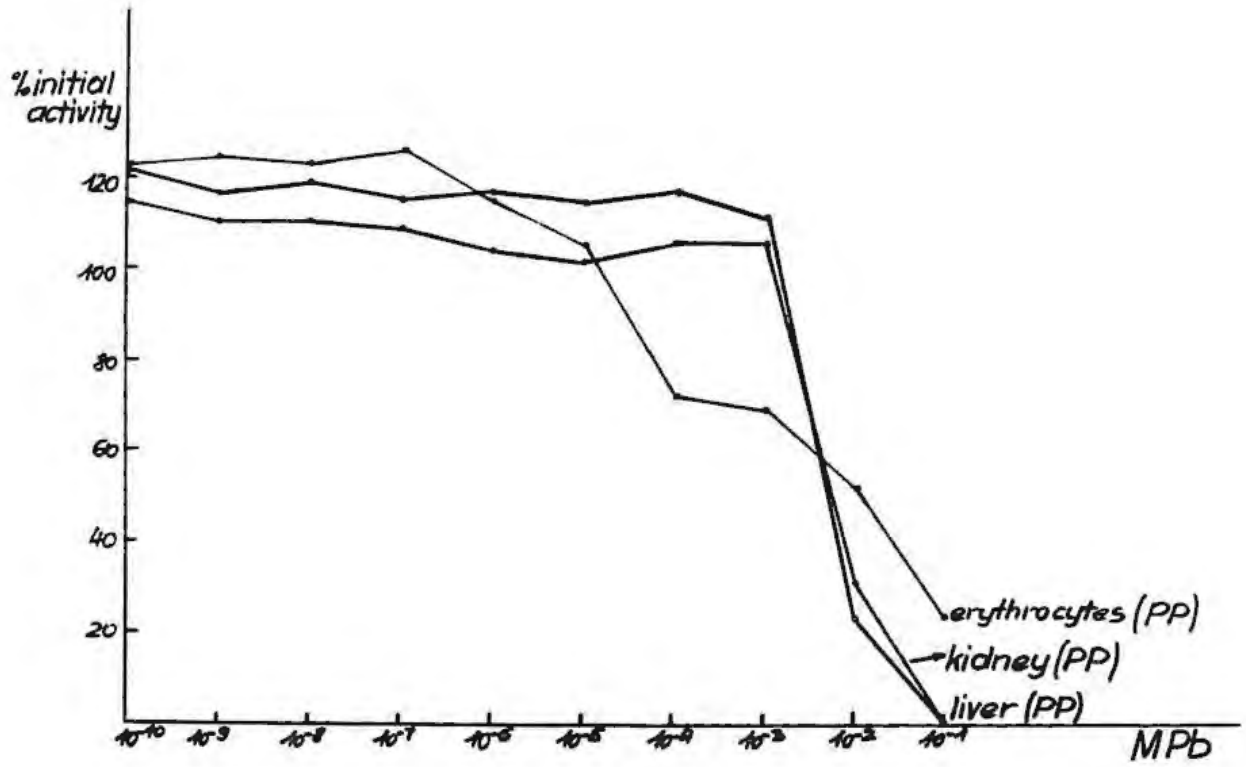

Figure 8. Protoporphyrin formation from delta-aminolevulinic acid in presence of lead.

