PROCEEDINGS

OF THE
SPECIAL FOREIGN CURRENCY PROGRAM
SYMPOSIUM

DUBROVNIK

OCTOBER 1970

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
HEALTH SERVICES AND MENTAL HEALTH ADMINISTRATION
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PREFACE

Via the Special Foreign Currency Program (Public Law 480) Research Agreements, the Health Services and Mental Health Administration, through the National Institute for Occupational Safety and Health, supported in Poland and Yugoslavia twelve different research projects on industrial health in 1970. This research was diverse in nature, sampling areas of toxicology, physical stress, chest disease and epidemiology, and utilizing a variety of laboratory, and field methods for attaining needed data. Sufficient progress had been made in each of these projects so that important information had become available and could be shared with other scientists working on similar or related problems. In order to accomplish this exchange of information a Symposium of Principal Investigators and U.S. Project Officers for the Research Agreements in Yugoslavia and Poland was held in Dubrovnik, Yugoslavia. Aside from providing new information on timely problems in occupational health, the Symposium served to promote cross-fertilization of ideas among specialists with different backgrounds working in the same project areas or different ones. This latter interaction offered excellent possibilities for radically new approaches to old problems or for crystallizing new research leads. The Symposium also afforded an opportunity to develop some unification in the overall program of PL 480 supported research concerned with industrial health.

The agenda consisted of Principal Investigators presenting papers summarizing progress on the twelve Research Agreements which were grouped into toxicology, heat stress, and other physical hazards, and dust and related chest diseases. A general introduction to each project was given by the designated U.S. Project Officer and time was allowed for discussion and comment following each presentation. The final day of the Symposium consisted of a series of round-table discussions on issues in different problem areas, raised in the course of the meeting, hopefully producing new research leads. The general format of the Symposium was developed by Dr. Alexander Cohen, and Dr. Milos Kilibarda during discussions in Belgrade in February 1970. All of the Principal Investigators communicated in English, so no translation was required.

The Proceedings of this Symposium include project presentation and relevant comments to them and the round-table discussions. In the compilation of the papers only minimal essential additional changes were made in order to maintain the sense and flavor of each Principal Investigator.

We are grateful to the authors of the papers, to all the participants for the discussion, to the staff of the Serbian Institute of Occupational and Radiological Health, to the staff of the American Embassy in Belgrade, to Dr. Milos Kilibarda, Dr. Alexander Cohen, Mrs. Joan Baugus for the many hours she spent on the manuscripts, and especially to Mr. August Lauman for his design of the cover sheet.

Austin Henschel, Ph.D.
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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.

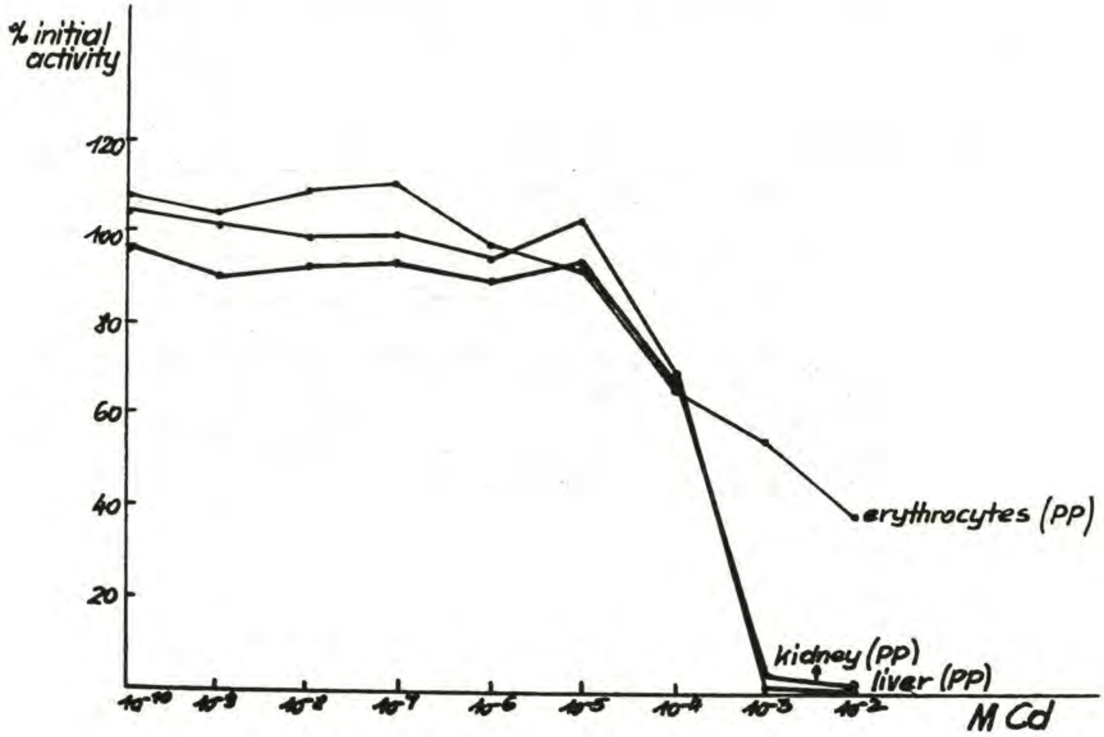

Figure 1. Protoporphyrin (PP) formation from delta-aminolevulinic acid in presence of different cadmium (Cd) concentrations.

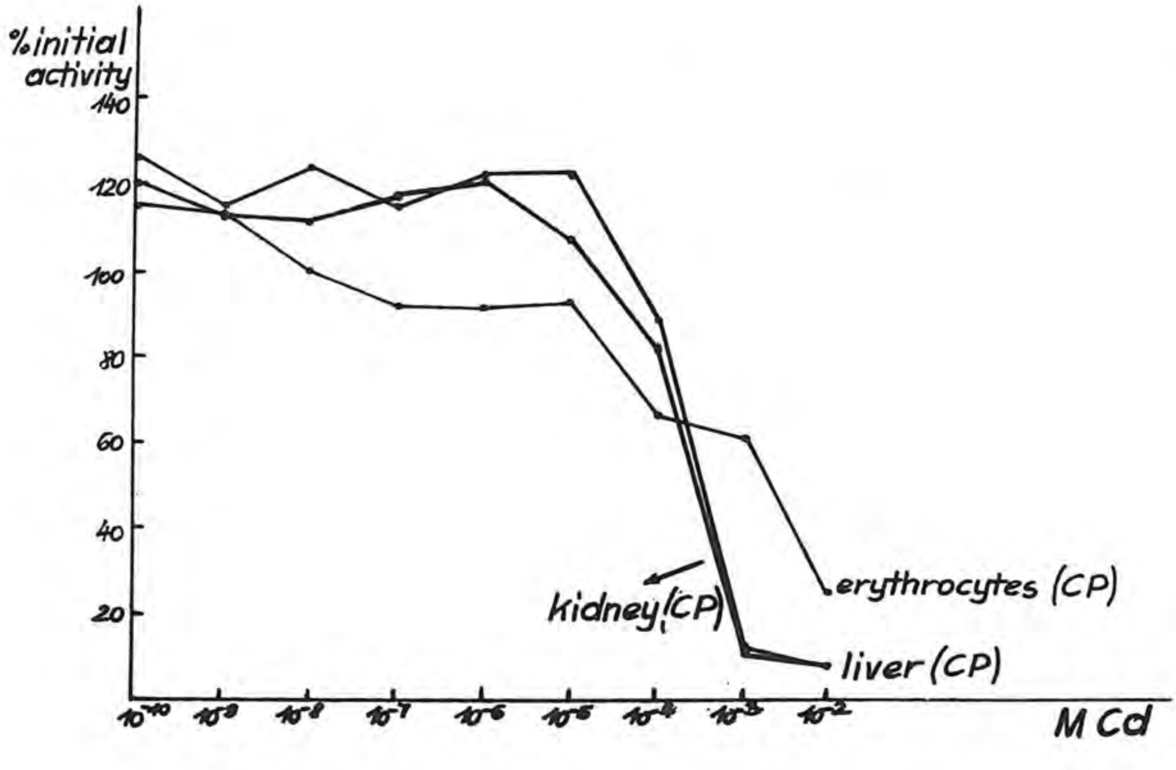

Figure 2. Coproporphyrin (CP) formation from delta-aminolevulinic acid in presence of different cadmium (Cd) concentrations.

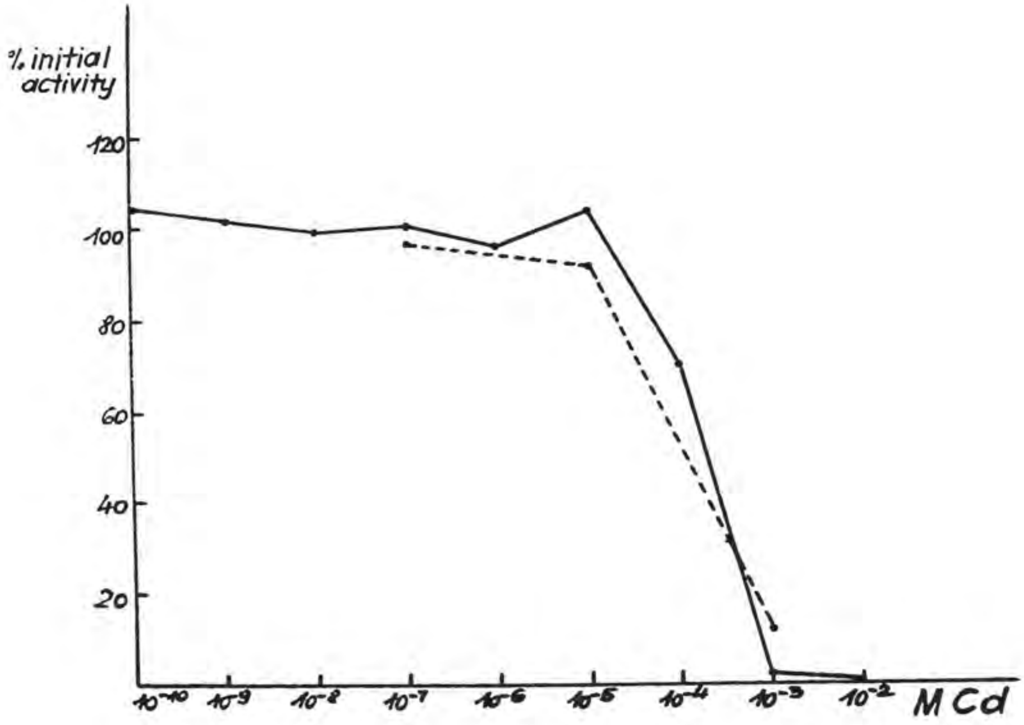

Figure 3. Protoporphyrin formation from delta-aminolevulinic acid in presence of different cadmium concentrations in livers of unexposed animals (continuous line) and animals previously administered cadmium parenterally.

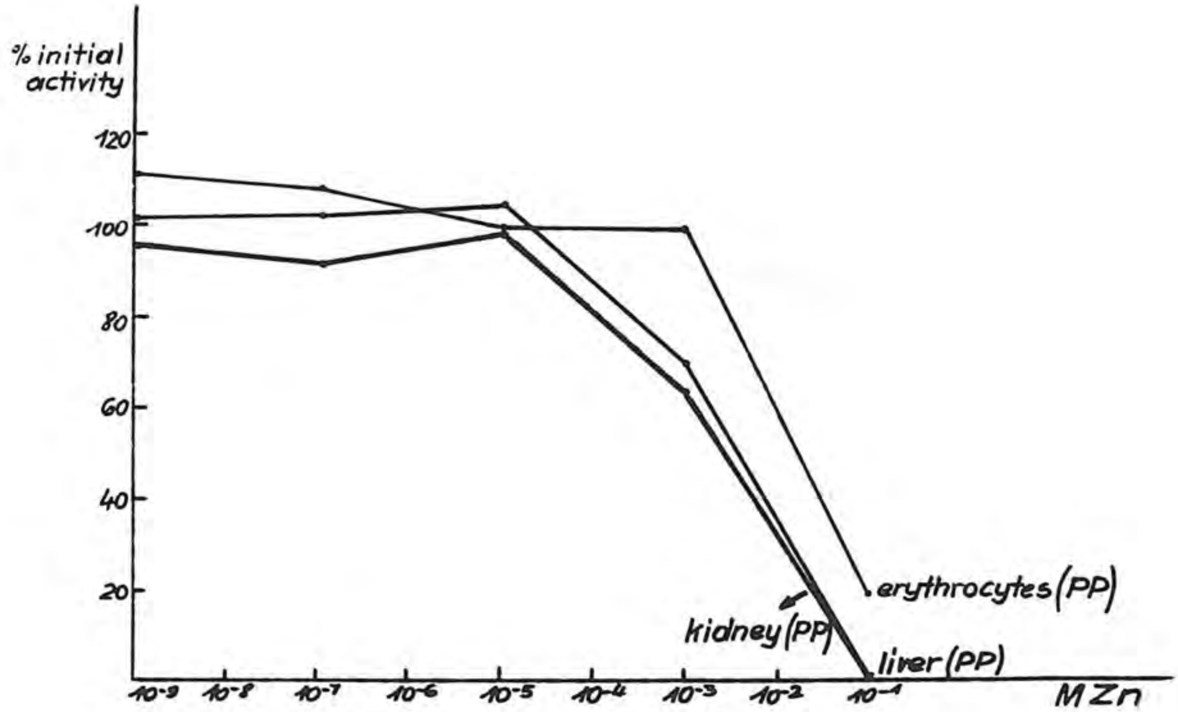

Figure 4. Protoporphyrin formation from delta-aminolevulinic acid in presence of zinc.

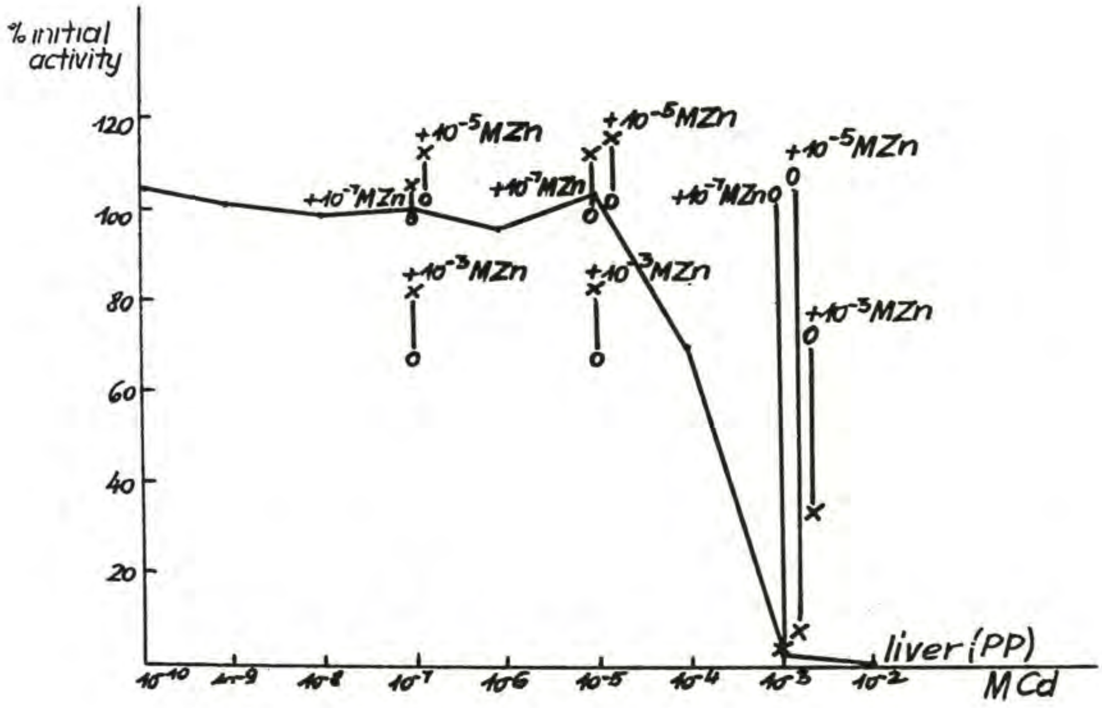

Figure 5. Protoporphyrin formation from delta-aminolevulinic acid in presence of cadmium and zinc.

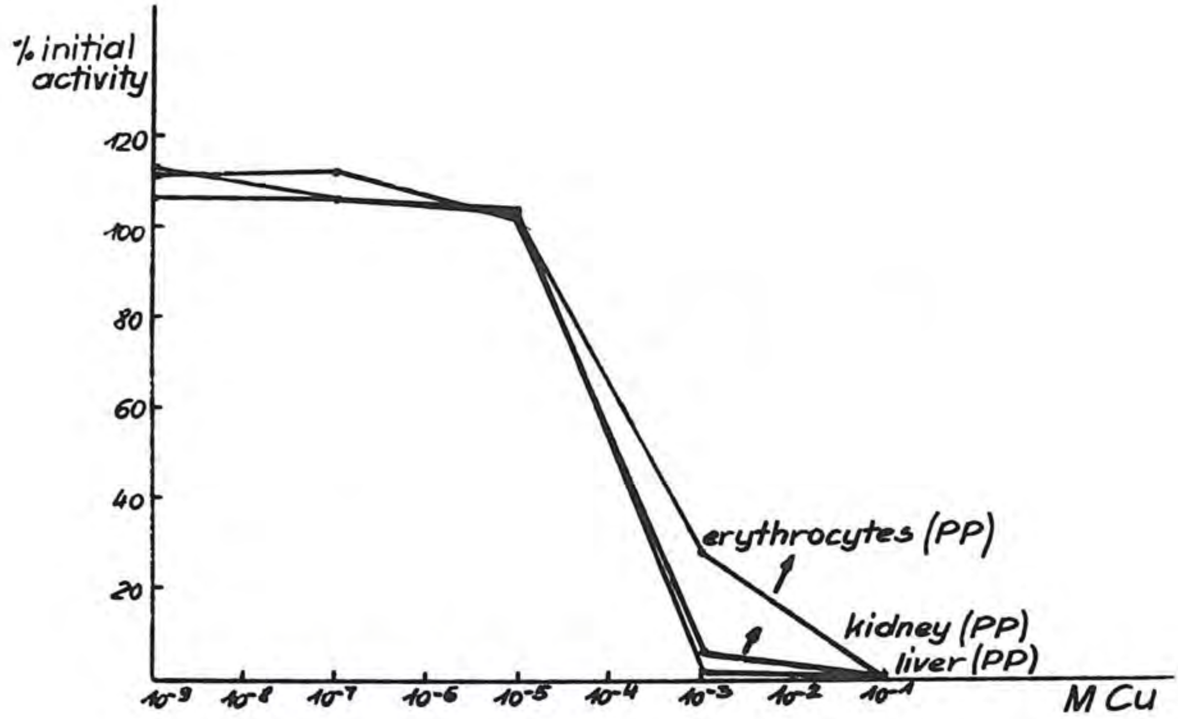

Figure 6. Protoporphyrin formation from delta-aminolevulinic acid in presence of copper.

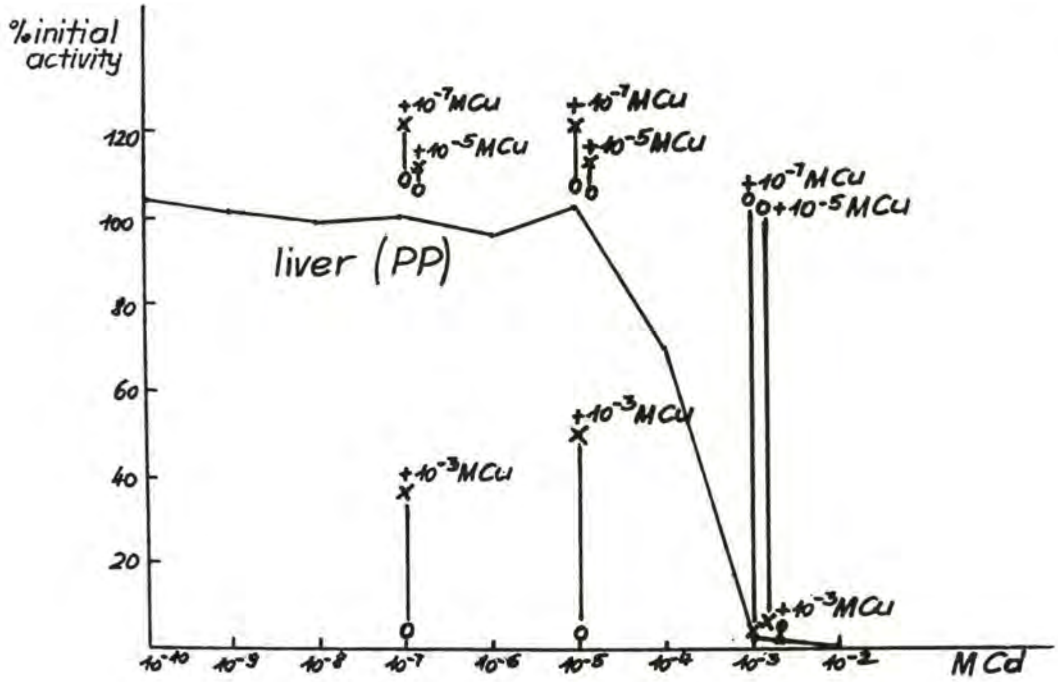

Figure 7. Protoporphyrin formation from delta-aminolevulinic acid in presence of cadmium and copper.

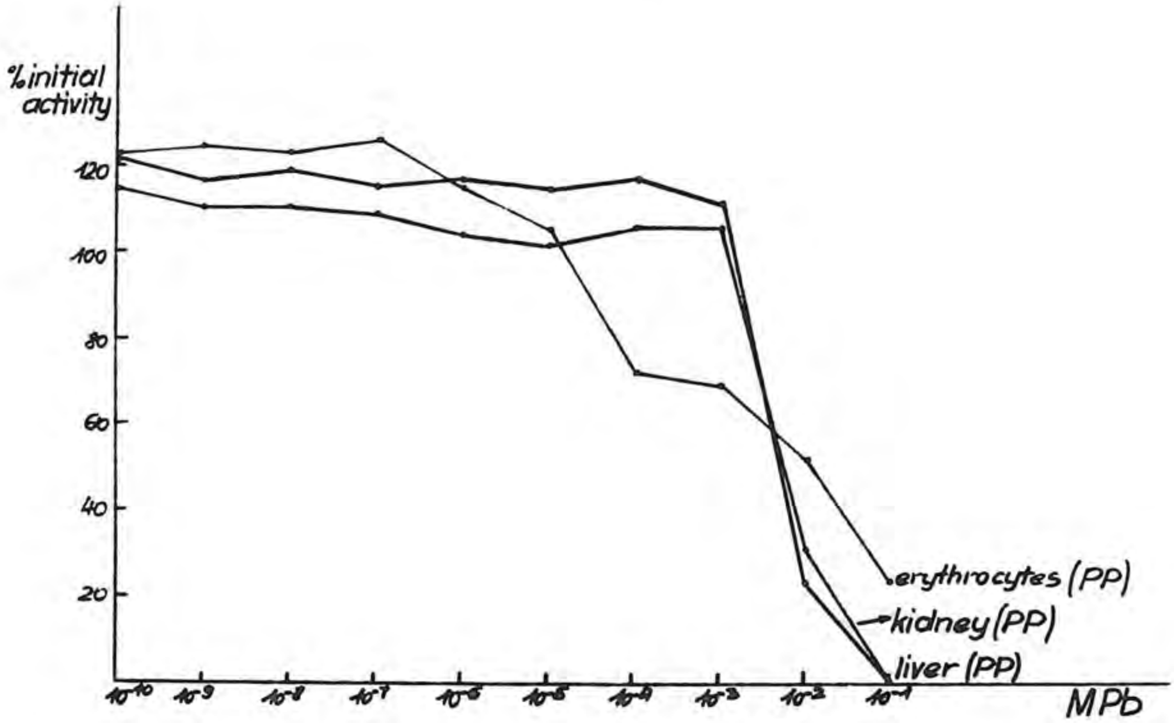

Figure 8. Protoporphyrin formation from delta-aminolevulinic acid in presence of lead.

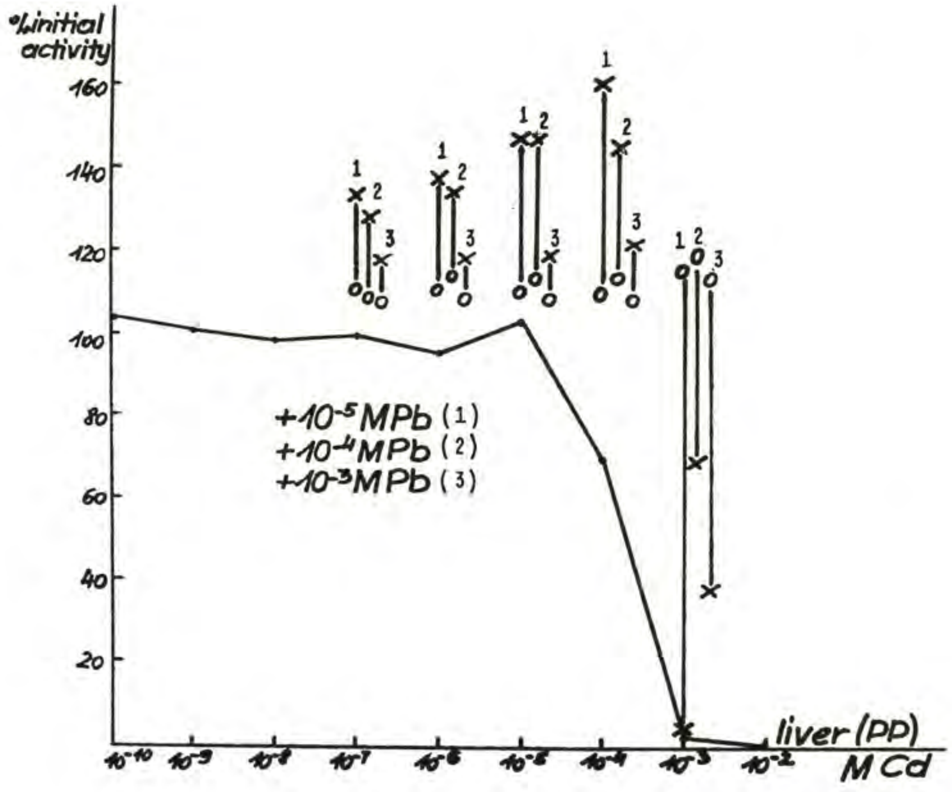

Figure 9. Protoporphyrin formation from delta-aminolevulinic acid in presence of cadmium and lead.