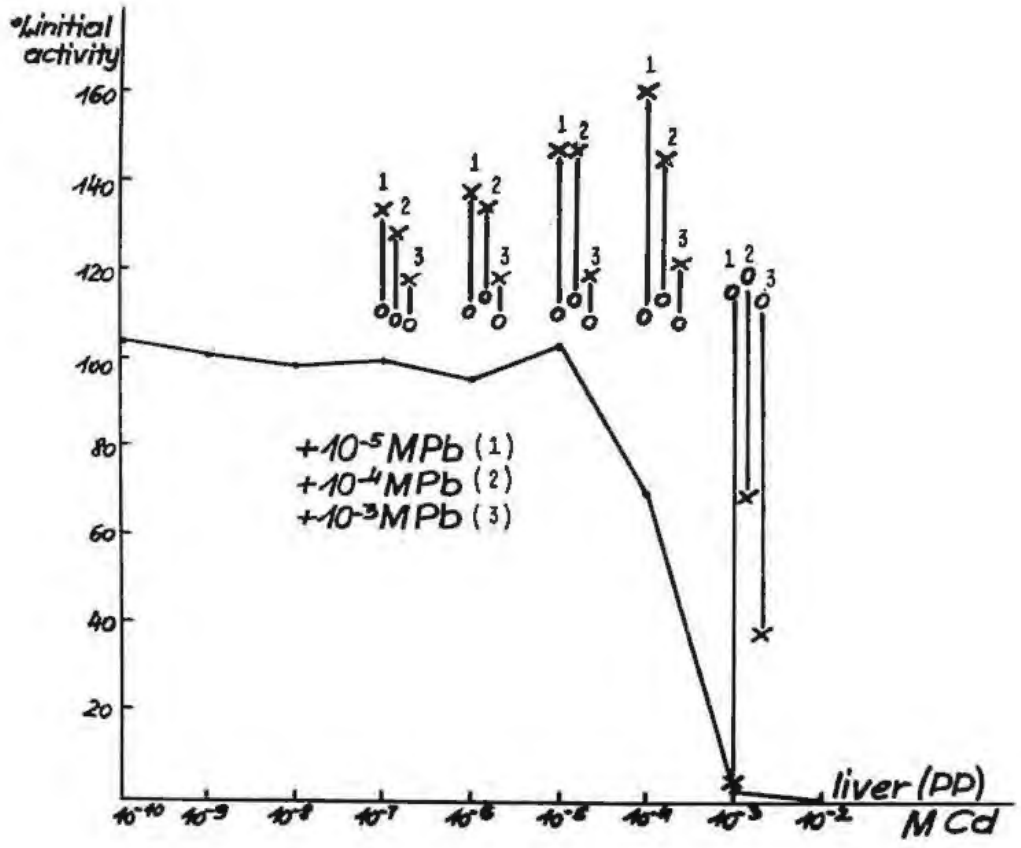

Figure 9. Protoporphyrin formation from delta-aminolevulinic acid in presence of cadmium and lead.

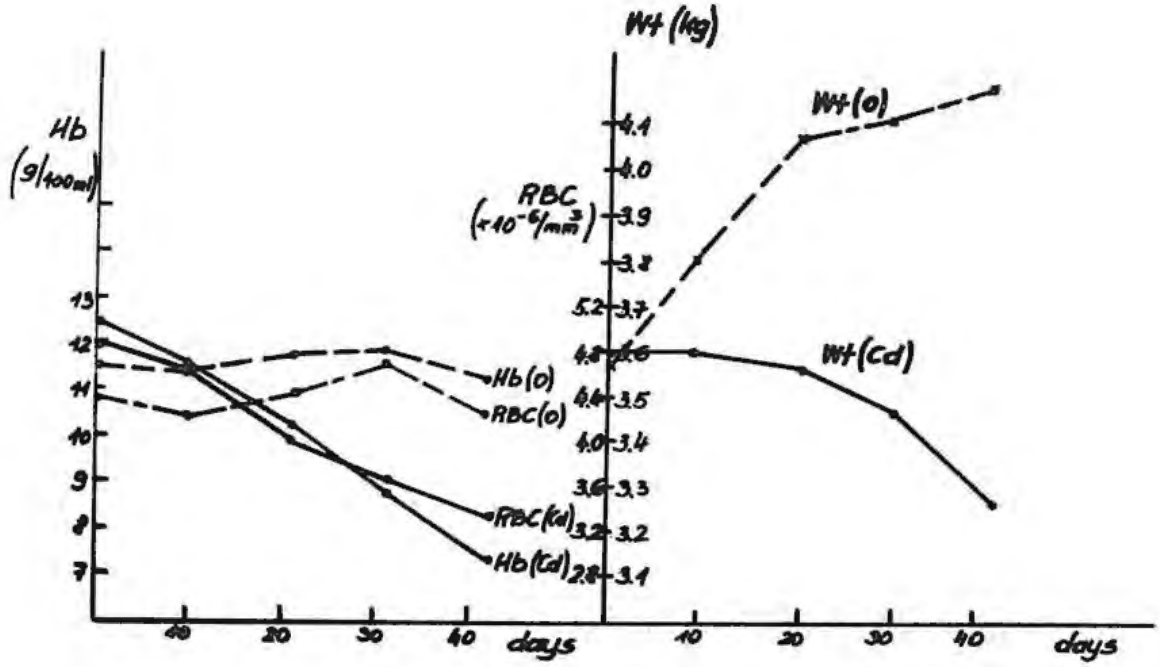

Figure 10. The hemoglobin content in peripheral blood, red blood count and weight in a group of animals intoxicated with cadmium (continuous lines) and the controls (dotted lines).