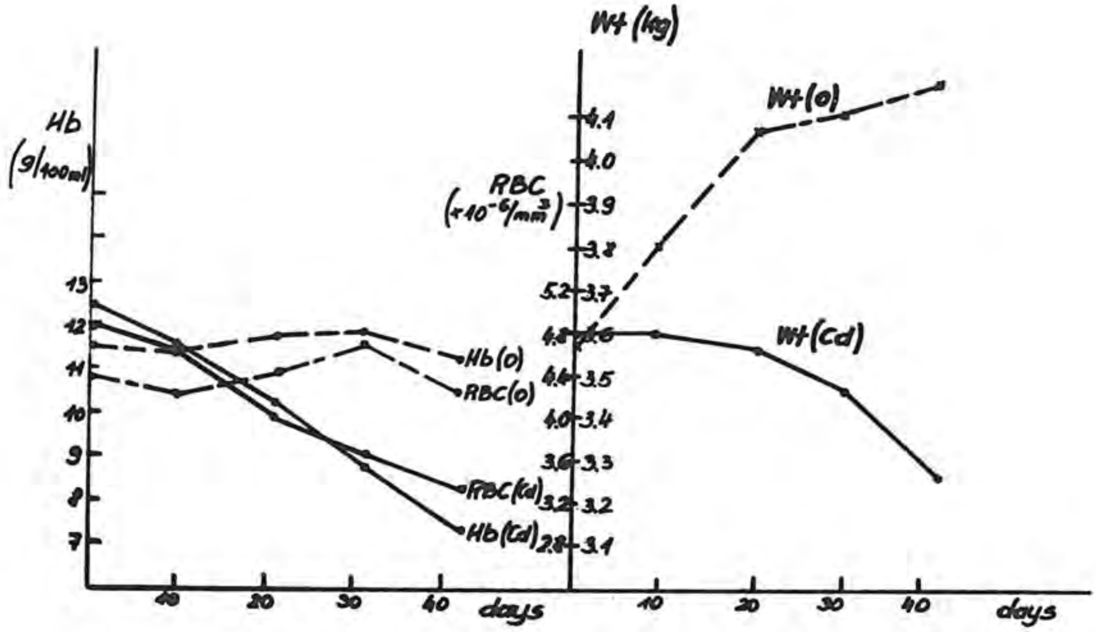

Figure 10. The hemoglobin content in peripheral blood, red blood count and weight in a group of animals intoxicated with cadmium (continuous lines) and the controls (dotted lines).

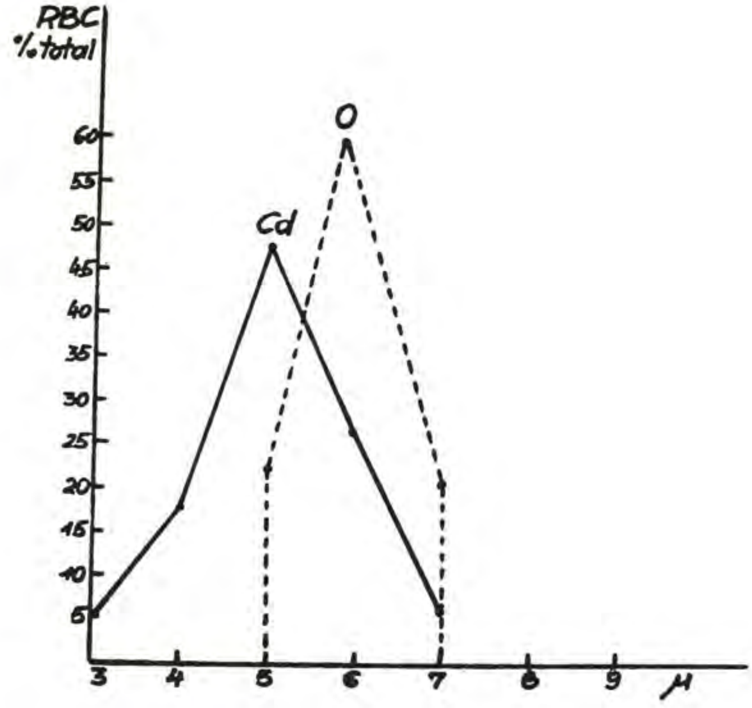

Figure 11. The Price-Jones curve in cadmium intoxicated animals and controls.

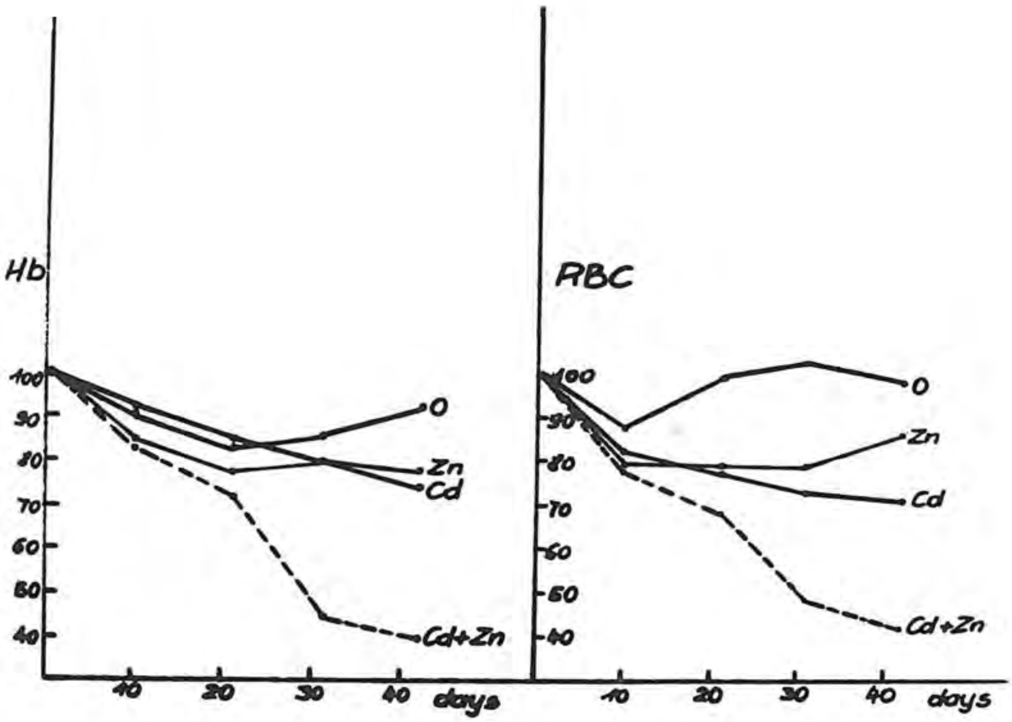

Figure 12. The hemoglobin content and red blood count in groups of animals receiving cadmium, zinc, cadmium and zinc and controls.

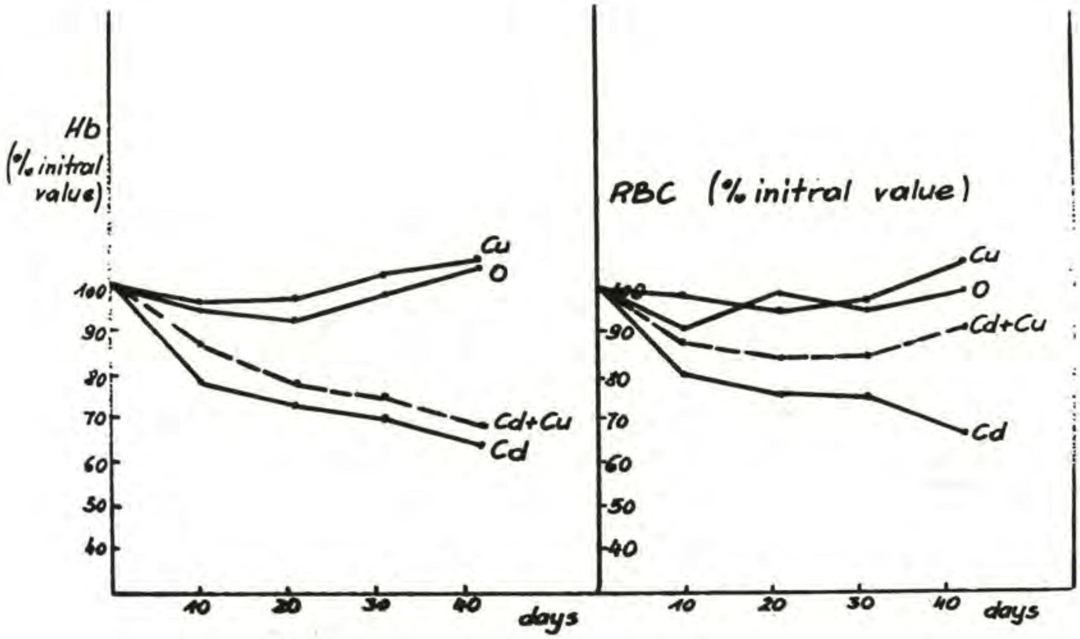

Figure 13. The hemoglobin content, and red blood count in groups of animals receiving cadmium, copper, cadmium, copper and controls.

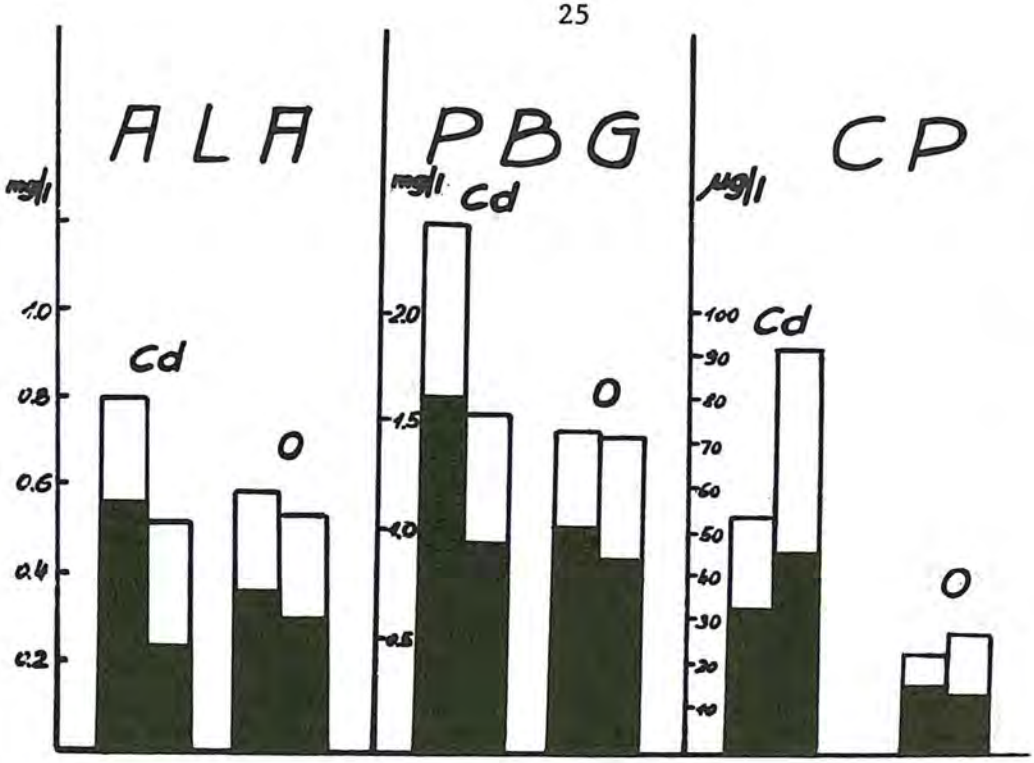

Figure 14. The urinary excretion of delta-aminolevulinic acid (ALA), porphobilinogen (PBG) and coproporphyrins (CP) in a group of cadmium-intoxicated animals. The black blocks represent mean group values, the white ones - the respective standard deviations. In each pair of blocks the first one is related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

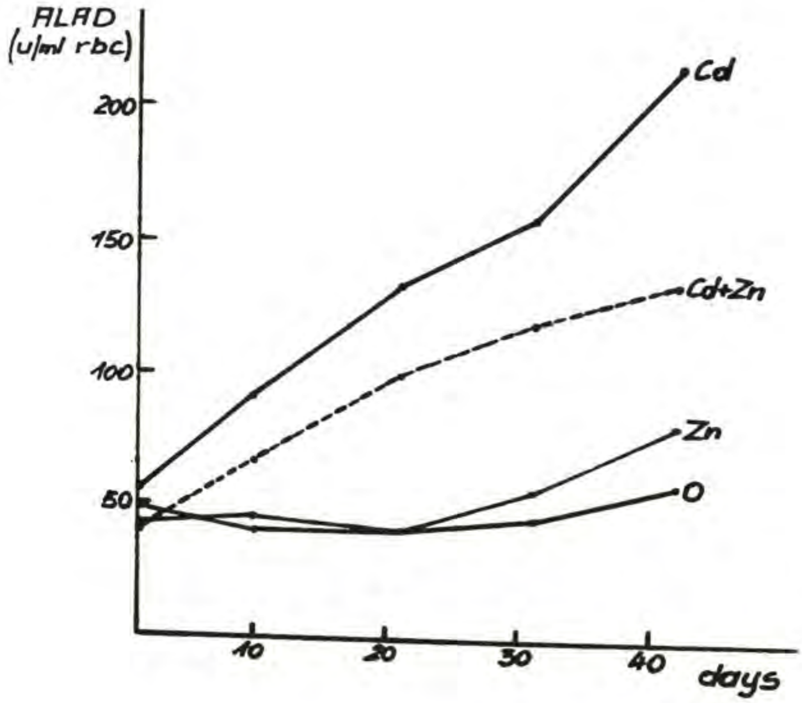

Figure 15. The activity of delta-aminolevulinic acid dehydratase in animals exposed to cadmium (Cd), cadmium and zinc (Cd + Zn), zinc (Zn) and in controls (0).

Serum		Alb.%	Globulin %					
			α_1	α_2	β_1	β_2	φ	γ
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).

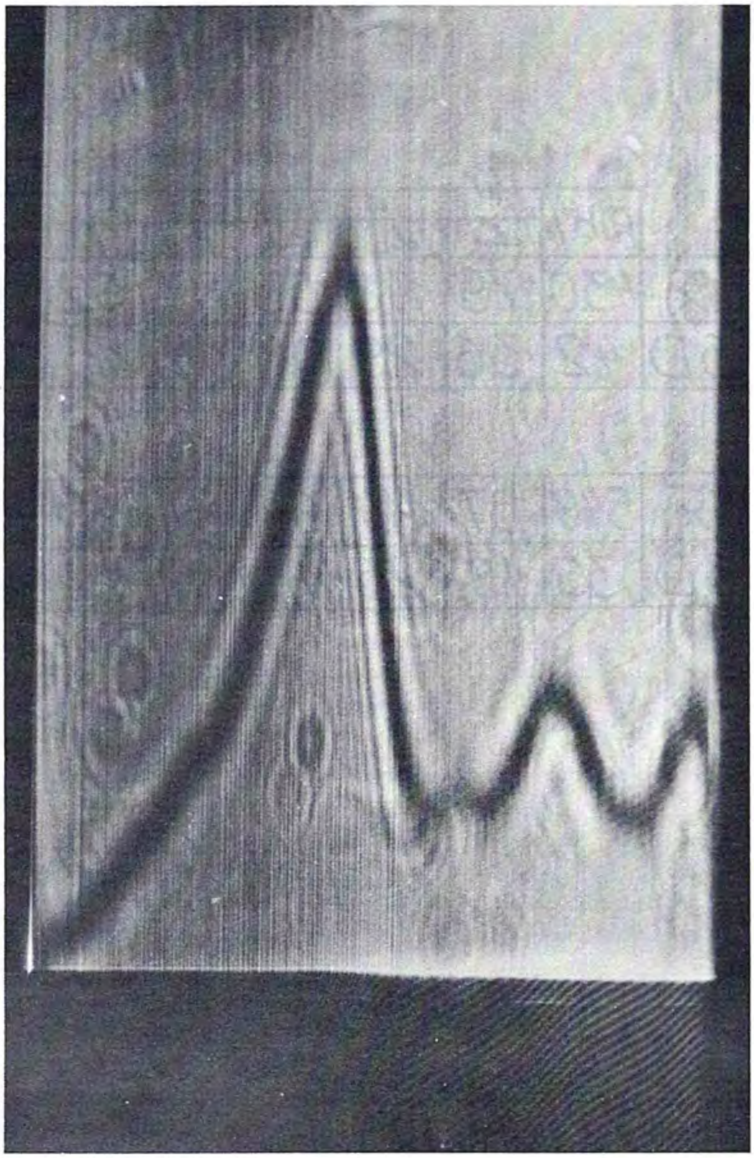

Figure 17. The electrophoretic pattern of water soluble proteins of the liver in a control animal.

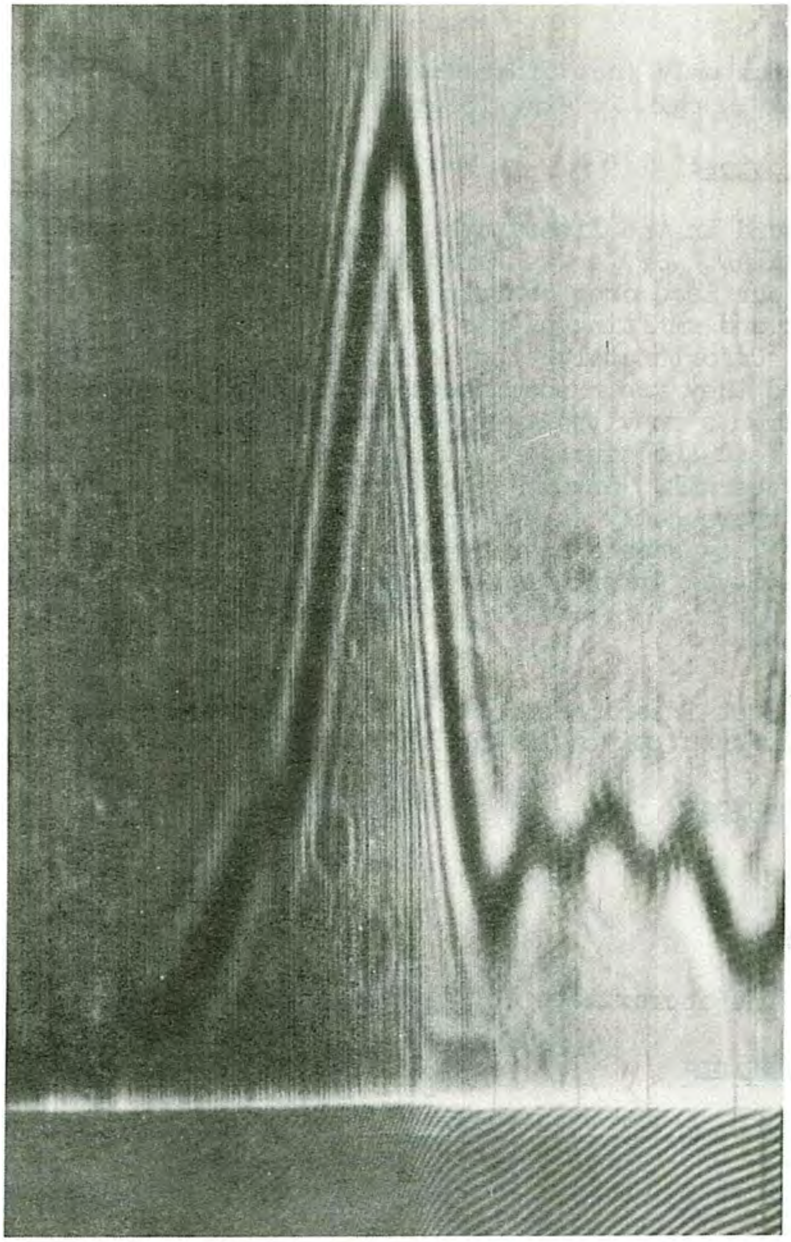

Figure 18. The electrophoretic pattern of water soluble proteins of the liver in a cadmium-intoxicated animal.

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 metalothionein 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.

LEAD CONTAMINATION OF ENVIRONMENT IN MEZA VALLEY
A Preliminary Report

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Recently great attention is being paid to the problems of lead contamination in the urban atmosphere due to emission from motor vehicles exhaust. Numerous studies are in progress on this subject, especially in the USA. The aim of these studies is to establish the lowest level of lead in the urban atmosphere that will produce a measurable change in the body due to exposure to respirable and absorbable lead particles.

On the other hand, in the world literature a great number of papers have been published in the last decades on occupational exposure in lead and lead intoxication.

In Yugoslavia there are two areas in which the population is exposed to higher levels of lead than urban populations are but lower than occupational exposures. These areas are around two big lead mines, and possessing and smelting plants.

The first mine is located in Slovenian Alps and the second one at Trepca on Kosovo, toward Albanian border. We started our ecological study on lead contamination in the biosphere and population exposure around lead mine "Mezice" in Slovenija. Later we intend to develop a similar study around the Trepca mine. In such a way we hope to fill the gap between studies on low lead exposure of urban populations and high occupational exposures of workers and lead miners.

<u>Location of "Mezice" lead mine and smelting plant</u>

The lead mine "Mežica" is located in the valley of river Meža, in Slovenian Alps on the Austrian border. The valley is situated on about 600 m. over sea level, but the surrounding mountains reach 1624 m. This results in a closed long space in the center of which the lead smelting plant is built.

The lead mine is about 300 years old, possessing ore with an average lead content of 6-6.5% in the form of lead sulfides (80%) and lead oxides (20%). The ore contains also 2-3% of Zinc, 0.01% of Arsenic, 0.3% Cadmium and 0.0003% of Bismuth.

In 1746 the smelting plant in Zerjav was founded. In the second half of 19th century it was producing about 2,500 tons of lead yearly. After 1900 production was increased until today the production is about 22,000 tons of lead and 11,000 tons of zinc a year.

The chimney of the smelting plant emits lead aerosols (lead oxides) which contaminate the valley. The lead aerosols from the air contaminate the soil, waters, plants and food. The flotation of smelting plant heavily contaminates the river Meza. Therefore, animals and population of this valley are exposed to lead due to inhalation and ingestion of contaminated air and food.

In the valley of river Meža near lead mine and smelting plant four main and many small settlements are located.

In the center of the valley Žerjav, where the smelting plant is located, in 1961 there were 1,053 inhabitants.

About 2 km. south from Žerjav the new settlement Rudarjevo was recently built with some hundreds of inhabitants. About 5 km. south-west the old settlement Crna with 2,198 inhabitants in 1961 is situated. About 10 km. north west from the smelting plant village Mezica is situated with 2,515 inhabitants in 1961.

In 1967 we started a broad study of lead contamination in plants, especially vegetables, and food.

We planned to perform a complete ecological study of lead contamination in the biosphere and man. That means we planned to establish lead contamination levels in all links of the ecological chain: air-water-soil-plants-animals-food-man. Now we wish to report some preliminary results obtained in 1967 and 1968. We are preparing some detailed papers to be published in the near future (1, 2, 3, 4, 5).

Lead in air

A group of experts in the area sampled the air daily at nine spots in the valley from summer 1967 through 1968 to determine lead and sulphur dioxide levels.

A team from our Institute performed grab sampling on microsorban filters determining lead by dithizone method in May 1967 and 1968. They also sampled using a thermoprecipitator to determine the particle size distribution at the various sites.

Geographic and topographic situation together with meteorological factors, especially north-south winds, produce contamination along the valley. For years the smoke from smelting plants emitted daily 15 m^3 per second without filters, dispersing lead aerosols. In the middle of 1969 the first filter (Lurgi) was installed which reduced the contamination a great deal.

In Table 1 some results of lead determination in the air on various days at the same sites are presented. It clearly shows the influence of meteorologic and topographic factors on daily distribution of lead. These results are obtained from J. Perman, Ch. E., chief of the group of experts following the level of lead in air.

Lj. Jablanov from our Institute performed determination of particle size taken by a thermoprecipitator. The results from the thermoprecipitator measurements are presented on Table 2 and show that with distance the percentage of particles sized under 1 micron is increased which is a very important fact from the toxicologic point of view (Table 2).

Lead in water, snow and soil

We established that the lead in drinking water of the local piping system is under MPC values MPC:0.05 mg/1. Also water samples from wells in various parts of the valley were analyzed showing values under 0.035 mg/1 of lead. All these results suggest that drinking water does not represent any dangerous contamination for man (2).

Creeks coming into the Meza River, and the Meza River before flotation, contain similar quantities of lead. Flotation at the smelting plant, however, represents an immense source of contamination of the Meza River. The level of lead in this river is continually decreasing until at the mouth of the big river Drava, a very low lead content is found (2). These results are presented on Table 3.

The lead content of the snow in the valley was also determined (3). Many factors influence the quantity of lead. In the first place, meteorologic and topographic factors are important, as in the case of lead in air and soil. But, duration of the snow cover in days is also extremely important. The snow sample was taken by a metal model with standard surface. In non-contaminated areas the lead content in snow is 0.002-0.02 mg/kg. In contaminated areas the results are between 0.13-262 mg/kg. depending on mentioned factors. Determination of lead in snow could represent a good control of determination of lead in air. Namely, this quantity represents integral measurements of the lead in the air that has settled out for duration of the snow cover. We are evaluating now these results.

Determination of lead in soil represents a very important point in our study: (a) lead content represents results of integral contamination with lead aerosols, (b) these results are important for the study of lead absorption by plants to establish absorption coefficients.

Therefore, we performed soil sampling on 33 sampling spots together with sampling of plants and vegetables. First determination of total lead content in soil was performed. Using results of lead determination in underground parts of vegetables to calculate absorption (discrimination) coefficient, we obtained very dispersed and unrealistic results. It was obvious that total lead does not represent a real factor of absorption into the plants. Therefore, we applied determination of so called AL-dissolved, the absorbable, physiologically active lead. Soil samples were extracted with mixture of 0.1 N ammonium lactate and 0.4 N acetic acid and lead determined in the extract. In Table 4 the results of total lead and AL-soluble lead determinations are presented.

It is obvious that only a certain percentage of total lead is absorbable into the plants (Table 4). Great differences exist ranging from 1.3% to 50% of AL-soluble Pb due to various factors. We assume that physicochemical properties of the soil, mechanical properties, quality, humidity, pH and other factors influence percentage of AL-soluble lead. It is evident that soil in Meža valley is highly contaiminated with lead, from a "normal level" to 25 grams per kg and that high levels of lead contamination of plants could be expected.