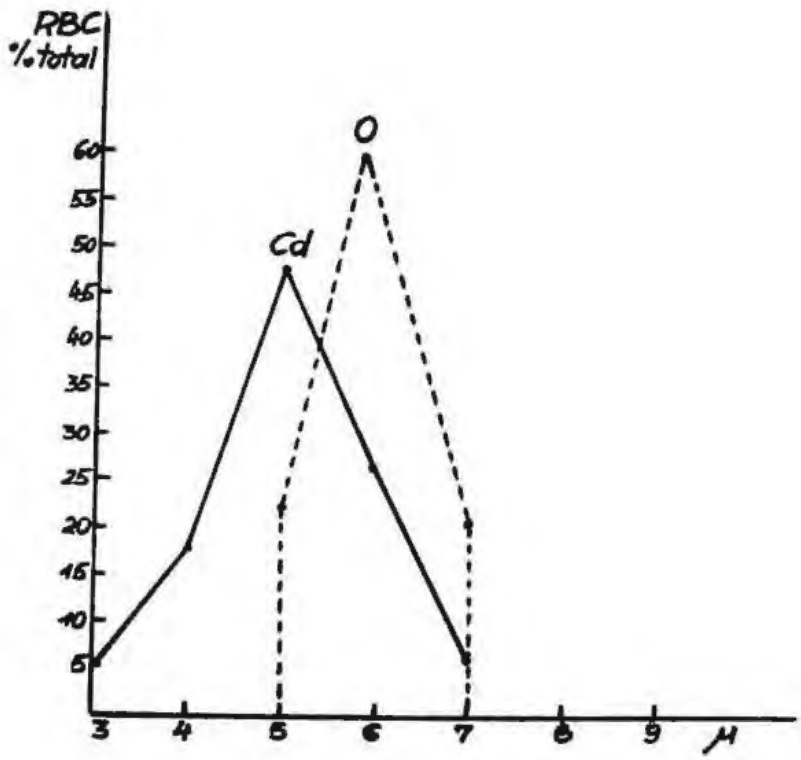

Figure 11. The Price-Jones curve in cadmium intoxicated animals and controls.