In January 1969 a filter was fixed and contamination through the chimneys dropped almost 50%. We are continuing lead determination in soil to see the consequences of this reduced input into the atmosphere.

Lead in plants and food

We mentioned that plant samples were taken on the same spots with the soil samples. We took above ground and under ground parts of the plants washed them well with tap and distilled water and dried, cut and used them for lead determination. Sampling was performed regularly at the end of July and at the end of October each year. Thousands of results are now tabulated but here we wish to give only first impressions.

Evaluation of these results on lead content in plants must be divided in two groups: above ground and under ground parts. Content of lead in above ground parts represents adsorption and absorption of deposited lead on the surface. In underground parts only absorption from the soil exists.

We determined lead in above ground parts of various vegetables and plants as endive, parsley, cabbage, common leek, onion, garlic, carrot, red carrot, turnip cabbage, garden rutabaga, bush beans, celery and grass. We found the greatest lead content in hay and grass. Therefore, these results could be used for evaluation of contamination by lead aerosols.

Under ground parts are of greater interest from the physiological point of view. In Table 5 some results are presented divided in 3 groups by level of lead content: high, medium, low. It is obvious that parsley, rutabaga and garden beet are not suitable for soil with high lead content because they will absorb higher lead quantity and represent a danger as human food. Common leek, red carrot, celery, onion, garlic and potato are more suitable due to lower absorption.

We also calculated absorption coefficients using Pb value of vegetables and total lead content of soil (C_T) and AL-soluble lead of the soil (C_{AL}). In Table 6 such coefficients are presented for parsley, red carrot and potato as representatives of mentioned three groups (Table 5).

It is evident that calculated C_{AL} coefficients are less dispersed than C_T (Table 6). In the case of red carrot we performed statistical evaluation and established much higher correlation between Pb content of this vegetable with AL-soluble Pb, than with total Pb. Statistical evaluation will be performed for other vegetables.

It is evident that AL-soluble Pb represent more authoritative data for the determination of absorption coefficient of Pb into the plants than total lead content. We are continuing this study to find out if some other kind of lead determination (ionized lead) will give better results.

Also we are evaluating results of some other food and cooked food lead content. In this way we hope to obtain data for evaluation of population exposure in the case of lead absorption due to (1) inhalation and (2) inhalation and ingestion, which is the case for the population in Meza valley.

Exposure of population to lead

To evaluate lead exposure we performed a broad epidemiologic study on persons who are not professionally exposured to lead, mostly women and children. In 1968 we performed a screening test using ALA determination in urine of 912 persons. Results of these determinations are presented in Table 7, together with results obtained on control group. For comparison we are giving also some results of miners and workers professionally exposed to lead.

All persons included in this study completed a long questionnaire about personal anamnesis, status, all complaints, nutritional habits and source of used food (from contaminated area or not) and many other relevant data.

In 1969 a detailed medical examination of these groups was performed together with many laboratory analyses as blood picture, determination of lead, coproporphyrine and porphobilinogen in urine. In some cases also determination of ALA-dehydrogenase in blood was performed. Naturally, in 1969 also determination of ALA in urine was performed.

We mentioned that in January 1969 a filter was fixed in the smelting plant and that contamination dropped on almost 50%. It is interesting to note a drop in ALA values in summer of 1969 in comparison with results from the summer of 1968. In Žerjav the drop was 48%, in Mežica 38% and Črna only 19%. Results are presented on Table 8 for same individuals in two successive years.

It is necessary to point this example as a possibility to evaluate technical measures by means of exposure tests performed on biological samples of exposed persons. Now evaluation of the data from populations is in progress. We intend to use analysis by computer technique to compare subjective complaints and objective findings.

Beside all these tests for exposure we would like to get some data about body burden of lead of exposed populations. Therefore, in the summer of 1970 we studied mobilization of lead by injection of Ca-EDTA in 160 exposed persons. We also determined excretion of lead before injection and in 24 hours sample of urine after injection, determination of ALA, and zinc in urine, determination of blood picture, etc. We obtained highly interesting results showing impressive body burden in women, children and men nonprofessionally exposed to lead.

In such a way we hope to elucidate exposure and body burden of exposed population and to find out possible consequences of chronic exposure to lead. It is necessary to keep in mind that the population of the valley is constantly exposed to lead from the beginning of this century, now for the third generation.

We hope also to calculate particular exposure only due to inhalation and to combination of ingestion and inhalation using results obtained by analysis of food.

Results and evaluation of results in two - three years will give some interesting conclusions on contamination of biosphere and population around this lead-zinc mine.

We hope to extend this study on environment around the lead mine "Trepca" in Kosovo region (near Albanian border). This environmental study will be more complicated due to the presence of other heavy metals: Zn, Cd, Bi and other trace metals.

Table 1

Lead in Air in Micrograms/m^3

Date	Mežica	Žerjav Center	Žerjav East	Črna Center	Črna East
10/4/67	1.3	13	30	7.6	4.2
10/11/67	15	84	68	36	25
10/18/67	9.6	18	66	3.2	4.2
10/25/67	24	-	68	60	29

Table 2

Particle Size Distribution of Lead Aerosols

Size in Microns	Percentage of the Whole Sample: At smelting plant-Žerjav		Črna Center	Črna East	Mežica
	5/25/68	5/30/68	5/29/68	5/29/68	5/29/68
0.3	35.48	44.08	43.88	55.00	43.25
0.8	23.26	22.06	26.33	25.00	26.14
1.0	12.85	14.40	12.28	7.50	12.61
1.5	5.50	4.23	5.26	5.00	4.50
2.0	2.75	3.38	3.50	2.50	3.60
2.5	3.66	1.70	1.75	2.50	1.80
3.0	2.75	0.84	–	–	0.90
4.2	2.75	1.70	3.50	2.50	2.70
5.6	5.50	3.38	1.75	–	1.80
6.9	5.50	4.23	1.75	–	2.70

Table 3

Lead Content of Meza and Drava Rivers
30-31 October 1968

Sampling Spot	Pb in mg/l
Various creeks coming to Meža River	0.0013-0.02
Meža 150 m. before flotation (Zerjav)	0.0068
Meža just after flotation (Zerjav)	685.12
Meža 300 m. after flotation	226.36
Meža in center of Zerjav	158.45
Meža in Polena	136.37
Meža in Poljana-10 km. from flotation	92.28
Meža at mouth to Drava-22 km. from flotation	2.89
Drava River from mouth of Meza to Maribor	0.0114-0.0055

Table 4

Pb in Soil in mg/kg

Location No.	Height in Meters (over sea level)	Distance in Meters from Chimneys	July 1968 Total Pb	July 1968 Active Pb	July 1968 %	October 1968 Total Pb	October 1968 Active Pb	October 1968 %
1	540	200	24 880	12 057	48.4	24 680	12	
2	540	500	3 960	1 251	31.6	8 508	2 395	28.2
3	540	230	4 624	924	20.0	5 084	1 905	37.5
4	540	520	2 696	542	20.1	3 147	374	11.9
5	540	1 100	3 584	-	-	2 576	909	35.3
6	540	2 200	2 478	712	28.7	4 916	542	11.0
7	700	1 850	1 320	232	17.6	1 455	78.9	5.5
8	550	3 500	1 228	397	30.8	955	184	18.5
9	500	3 980				582	186	32.0
10	620	1 620	1 183	243	20.5	1 140	252	22.1
11	600	1 950	696	275	39.5	1 023	89.9	8.8
12	630	2 570	384	155	40.4	938	112	12.0
13	580	5 800				408	39	9.6
14	480	4 520	387	25.1	6.5	365	43.4	11.9
15	640	2 000	784	30.0	4.0	658	45.4	6.9
16	650	2 030	975	31.8	3.3	1 000	50.0	5.0
17	580	3 010	1 430	241.0	16.9	1 077	317	29.4
18	650	3 520	1 974	26.8	1.4	745	26.4	3.5
19	550	4 420	661	27.6	4.2	371	38.8	10.5
20	700	4 100	643	32.9	5.1	397	29.9	7.5
21	730	3 500	360	13.6	3.8	204	17.2	8.4
22	710	4 750	3 538	224.0	6.3	3 718	93.2	2.5
23	600	970	1 052	183.0	17.4			
24	600	1 750	237	15.2	6.4	222	18.4	8.3
25	700	3 950	375	20.6	5.5			
26	750	1 500	531	45.7	8.6	595	39.7	6.7
27	930	2 250	295	14.4	4.9	191	12.8	6.7
28	970	7 170	213	9.6	4.5			
29	450	7 500	185	10.8	5.8			

Table 4 Continued

Location No.	Height in Meters (over sea level)	Distance in Meters from Chimneys	July 1968			October 1968		
			Total Pb	Active Pb	%	Total Pb	Active Pb	%
30	450	7 250	563	13.9	2.5	363	40.4	11.1
31	450	9 600	66.5	0.9	1.3	35	2.1	6.0
32	400	9 350	39	1.8	4.7	24.6	1.5	6.1
33	364	17 100	28	2.1	7.5	36.5	3.0	8.2
Noncontaminated Area - 12 samples			10.38 (2.6-20.8)	1.1 (0.4-2.2)	10.5			

Table 5

Lead Content of Underground Parts of Vegetables

Plant-Underground Part (peeled or scraped)	mg Pb/kg Maximum Value	Minimum Value	Non-contaminated Area (Maximum)
Parsley (Petroselinum Hortense Hoffm.) (October 1968)	51.12	0.80	0.22
Rutabaga (Beta Vulgaris L.)-nonpeeled	39.72	0.80	0.22
Gardenbest (Beta Vulgaris L., SSP Esculenta)	33.45	0.22	0.14
Common Leek (Allium Ampeloprasum L.)	17.60	1.41	0.21
Red Carrot (Daucus Carrota L.)	17.39	0.61	0.29
Parsley (July 1968)	16.18	0.52	0.45
Celergy (Apium Graveolus L.)	13.20	2.25	0.53
Onion (Allium Cepa L.)	4.53	0.22	0.30
Common Garlic (Allium Sativum L.)	1.52	0.11	0.30
Potato (Solanum Tuberosum L.)	1.01	0.14	0.12

Table 6

Pb Content of Vegetables and Absorption Coefficients

Location #	Parsley (October 1968)			Red Carrot (July 1968)			Potato (October 1968)		
	Pb mg/kg	C_T	C_{AL}	Pb mg/kg	C_T	C_{AL}	Pb mg/kg	C_T	C_{AL}
1	51.12	0.21×10^{-2}	0.42×10^{-2}	17.39	0.7×10^{-2}	0.14×10^{-2}			
2	40.12	0.47	1.68						
3	22.14	0.44	1.16				0.01	0.02×10^{-2}	0.05×10^{-2}
4	17.14	0.55	4.58	6.55	0.24	1.21			
5	13.80	0.54	1.52	10.87			0.89	0.03	0.09
6				17.25	0.69	2.42	0.67	0.01	0.22
7	5.46	0.38	6.92	3.15	0.24	1.36	0.40	0.03	0.51
8	12.66	1.27	6.88	3.52	0.27	0.89			
9							0.63	0.11	0.34
10	4.80	0.42	1.90	14.04	1.19	5.78	0.35	0.03	0.14
11	1.73	0.17	1.92	13.45	1.93	4.89	0.37	0.04	0.41
12	2.60	0.28	2.32				0.21	0.02	0.19
13							0.63	0.15	1.61
14	2.94	0.81	6.77	1.34	0.35	5.34			
15	1.60	0.24	3.52				0.22	0.03	0.48
16	4.73	0.47	9.46	2.01	0.21	6.32			
17	10.33	0;96	3.26				0.23	0.02	0.70
18							0.65	0.09	2.46
19	2.73	0.74	7.04	1.69	0.26	6.12			
20	7.23	1.82	24.18				0.61	0.15	2.04
21				0.61	0.17	4.49	0.19	0.09	1.10
22	6.20	0.17	6.65						
23				2.67	0.25	1.46			
24	2.23	1.0	12.11	1.28	0.54	8.42	0.30	0.13	1.63
25				3.46	0.92	16.79	0.55		
26	7.14	1.20	17.98	1.98	0.37	4.33	0.30	0.05	0.76
27	1.27	0.66	9.92	1.05	0.36	7.29	0.21	0.11	1.64
28				1.01	0.47	10.50			
29				0.98	0.53	9.07			
30				2.45	0.44	17.62			
31	0.80	2.28	38.09				0.14	0.40	6.66
32				0.89	2.28	49.44			
33	1.03	2.93	35.66	1.27	4.53	60.47			

Table 7

Determination of ALA in Urine of Exposed Population in mg/l in Summer 1968

Settlement	No. of Samples	Minimal Value	Maximal Value	Average Value
Lamprečе	55	2.5	21.2	9.82
Črna	273	3.0	27.0	11.10
Glančnik	12	3.7	18.0	11.16
Rudarjevo	138	2.1	35.9	11.70
Polena	165	0.7	44.0	11.94
Žerjav	269	1.4	42	12.97
Total	912			
Control Group	50	0.35	8.55	5.20
Lead Miners	7	24.5	50.4	31.80
Lead Smelters	70	7.5	117.0	54.40

Table 8

ALA Values in the Urine of Inhabitants of the Meza Valley

Settlement	No. of Inhabitants Examined	Average ALA Values in mg/1 Summer 1968	Summer 1969
Zerjav	99	19.6	10.16
Crna	130	16.0	13.0
Mezica	60	16.7	10.66
Control Group			
Mislinja	51	5.20	

Decrease of average ALA values in 1969 in relation to 1968

Žerjav for 48%
Črna for 19%
Mežica for 38%

References

Following papers are prepared for publications:
"Lead Contamination of Environment in Meza Valley"

1. Djuknić, V., Lj. Jablanov, and D. Djurić: Determination of Lead in Air.
2. Kerin Ž., and D. Djurić: Determination of Lead in Waters.
3. Kerin, Ž.: Determination of Lead in Snow.
4. Kerin, Ž., and D. Djurić: Lead Content of Overground Parts of Plants.
5. Djurić, D., and Ž. Kerin: Some Considerations on Lead Content of Soil and Plants.