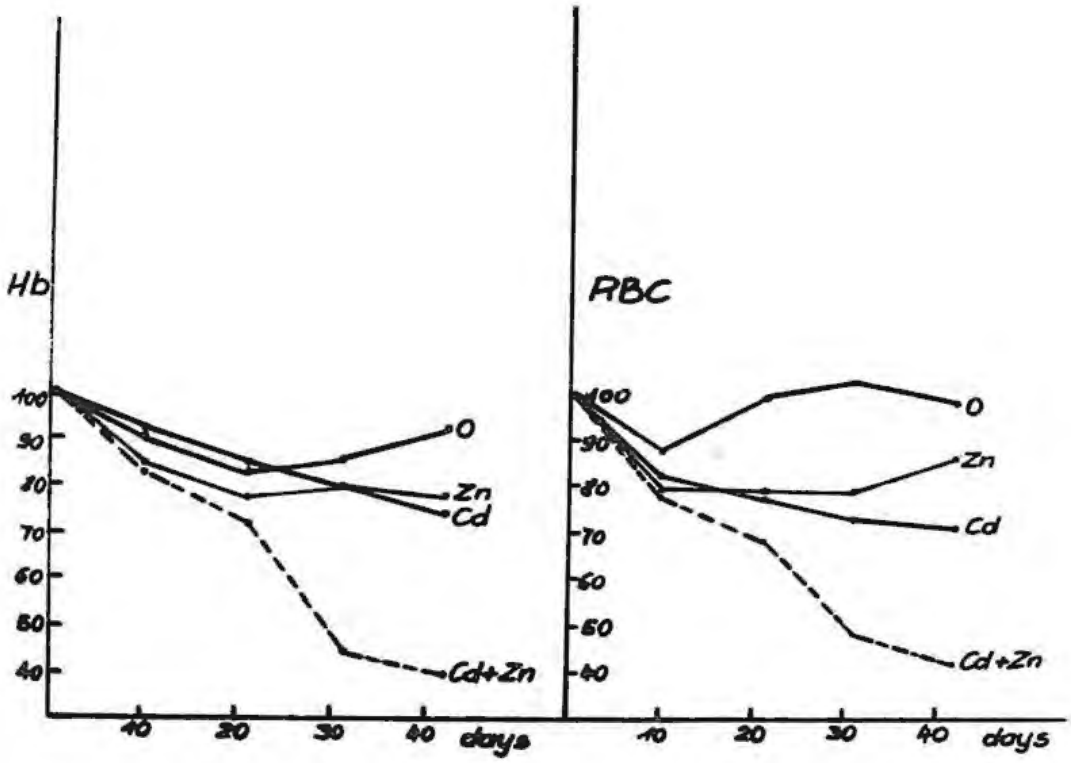

Figure 12. The hemoglobin content and red blood count in groups of animals receiving cadmium, zinc, cadmium and zinc and controls.

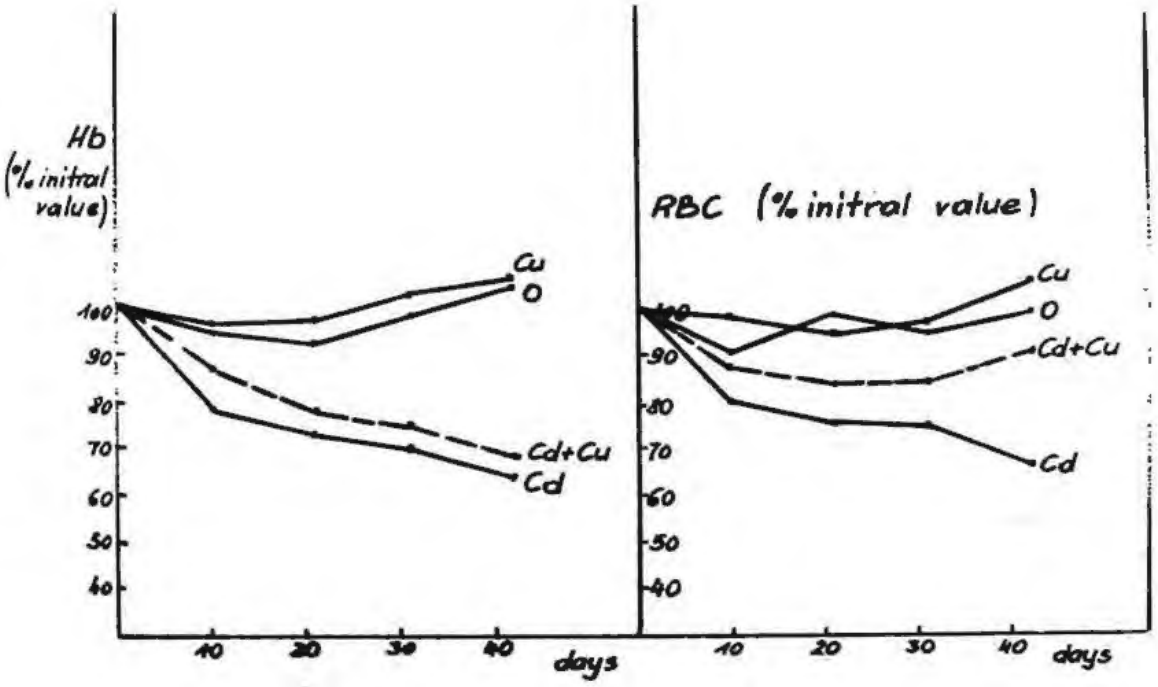

Figure 13. The hemoglobin content, and red blood count in groups of animals receiving cadmium, copper, cadmium, copper and controls.