STUDIES ON CARBON DISULPHIDE TOXICOLOGY IN YUGOSLAVIA 1963 - 1970
A Review

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Medical Service of Factory "Viskoza", Loznica

From the beginning of work in the viscose factory "Viskoza" in Loznica in 1957, some problems in industrial hygiene and occupational health developed.

In the viscose stapple-rayon production operation, serious microclimatic situation (relatively high humidity and high temperature) was connected with carbon disulphide (CS_2) levels far over the maximum allowable concentration (MAC) values. The concentration of hydrogen sulfide (H_2S) was at MAC level or a little over. Therefore, this situation produced a series of carbon disulphide intoxications after few years of exposure. In viscose rayon operations the concentration of CS_2 was under MAC values but the H_2S a little over. Here some troubles with glaucoma of the eyes occurred.

Taking in consideration this situation we decided to pay great attention to the problems of CS_2 exposure and intoxication. It was necessary to choose the most suitable methods for evaluation of exposure and detection of early signs of intoxication.

In September 1963, a five year research study (1963-1968) was initiated between the Institute of Occupational Health in Belgrade, and U.S.A. Public Health Service, Division of Occupational Health. All papers mentioned in this review are based on the work performed under this Research Agreement. In January 1969, the Agreement was extended for another five year period (1969-1973) with an envifonmental study on lead included.

Biochemical and Toxicological Research

In the beginning we paid attention to hygienic problems. For determination of CS_2 and H_2S in the air we applied standard methods with good results. Also determination of microclimatic conditions, including air motion, was performed (1).

At the same time we tried to find a good exposure test based on bioassay. Determination of CS_2 in blood was technically complicated and obtained results were not reliable (2,3). Also determination of CS_2 in the urine did not show good correlation with exposure of workers to CS_2 (2,3). In 1963 Dr. Vašak (Prague, Czechoslovakia) reported the iodine-azide test as an exposure test for CS_2. Immediately in 1964 we used this test in our viscose factory and obtained very satisfactory results. By determination of iodine-azide test (IAT) in the urine of exposed workers before and at end of work, we found a good correlation with integral exposure during 8 hours work for that particular day (4).

On the other hand, this study revealed the existence of "recovery" and "non-recovery" aspects in the excretion of CS_2 metabolites, causing iodine-azide reaction. For the next two years we studied the phenomenon of "non-recovery" and established that it represents an early sign of intoxication, preceding clinical signs for some months. We are assuming that "non-recovery" represents the consequences of a biochemical injury of the enzymatic system metabolizing CS_2 but further studies are needed to elucidate this problem (5).

We also used iodine-azine test to evaluate efficiency of the respirator and filter "A" (produced by Yugoslav factory "Miloje Zakić") which is a respiratory protection device for workers exposed to CS_2 in the viscose factory. We analyzed for one week the urine of workers using this device by IAT and established the capacity of the filter for CS_2 (6). These experiments, in real operating condition for only one week, gave answers on a very complicated question for which many months of laboratory research would be needed (7).

Developing our biochemical research, we paid great attention to carbon disulphide metabolism, which has not been satisfactorily elucidated (8,9). Elimination of sulphates in the urine of exposed workers was studied and an increase in the elimination of the organic fraction established (10). In a few cases excretion of total sulphates was increased with no correlation to CS_2 exposure.

Concentration of CS_2 in expired air of exposed workers was followed for 8 hours of work. During the first hour of exposure an equilibrium was reached with a constant retention of CS_2 of about 45% (11).

Due to absorption of CS_2 into the organism, the dithicarbaminic acids, possessing free SH-groups, are produced in the blood. We followed levels of free SH-groups in the blood of exposed workers but no linear correlation with exposure was established (12). Such compounds represent good chelating agents for heavy metals and we continued to study excretion of copper (Cu) and zinc (Zn) in exposed workers. In workers constantly exposed to CS_2 no changes in Cu excretion was found (13). In workers after vacation on the first and third day of exposure we found some increase in excretion of Cu and Zn suggesting chelating effects of CS_2 metabolites (14, 15, 16, 17).

Also a very interesting study on excretion of diethyldithiocarbamate in the urine of alcoholics treated with TETD (antabuse) was performed (18) and an increase established. In the majority of cases the iodine-azide test was positive, but not in all.

A complicated study on isolation and identification of CS_2 metabolites, especially those causing JAT, was developed. Until now two metabolites were identified: mercaptothiazolidone and thiourea, and a third one traced (19). Now similar study in the urine of alcoholics treated with TETD is in progress.

Hormonal Studies

In relatively young workers (average age 32 years) exposed to CS_2 up to 5 years, we performed determinations of total 17-ketosteroids, 17-hydroxicorticosteroids, androsterone and etiocholanelone. Also a group of disabled "invalids," transferred from CS_2 exposure after one or more acute intoxications, was included. This study was performed together with a team from Institute of Occupational Health in Pavia, Italy (Director Professor S. Maugeri).

Statistical evaluation of total 17-ketosteroids excretion showed that a decrease exists which is linearly proportional to the duration of exposure (20, 21), with lowest excretion in "invalids." The results of 17-hydroxicorticosteroids determination showed similar results with no linear correlation with exposure duration (22). Androsterone and etiocholanelone as catabolites of testosterone, showed different results (23, 24). Androsterone excretion was decreased with no correlation to exposure duration. No decrease in etiocholanelone excretion was established. Further studies are needed to elucidate where is the cause of these changes: injury on the level of testes, suprarental glands or hypothalamus.

Clinical Studies

For many years the health of workers exposed to CS_2 has been followed. In well developed chronic poisoning the clinical picture was rather uniform. More than 90% of observed cases complained of general weakness, especially of the lower limbs, vertigo, sexual disturbances and gastric troubles. The most frequent objective findings were asthenia, neurasthenic syndrome, polyneuritis (especially of lower limbs) with disappearance of Achilles reflex, decrease of gross motor strength, disturbances of sensibility and hypo or anacid gastritis. In spite of relatively great experience, the diagnosis of initial sulfocarbonism, that means condition with many functional disturbances without organic lesion, even today presents a problem.

A team of specialists (occupational health specialist, neurologist, ophthalmologist, specialist in internal medicine) regularly perform medical examinations of exposed workers. Many clinical methods of examination were used with special attention to condition of the neuromuscular and vascular systems.

Examination of Neuromuscular System

These examinations were performed on a group of workers with expressed signs of intoxication and a group exposed to high concentration of CS_2 without clinical signs. Classical electrodiagnostic methods were used (with farad and galvanic electric current). All 100% of intoxicated workers showed hypoexcitability to galvanic and farad impulses while 84% of exposed workers without clinical signs did. Slow contraction of the small muscles of the hand and thumb was established in 85% of the cases with polyneuritis. Control measurements are made every 2-3 months to follow evolution of changes. In about 75% of followed cases, a correlation between clinical and electrodiagnostic findings was evident (25). Therefore, methods of classical electrodiagnosis could be used as auxiliary methods for diagnosis of sulfocarbonism.

Results of measurements of chronaxia, I/T curve and speed of conduction showed that these methods are not suitable for early diagnosis of sulfocarbonism (26). In workers with polyneuritis, pathologic parameters are increased in relation to others.

Dynametric determination of muscle strength showed differences with lowest values in the intoxicated group (27). Similar results were obtained with registration of muscle tonus (miotonometry). Measurement of neuromuscular reaction (using light and noise signals) showed that it is slowest in intoxicated groups.

All mentioned methods for measurement of neuromuscular system changes gave good results in cases where examination revealed signs of intoxication. Therefore they can be used as auxiliary methods to complete diagnosis.

Studies on Cerebral and Peripheral Blood Circulation

Together with a team from Institute of Occupational Health in Pavia in 1965, we studied cerebral and peripheral blood circulation using rheography, oscillography, plethysmography and peripheral rheography. Vascular changes were studied on a group of relatively young workers (average age 32 years). The results (28, 29) suggest that CS_2 has vasotropic effects causing functional and organic alterations in central and peripheral blood vessels. Organic alterations on cerebral circulation are registered in 8% of exposed workers (exposure more than 5 years) and in 21% of invalids. Organic changes in peripheral blood vessels were evident mostly on upper extremities in 8% of exposed workers and 10% of invalids. In 1969, we performed again plethysmographic determination on peripheral blood vessels and found an increased number of cases (55%) in workers exposed more than 10 years to CS_2. Also we measured arterial tension and after statistical evaluation found an increased tension in the group exposed over 10 years in relation to control group (30). In connection with this we also performed an electro-skin test by application of adrenalin and histamine by electrophoresis (30). The main characteristic was absence of skin reaction on adrenalin. The frequency of this phenomenon was correlated with exposure duration. In the group with more than 10 years exposure this phenomenon appeared in 34% of examined workers, in 5-9 year exposure group 30%, under 5 years 16% and no one case in the control group. In all workers we performed electrocardiographic (EKG) examinations with no remarkable findings on coronary vessel involvement.

Ophthalmologic Investigations

In 1963-64, ophthalmologic examinations of exposed workers were performed with the aim to establish if the changes in the eye are due to primary effects of CS_2 on nerves or on the vascular system (31). Very often alterations in reactivity of pupil to light was established; in rare cases retrobulbar neuritis and paralysis of oculomotor nerves were observed.

In 1969, a study performed with a team from Osaka University (Japan) using fluorescent angiography and direct ophthalmoscopy revealed microaneurismic alterations in exposed workers similar to those in diabetes mellitus (32). Pathologic changes were related to exposure duration. By ophthalmodynamometry a significant increase in tension of arteria ophthalmica was established in relation to the control group.

In the operation of "silk" viscose-rayon and less in stapple rayon operation, a great number of glaucoma was established (33). It is supposed that H_2S is a main cause of this trouble and practical recommendations were developed.

Hematological and Biochemical Investigations

In pathogenesis of the vasculopathology due to CS_2 the disturbances of lipid metabolism are often cited as the cause of changes in blood coagulation. Therefore we determined lipoproteins, total cholesterol and some factors of blood coagulation in 1965 and 1969. In 1965 workers exposed to CS_2 for 6 years, we did not find any changes in lipoproteins and cholesterol metabolism (34). In 1969, we established an increase of lipoproteinic index in 50% of examined workers exposed to CS_2 over 10 years (35); which is statistically significant in relation to the control group. A small increase in cholesterol was found in both studies (34, 35). In 1969, determination of esterified cholesterol was performed and an increase was established with exposure duration (35).

Together with the team from Institute of Occupational Health in Pavia very detailed investigations of some parameters in blood coagulation were performed in workers (36, 37, 38, 39, 40) and rats (41). In 1965, time of coagulation, Rumpel-leed phenomenon, prothrombin time, fibrinolytic and antiheparinic activity and thromboelastrogram were measured. The results suggested disturbances in blood coagulation with a protracted time of the coagulation processes, especially in the beginning of exposure duration with tendency for the change to disappear later (repeated investigation in 1969). On the other hand fibrinolytic activity increased with duration of exposure. It seems that disorders of blood coagulation represents characteristics of early exposure with a tendency to disappear with longer duration.

The mentioned vascular alterations showed a tendency to appear after at least 10 years of exposure. It is necessary to stress again the fact that the examined workers were relatively young and without diseases which could produce the mentioned effects. Taking in consideration working conditions and risk in the working place, it could be concluded that CS_2 represents the main etiologic factor in the development of the vascular alterations. Changes in lipid metabolism are of primary importance in pathogensis of vasculopathia sulfocarbonica.

Study of the vascular system and the metabolic disorders will represent an important field of our further studies on sulfocarbonism.

In 1969, we performed together with the team from Osaka University (Japan) a study to confirm their hypothesis and investigations that CS_2 causes retinal microaneurisms and metabolic disturbances. For this purpose we applied Prednisolon augmented and glucose tolerance test on exposed workers (42). This first investigation gave interesting results but it will be necessary to extend them on greater number of workers.

In collaboration with Dr. Tintěra (Czechoslavakia) a study on changes in vitamin B_6 metabolism showed an increase in excretion of tryptophane in exposed workers (43).

It should be mentioned also that in a large percentage of examined workers, gastric disturbances were evident (44). Chemical and radiologic pictures were studied and evidence of hypo or anacid gastritis was found in a majority of the workers with expressed clinical picture of CS_2 intoxication. Taking into consideration the structure of the national habits in nutrition, CS_2 seems to be contributing, but not the main etiologic factor causing gastric disturbances.

Conclusions

This review represents a very short report on numerous investigations performed from 1963-1970 in the field of sulfocarbonism. Majority of results are published and some will be published soon.

Numerous methods and techniques were applied in these investigations bringing some solutions or helping to elucidate some specific problems for our conditions.

The majority of our investigations gave results of immediate practical value. After their evaluation we prepared numerous recommendations to the Directory of the factory and Medical Services of the factory. In such a way some preventive measurements were developed especially in the working conditions of exposed workers, working arrangements, way of nutrition, and control of exposed workers.

In spite of great efforts and results, many problems are still unsolved requiring our full engagement in the future. The diagnosis of initial sulfocarbonism has to be pointed out as one of the problems of great importance.

We hope to introduce new methods of electroencephalographic investigations which will help us in early diagnosis of lesions due to CS_2 effects.

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Discussion after Drs. Djuric and Graovac-Leposavić's paper.

Dr. Bessey: According to the one slide we saw the differences between vegetable products and animal products contaminated with lead. Did you find any symptoms of intoxication of livestock?

Dr. Djurić: Farmers complained that the cattle are not reproductive, but until now we can not say for sure what it is. We performed some analyses of lead in bones, and it is very high, but it is very high in people. It is for sure that some effects are there because these cattle take food only in the contaminated area and, as I said, hay has about 300 milligrams of lead per 1 kg, and therefore, I think that ingestion of lead by cattle is very high. Insects are dying, especially bees, but I am not sure if it is due to lead or due to sulphurdioxide. Also hens are dying out, but we assume that it is mostly due to lead. It is for sure that animals in these areas are very exposed and that something happens to them. But we did not study it in detail.

Dr. Szymczykiewicz: I have one question for Dr. Leposavić. Did you observe in the women exposed to CS_2 some pregnancy abnormalities? For instance, in our country we observed a lot of pathological changes in the women exposed to CS_2.

Dr. Graovac-Leposavić: In our country it is forbidden for women to work in the operations where they could be exposed to CS_2.

Dr. Kilibarda: In this figure what you showed, about 200 tons pollution of lead, is that per year? I don't wonder that is so high because we know some similar conditions, but to avoid any mistake, are your calculations about 200 tons of lead all right?

Dr. Djurić: It is not our estimation, it is calculation of the factory experts and they assume that is about this figure, and they like to avoid the loss of lead also.

THE EFFECT OF CARBON DISULPHIDE ON THE METABOLISM OF PYRIDINE NUCLEOTIDES AND NICOTINAMIDE

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The toxic action of carbon disulphide is many sided. For example, it has been known, that, depending on the severity of exposure, the prevailing symptoms may be the result of a neurotoxic action (at higher concentrations) or a vasculotropic one (at lower concentrations). Similarly, various known biochemical disturbances in the organism may be caused by this compound. At present, it seems difficult to answer the question whether the different disturbances appear independently each from the other or as a center of initial action with derivative symptoms which are being actually observed.

Our present studies on the metabolism of nicotinic acid in the course of carbon disulphide intoxications were started on the basis of the following earlier observations:

1. Exposure to CS_2 brings about disturbances in the urinary level of the nicotinic acid, manifested by an increase of the N^1-methylnicotinamide. That was first observed by Liniecki (1) and later confirmed by Wrońska et al. (2) to occur in rats. However, similar disturbances were also found in workers exposed to CS_2, in the viscose rayon industry (3). Moreover, some quantitative relationship was observed between the elevation of N^1-methylnicotinamide and the actual concentration of CS_2 in the air (Fig. 1), (4). It was found in rats exposed to a concentration of 0.9 mg/1 of CS_2 that increase of N^1-methylnicotinamide in the urine takes place already at the beginning of chronic exposure (after 2 days), reaching a steady state of the elevated level after one week of exposure **(Figure 2) (4).**

2. Some biological importance of the above disturbances seem evident if one considers the influence of nicotinic acid on the pathways of the lipid metabolism: Chronic exposure to CS_2 results in changes of the lipid metabolism consisting of an increase of the cholesterol, phospholipids and triglycerides in blood. These changes could be prevented by feeding of the animals with nicotinic acid (5).

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The biochemical background of the above phenomenon is still obscure. With respect to the changes in the metabolism of nicotinic acid it seemed to be interesting to find out the sources of the excess of nicotinic acid appearing in the urine in form of the N^1-methylnicotinamide, if constant uptake of nicotinic acid from the food is accepted.

The existing data point to the fact that the bulk of the systemic nicotinamide is present in the body in the form of nicotinamide adenine nucleotides. Therefore, theoretically at least, the only source for the elevated levels of nicotinic acid could be the pool of the nucleotides; carbon disulphide could, eventually, increase their degradation rate, resulting in an increased excretion of the nicotinamide (as a methylated derivative or nicotinic acid). If this hypothesis is taken as a basis, two possibilities seem to have existed: (a) the increased degradation rate of nucleotides has been accompanied by similar increase in the rate of synthesis (increased total turn over rate), allowing the actual absolute level of nucleotides to remain unchanged; (b) otherwise, when there was no increase in the synthesis of nucleotides, the elevated excretion would result in a depletion of the systemic levels of the nucleotides if the exposure to CS_2 had persisted for a sufficiently long time.

In the present studies attempts were undertaken to answer the following questions:

a. Whether, apart from disturbance of N^1-methylnicotinamide, also **the excretion of other metabolites of nicotinic acid is disturbed** by exposure to CS_2;

b. Whether the elevated excretion of the nicotinic acid metabolites is reflected by a drop in the level of systemic nucleotides, and

c. What is the biochemical background of the disturbances in question. Attempts to approach the above problems are given below in detail.

I. The nicotinamide metabolites in the urine of rats exposed to CS_2.

The underlying data on the excretion of the metabolites of nicotinic acid were fragmentary only and were based entirely on the fluorimetric determinations. Therefore, we decided to check once again the accepted assumption using methods which differ from those applied **previously and which allow the estimation of N^1-methylnicotinamide** as well as nicotinic acid and nicotinamide.

The evaluation of the excretion of the nicotinic acid metabolites has now been based on: (a) quantitative determination of the metabolites by chemical methods, and (b) on the measurement of activity of individual metabolites, following administration to rats in vivo of nicotinic acid labelled with ^{14}C in carboxyl group. This was done in separate experiments in the following way:

1. The rats exposed to CS_2 and the controls were placed in metabolic cages and in the daily urine collected the following metabolites were determined: (a) N^1-methylnicotinamide (fluorimetrically after Huff et al. (20)); the "total nicotinic acid" colorimetrically and the nicotinamide, both after Wrońska et al. (21,22). The content of the free nicotinic acid was calculated from difference between "total nicotinic acid" and nicotinamide.

2. In the other series of experiment, rats (exposed and controls) were given intraperitoneally nicotinic acid or nicotinamide (2 μmoles of total activity 20 μC per rat) and the daily volume of urine was collected. The metabolites of nicotinic acid were separated by electrophoresis (23) or paper chromatography in the system 80% n-propanol or n-butanol: aceton: water (9:1:10). The measurement of the activity was done on the Nuclear-Chicago flow-counter.

The results presented in Table 1, confirm our view on the increased excretion of the nicotinic acid metabolites in exposed rats. N^1-methylnicotinamide was increased by 126%, the nicotinic acid by 34%, only the nicotinamide was not affected. The same trend is evident also from the isotopic studies (Table 2).

The new element of this study is that the discussed disturbances are not limited to elevated excretion of N^1-methylnicotinamide but also concern the second metabolic product of nicotinamide, namely nicotinic acid itself.

II. Studies on the levels of the nicotinamide-adenine dinucleotides in the tissues of rats exposed to CS_2.

A. Methods of determination of the nicotinamide-adenine nucleotides in the tissues.

The methods most often recommended for the quantitative determination of the nicotinamide-adenine dinucleotides include enzymatic procedures which are based on preparations that are hardly available in Poland, and also very expensive. The study therefore, had to be started on the development of simple modifications in which the above obstacle would be eliminated. Consequently, these attempts had to be extended to cover the specific demands for analysis of different kinds of tissues which were to be investigated.

The basic principle underlying the modifications was that of fluorimetric procedure described by Lowry et al. (6) and Bassham et al. (7). It appeared necessary to use a somewhat different procedure of determination when performed on the liver, blood and muscles than on the brain.

Liver, blood, and muscles*: The elaborated procedure (Figure 3) consists of 3 basic stages:

1. Selective extraction from the tissues of the reduced and oxidized forms, respectively, using conditions differing principally in pH and temperature: hot 0.1 sodium carbonate and cold 5% TCA.**

2. The separation of the phosphorylated forms was performed with the oxidized forms only: therefore, the reduced ones had to be first oxidized, and this was achieved by using phenazonium methosulphate. From the obtained NAD and NADP solutions the former was reduced in the system containing ethanol and alcohol dehydrogenase. In this way the two compounds differing initially by the degree of phosphorylation were converted into those differing in the redox state and as such could be separated using the initial procedure of acidic - alkaline destruction of one component.

3. The fluorescence was measured after proper incubation in 6 N NaOH at 37°C., 60 min, using "Farrand" fluorimeter (Model A), primary filter: 5860, and the secondary filters: No. 4308 and 3389 or "Opton" spectrofluorimeter (Model PMQ-II - excitation 370 nm and transmission 460 nm).

The recovery of standards (NAD, NADP and NADH) added to the tissue was almost complete and the standard error was ± 10%. The sensitivity was about 50 μmoles/g of liver.

The low content of the nucleotides, and especially of NADP in blood and muscles, was the cause for which in these tissues the procedure had to be limited to the determination of sums of the oxidized (NAD+NADP) and reduced forms (NADH+NADPH). The recovery of standards was satisfactory also with these tissues (for NAD-95%).

Brain***:In the way described above the content of the nicotinamide adenine dinucleotides may be also determined in fresh brain tissue. It has been shown, however, that the post-mortem changes of the

* For details see: Sokal et al. (13); Sokal and Wrońska-Nofer (12).

** TCA - trichloroacetic acid.

*** Sokal - in preparation.

content (Figure 4), and specially of the redox state of the nucleotides, are very rapid resulting in values that are erroneous with respect to the state existing in vivo. On the other hand, if the described procedure was applied to frozen brain tissue, artifact took place resulting in lowering of the levels of the reduced forms. Therefore, the extraction procedure had to be modified once again by introducing the basic principle of Burch et al., (8). From the latter the presence of an antioxidant (cysteine) and a low temperature (0°) appeared to be essential to counteract the oxidation of the reduced forms.

Using the methods presented above the levels of nicotinamide-adenine dinucleotides were determined in animal tissues, and the obtained levels are presented in Table 3.

B. The influence of the prolonged exposure to CS_2 on the levels of nicotinamide-adenine nucleotides in the tissues of rat*.

These studies were performed in order to answer the question whether the increased excretion of the nicotinic-acid metabolites occurs at the expense of the systemic pool of nucleotides. As mentioned formerly, this seemed probable since the nicotinamide-adenine dinucleotides represent the main pool of the systemic nicotinamide (for instance in the liver, about 80%; see Ricci and Pallini (9)). On the other hand, prolonged elevated excretion of the nicotinic acid metabolites in a degree found experimentally if kept over sufficiently long time-period, would consume an excess of nicotinamide in the amount comparable with their content in the whole body of the rat.

The experiment was kept over 5 months on rats exposed daily to CS_2 in concentration of 1.8-2 mg/l. The levels of the nucleotides were determined in groups of animals sacrificed after different time-periods of exposure, and the determinations were performed on blood, liver and muscles; these tissues contain jointly about 80-90% of the systemic pool of nucleotides (10). White female rats of the Wistar strain were used. The animals were fed standard ISM diet.

The results are shown in Tables 4 and 5. From Table 3 it follows that within the period of 5 months only subtle changes in the levels of nucleotides could be found in the tissues; in liver a slight increase occurred both of the reduced and oxidized form, resulting chiefly in the form of an increase of NAD and NADPH levels (Table 5). The reverse

* For details see Wrońska-Nofer et al. (10); Sokal (24); part of the data are in preparation.

was found in blood and muscles. The above changes were reached, after 5 months duration of exposure, about 20% different from the controls. The drop of the oxidized form of nucleotides in muscles was lower by about 9%.

The above had to be compared with the predictions resulting from the increased output of nicotinamide metabolites. The latter have not been determined parallely in this experiment but the data have been taken from the previous experiments as sufficient for such comparisons. From those it could be calculated that the excess excretion of nicotinic acid metabolites amounted in rat to 0.38 μmole/day (2,14). If such a daily excess is summed up with time and the result is substracted from the whole systemic pool of nicotinamide-adenine nucleotides, the predicted drop of their systemic level is obtained (Figure 5). After 5 months of exposure, the latter should drop to only 25% of the initial (control) value. The actual decrease, however, has not exceeded some 15%, a value far from that resulting from the predicted hypothesis. It seems justified, therefore, to exclude the assumption that the increased output of nicotinamide metabolites in rats exposed to CS_2 occurs at the expense of the systemic levels of nucleotides.

The above does not apply to later periods of exposure, when severe injury is already observed (limb paralysis, drop of body weight and especially of the muscles). These severe symptoms are accompanied also by some changes of the nucleotides in the tissues.

The results of experiments with such a prolonged exposure given in Table 6, show that at that time there are still no essential changes in the level of nucleotides in the brain. In the muscles, however, there is an essential drop of the total level of nucleotides reaching about 50%, after 8 months of exposure. In this type of a very prolonged exposure, apart from the total tissue levels, also the distribution among subcellular fractions of liver was determined. Whereas, total deviations found in the liver are not very pronounced, of some interest may be the drop of the nucleotides in the mitochondrial fraction (Table 7). This may be connected with some damage to these subcellular elements as found by Woyke (15).