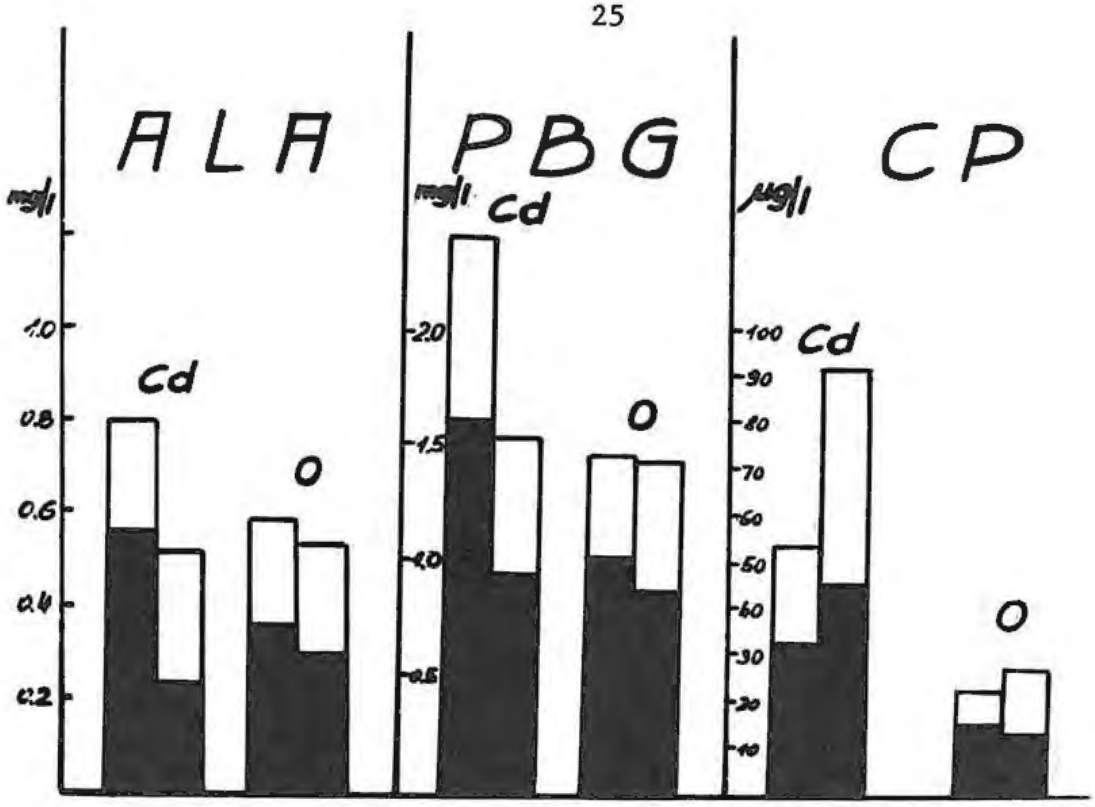

Figure 14. The urinary excretion of delta-aminolevulinic acid (ALA), porphobilinogen (PBG) and coproporphyrins (CP) in a group of cadmium-intoxicated animals. The black blocks represent mean group values, the white ones - the respective standard deviations. In each pair of blocks the first one is related to the beginning of the experiment, the second one - to six weeks' exposure.

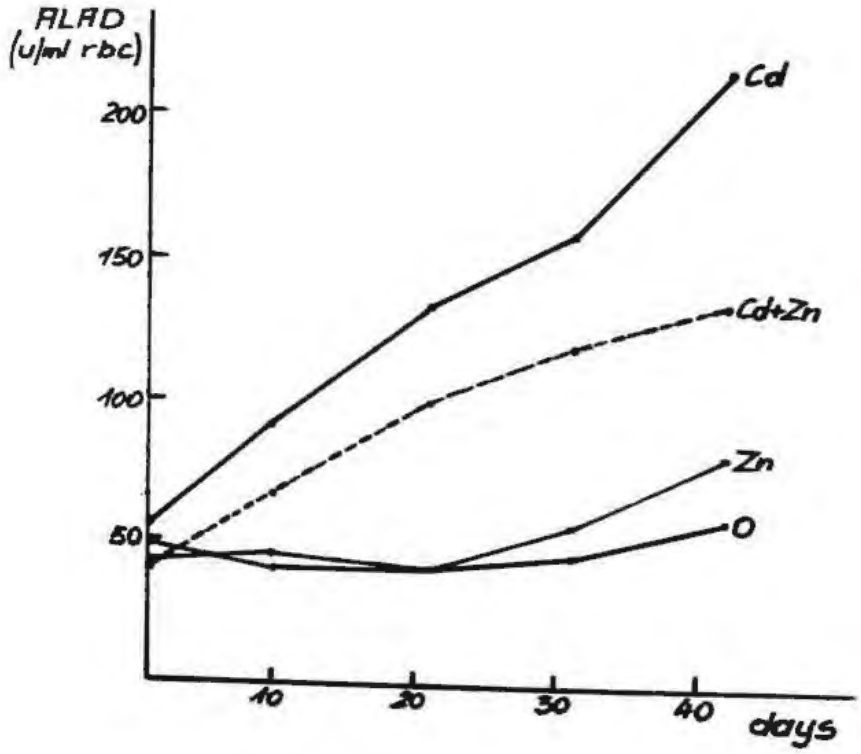

Figure 15. The activity of delta-aminolevulinic acid dehydratase in animals exposed to cadmium (Cd), cadmium and zinc (Cd + Zn), zinc (Zn) and in controls (0).

Serum

		Alb.%	Globulin %					
			α_1	α_2	β_1	β_2	γ	δ
Cd	$\bar{x}$	43.0	7.9	14.8	5.3	9.7	6.2	11.9
n=7	S.D.	4.2	3.6	2.9	0.8	1.4	1.5	2.3
0	$\bar{x}$	54.9	5.7	11.4	6.4	8.7	5.0	8.0
n=13	S.D.	3.5	1.9	2.5	1.6	3.2	1.2	2.4

Figure 16. The electrophoretic pattern of serum proteins of cadmium intoxicated animals (Cd) and controls (0).

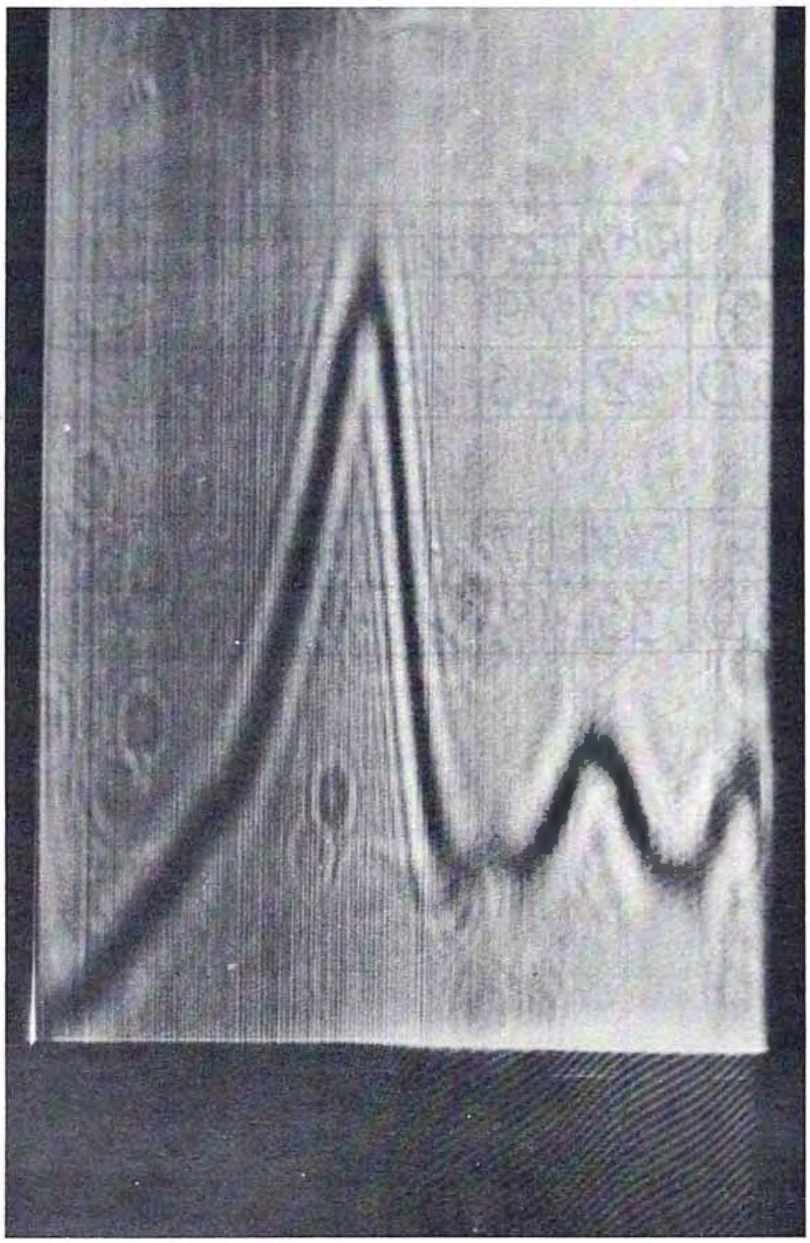

Figure 17. The electrophoretic pattern of water soluble proteins of the liver in a control animal.

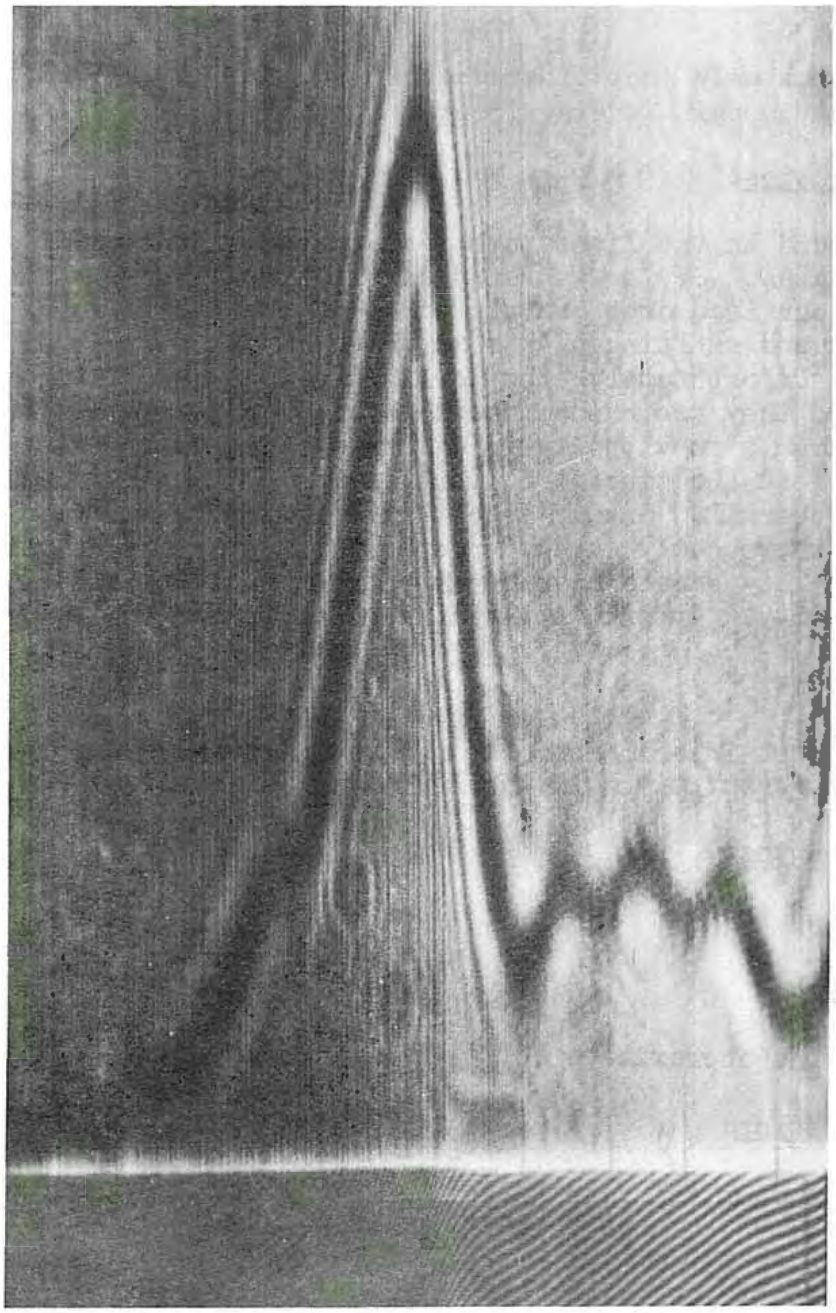

Figure 18. The electrophoretic pattern of water soluble proteins of the liver in a cadmium-intoxicated animal.

DISCUSSION AFTER DR. URBANOWICZ'S PAPER:

Dr. Bessey:

Does cadmium only (not other metals) increase activity of these enzymes (ALAD) and is this in experimental animals only?

Dr. Urbanowicz:

I have found in the literature that rhubidium and manganese also activate ALAD. So far we found it only in animals. As to human exposure our lead ores contain zinc and cadmium as well. We have big mines and smelting plants in which many thousands of workers are engaged. Unfortunately, they are shifted around different departments, and they are exposed both to lead and cadmium. As I have shown in experiments in vitro there exists antagonistic effects between cadmium, lead and zinc. An overall effect of mixed exposure may happen to be nil. Anyway, I just visited a new cadmium plant in Finland, where newly engaged people have never been exposed to lead or zinc. They should have been tested by now, as to their erythrocytic ALAD activity. Unfortunately I have not gotten the results.

Dr. Bessey:

Is the effect in animals quite uniform--that is from animal to animal and at different times in the same animal?

Dr. Urbanowicz:

Yes, it is.

Dr. Bessey:

What was the approximate level of human exposure in Finland?

Dr. Urbanowicz:

I have not been told about that. I don't think it was great, because the factory was almost completely automatized. Anyway, the doctor who was in charge of the workers had some troubles with them, and suspected that they might have become intoxicated.

Dr. Piotrowski:

I wanted to ask you if the concentrations which you obtained in the in vitro experiments and on the prolonged exposure to cadmium day by day may be in some way compared?

Dr. Urbanowicz:

Yes, they can. We got 0.5 milligrams per 1 gram fresh liver in the in vivo experiments. That meant in the incubation procedure a 4×10^{-4}M concentration.

Dr. Piotrowski:

At this level you got the effect of inhibition in the process of biosynthesis of protoporphyrin.

Dr. Urbanowicz:

That's right. But the biosynthesis of protoporphyrin is complex and the reaction catalyzed by ALAD is only one small step. Another disturbing factor for the comparison of results obtained in the in vivo and in vitro is the tissue deposition of cadmium as inactive compounds. I showed a slide reflecting results of experiments in which tissues of animals previously intoxicated have been tested by the in vitro procedures. There was no difference between the liver of those animals and unexposed ones. That means that cadmium administered in vivo was deposited as an inactive compound, and did not influence the experiment in vitro.

Dr. Piotrowski:

This is interesting because of the first results obtained in the "in vitro" experiments which, I think, I do understand. I will show you tomorrow some pictures which I think explain the difference between the in vitro and in vivo exposure to cadmium with respect to the binding of cadmium. I am talking about binding with metallothionein which almost selectively binds cadmium in liver. I understand that cadmium, previously deposited in the liver, had no effect on the concentration of cadmium added "in vitro" which was necessary to produce the inhibition comparable to "in vivo" inhibition. The final concentration necessary to exert the effects as I calculated them would be 10 to minus 5, you said to minus 4, is that right?

Dr. Urbanowicz:

There is a dilution by a factor of 10^{-1} in the "in vitro" experiment. That makes the difference.

Dr. Piotrowski:

And this is the question, because in all the "in vitro" experiments you got concentrations which inhibited the biosynthesis at 10^{-3} and now in the "in vivo" experiments the effective concentration would be lower by, at least, one order of magnitude. Moreover, this is cadmium deposited in an "inactive" form. Thus, can the mechanism be the same?

Dr. Urbanowicz:

I think I have to know the data of your experiment you just mentioned before this question can be settled.

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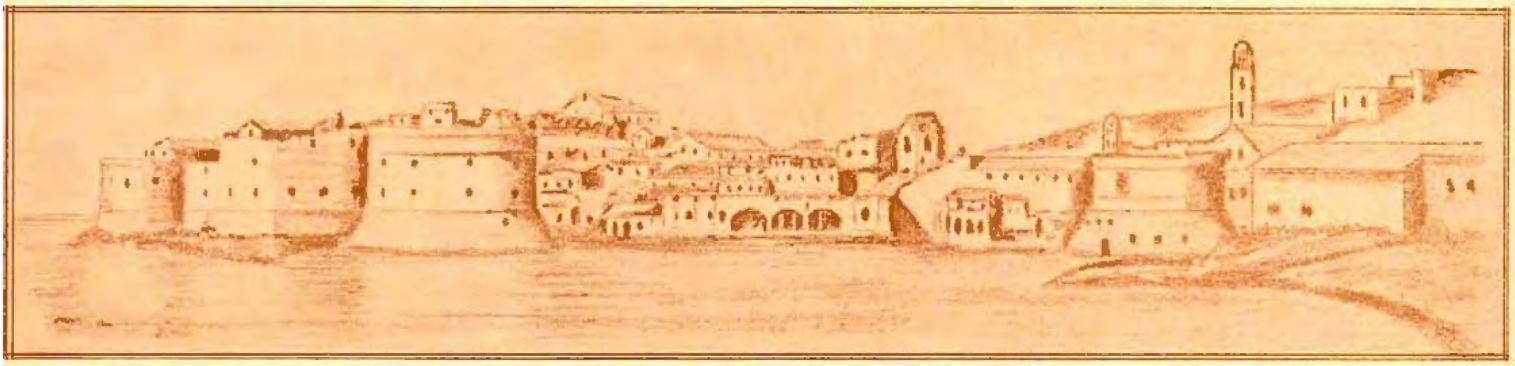

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