III. The turnover of the nicotinamide-adenine nucleotides in the liver of rats exposed to CS_2.

From the former data it follows that the exposure to CS_2, if limited to the time period of half a year, does not influence considerably the levels of the nicotinamide-adenine nucleotides in the tissues. Therefore, it had to be expected that increased excretion of N^1-methylonicotinamide is accompanied not only by the higher degradation rate of the nucleotides but that the whole turnover rate is accelerated.

It is known that into the nicotinamide-adenine nucleotides are incorporated both, nicotinic acid and nicotinamide, the latter after previous desamidization. The studies on both these processes are presented below.

A. The incorporation of nicotinic-acid into the nicotinamide adenine nucleotides.

The experiment was performed on 29 rats (in this number 15 controls). The rats were given intraperitoneally 2 μmoles of nicotinic acid labeled ^{14}C (20 μC). The rats were killed 15, 30, 60, and 240 minutes after injection of nicotinic acid. Immediately after sacrification, the oxidized forms of nucleotides were extracted from the liver with cold 5% TCA and precipitated with acetone. The precipitates were further dissolved in water* and the two forms, NAD and NADP, were separated by column chromatography (column filled with Dowex 1x8, 200-400 mesh) using continuous concentration gradient of formic acid**. The final determinations were done fluorimetrically (as described formerly); the radioactive NAD, collected in the effluent of the column, was applied on the planchettes and the measurement of B-radiation was done on the Nuclear-Chicago Flow-Counter.

The results are presented in the Figure 6. The specific activity of NAD rose within 30 minutes after injection in both groups and later it decreased. However, the dynamics seemed to differ essentially in both compared groups. In rats exposed to CS_2 both the rise of specific activity in the first period and the drop in later stage were more rapid than in controls. This confirms our assumption of an increase of the total turnover rate of the nucleotides in carbon disulphide exposure.

B. Deamidization of the nicotinamide.

To assess the changes in the process of deamidization of nicotinamide its activity was measured in vitro in the liver homogenates. The activity of the nicotinamide amidohydrolase was measured using the method of Kirchner et al. (18) with ^{14}C labeled nicotinamide as substrate. This method is based on the determination of nicotinic acid liberated during incubation at 37°C in medium consisted of liver homogenate in 0.033 M phosphate buffer, pH 8.6 and labeled ^{14}C nicotinamide. The results were presented in μmoles of nicotinic acid per mg of protein of the homogenate, per hour.*** This experiment was performed on 19 rats (in this number 9 control rats).

* This stage according to Gordon (16). In control experiments practically complete recovery (over 95%) of the oxidized nucleotides from the acid extract was obtained by acetone precipitation.

** This procedure after Hurlbert et al. (17). In control experiment almost complete recovery (ab. 90%) was found of the exogenous nucleotides added to the acetone precipitate.

*** The protein content of homogenate after Lowry et al. (19) using fraction of the bovine albumin as a standard.

The results listed in Table 8 point to an increase of activity of the nicotinamide-amidohydrolase in exposed rats by about 60%. This increase seems to act in favor of the accelerated turnover rate of the nucleotides giving more direct substrate for the incorporation process of nicotinic acid. On the other hand, this trend can hardly be explained as being in favor of the increased methylation of nicotinamide. In this way, a new gap arose in our hypothesis, where the increased excretion of N^1-methylnicotinamide was accepted as a basic assumption.

Conclusions

The chief aim of the present study was to elucidate the mechanism by which carbon disulphide influences the increase of urinary excretion of N^1-methylnicotinamide. The present data allow the following conclusions to be drawn:

a. The exposure to carbon disulphide results in elevated urinary excretion not only of N^1-methylnicotinamide but also of nicotinic acid and of the total pool of its metabolites.

b. The above increase of the urinary excretion of the nicotinamide metabolites does not occur at the cost of the systemic pool of the nicotinamide-adenine dinucleotides.

c. The increased rate of incorporation of nicotinic acid into nucleotides, as well as increased rate of deamidization of nicotinamide points to the fact that the elevated excretion of nicotinamide metabolites reflects an increase, often whole turnover rate, of the nicotinamide-adenine dinucleotides in CS_2 exposure. Whatever the mechanism of this phenomenon, it is responsible for keeping the pool of nucleotides on unchanged level.

d. The data do not allow, yet, to draw any final conclusions as to the mechanism of the duscussed phenomena. Obviously further studies would be necessary including those of the systemic levels of the different forms of nicotinic acid. Also, there are no data on the methylation process itself, as well as on the level of the donors of the methyl-groups. The basic question which remains to be answered, however, is that of sources of excess nicotinamide for its increased turnover. While the assumption of increased synthesis via tryptophan seems most probable it still remains to be proved.

e. It is not clear whether the discussed disturbances in the metabolism of nicotinamide represent a phenomenon specifically conditioned upon the exposure to CS_2 or not. The recent reports on similar disturbances occuring in animals exposed to X-rays let us suspect that the mechanism in question may be entirely nonspecific. This however, needs further detailed study.

IV. The evolution of morphological changes in rats in the course of prolonged exposure to carbon disulphide.

Histopathological studies in rats intoxicated with CS_2 have been undertaken independently of the biochemical one which aimed at explanation of the mechanism of disturbances in the metabolism of nicotinic acid in CS_2 intoxication.

In rats exposed for 8-10 months to CS_2 vapours, onset of the paresis of the hind limbs of a various degree developing into paralysis and muscle atrophy was observed. In the atrophied muscles a reduction of the level of nicotinamide adenine nucleotides approximately to one half of control values, was detected. With an anticipation to trace the relationship between the biochemical disturbances and the grade of structural lesions induced by CS_2, separate experiments were undertaken. It was aimed as well at following the process of evolution of the histopathological changes in muscles, peripheral nerves and central nervous system, as at determining in parallel concentration of the nucleotides muscle tissue in the course of maintained exposure to carbon disulphide. White rats of Wistar strain, controls and exposed to CS_2 at concentration of 1.5-1.8 mg/l over the period of 2-14 months, were used in this study.

For investigations of the muscles, quadriceps femoris, gastrocnemius and tibialis anterior were chosen. The material suitable to biochemical procedures was dissected from the femoral quadriceps at the moment when sacrifice of each animal was just accomplished. Wet tissue weight of the muscles (excluding quadriceps) was recorded after dissection. The muscles were then cut into transverse and longitudinal pieces for further histological and morphometrical assay. Hematoxylin and eosin and PTAH (Mallory's phosphotungstic-acid hematoxylin) stains and the McManus periodic-acid-Schiff (paS) procedure were employed using paraffin sections.

In the morphometric estimations transverse sections of gastrocnemius were used. The diameter of individual muscle fibers was recorded at random, the obtained values divided into eight classes in the range between 20-100 μ of thickness and the percent distribution expressed in the form of histograms.

From the general observations in this group of experimental animals first signs of muscular weakness were noted within the seventh month of the intoxication. Two months later paresis of hind extremities was sufficiently evident developing into paralysis at the fourteenth month of exposure.

For the comparative analysis wet muscle weight was expressed in direct values (absolute weight) and in relation to the body weight (relative weight).

Over the whole period of 14 months the weight of gastrocnemius and anterior tibial muscle of the intoxicated animals was constantly lower than the controls (Figure 7). Through the fifth month of exposure the difference was due to the retardation of muscle growth under the influence of carbon disulphide, and it was seen in the absolute as well as in the relative values. Later the muscle weight (both absolute and relative) in controls remained unaltered, whereas in the experimental group its gradual fall was observed. The latter was very slow in the fifth month, it became faster in the seventh, to become particularly pronounced within the 12-14 month of the exposure.

In the microscopic picture the presence of atrophied muscles fibers was a constant finding from the sixth month up to the final stages of the experiment. The grade of atrophy is nonuniform in various areas of the same sections. Dispersed across the whole thickness of the muscle are small groups of thin slightly basophilic muscle fibers (Figure 9). Their borders are indistinct and the number of nuclei is excessive. Within some of the bundles solitary hypertrophied fibers are represented (Figure 10). In the remaining muscle except for a slight hypertrophy no apparent morphological lesions can be seen.

Although the typical signs of atrophy were absent from the histological picture of muscles prior to the sixth month of exposure, some quantitative changes in the diameter of muscle fibers were detected by means of the morphometric procedures. They can be seen as a shift toward the extreme classes in the histogram of the gastrocnemius muscle of rats exposed for 5 months to carbon disulfide (Figure 8). The shift represents a simultaneous increase in the number of both the thinnest and the thickest (presumably hypertrophic) muscle fibers. In the following months, when the typical signs of muscle atrophy appear in the histological picture, a reliable count of thin fibers can be done, but the shift to large diameters is still evident.

From the nervous system the brain, cerebellum, brain stem, spinal cord and two peripheral nerves, ischiadic and femoral, were subject to histological study. Routine methods of fixation, embedding and staining, the latter consisting of hematoxylin and eosin, PTAH and the Luxol Fast Blue stain with cresil violet counterstanding (36) were employed in serial sections. In addition portions of the ischiadic and femoral nerve fixed in 2% glutaraldehyde were impregnated subsequently in osmium tetroxide, stained with Pal-Kulczycki's hematoxylin and embedded in Epon 812. From this material sections 1 u thick were cut on an ultramicrotome for the ordinary microscopic examinations.

In the neuronal cells of the central nervous system no apparent lesions were encountered. From all regions of white matter subjected to investigation porosis of a small degree was noted in the anterior and lateral funiculi of the spinal cord of the experimental rats sacrificed after 12 and 14 months of exposure. Within the somewhat spongy structures a few globular or polyglonal hyaline clumps could be found. They corresponded, in all probability, to the swollen axis-cylinders.

No definite conclusions about the actual condition of the peripheral nervous fibres could be drawn from the tissues prepared according to the routine procedures, whereas in the semi-thin sections after osmium impregnation signs of degeneration in separate fibres were detected as early as 4 months after beginning of exposure. With the prolonged intoxication very distinct alterations in the diameter of individual fibres, more abundant interstitium and thickening of the walls of intraneural blood vessels were also observed (Figures 11, 12). Mylein sheets were spared in the majority of the fibres, with occasional small defects or partial lamination. Complete loss of mylein sheet was apparent in a small percentage of fibers, evidently due to their total degeneration (Figure 12).

Investigations of the level of nucleotides in the femoral quadriceps muscle were conducted from the 1-12 months of exposure. Determinations were performed according to the fluorimetric method of Sokal et al. (8).

Seven months of exposure to carbon disulphide remained without an appreciable effect on the concentration of nucleotides in the muscles; during the following months its slight decrease was recorded. A considerable loss of the muscle nucleotides occured in the final stages starting at the end of 10 months of carbon disulphide intoxication (Table 9).

Conclusions

It seems that results of our investigations do justify the conclusion that muscular changes do belong to the syndrome of chronic carbon disulphide poisoning, produced under the experimental conditions described above.

The proof seems to lie in the presence of lesions of peripheral nervous system and in the fascicular character of muscle atrophy (32,34,38). Prior to appearance of the latter the morphometric procedure was effective in showing that initial stage of muscle atrophy was represented by characteristic quantitative deviations in fiber diameters. The important part of these changes could be characterized as hypertrophy of some fibers parallel to initial atrophy of the others. Furthermore, no appreciable morphological alterations were detected that would be suggestive of any diffuse muscle lesion pointing to a direct influence of CS_2 or its metabolites on the muscle fibers.

It should be emphasized that a prolonged exposure to carbon disulphide was necessary to produce a substantial decrease in the amount of nicotinamide-adenine nucleotides in the muscles.

At that stage of intoxication the fall of muscle weight amounted to 50% of the control values and the histological evidence of atrophy was already unequivocal. The changes in the level of nucleotides should be accepted therefore as an effect of neurogenic atrophy, due to the partial denervation of the muscle in chronic carbon disulphide poisoning.

Development of the syndrome of chronic intoxication was slow although a fairly high concentration of CS_2 was employed. The histological changes in the central nervous system were very slight as compared with the lesions produced by CS_2 in dogs (33) and rabbits under the conditions of experimental intoxication (35,37).

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Table 1

The Content of Nicotinic Acid Metabolites in the Urine of Rats Exposed to CS_2*

Group of Animals	Number of animals	Nicotinic Acid		Nicotinamide		Total nicotinic Acid		N^1-methlylonicotinamide	
		μg	%	μg	%	μg	%	μg	%
Control	13	29.8±9.9	100	30±9.9	100	59±10.9	100	157+71	100
CS_2	13	40±13.6**	134	32.5±7.05	108	72+13.7**	122	355+172	226

* The values given in the Table present μg of metabolites excreted in the 24 hours urine ± S.D.

** Difference statistically significant as compared with control: $P<0.05$.

Table 2

Activity of Nicotinic Acid Metabolites in the Urine of Rats.

Group of Animals	Nicotinic Acid C.P.M. x 10^{-4}	%	Nicotinamide C.P.M. x 10^{-4}	%	N^1-Methylnicotinamide C.P.M. x 10^{-4}	%
Control	13.06	100	2.62	100	13.05	100
CS_2	14.93	114	2.25	86	38.19	292

The values given in the Table present activity of nicotinic acid and its metabolites excreted in the daily urine of rats 24 hours after intraperitoneal injection of 20 uC of nicotinic acid (carboxyl $-C^{14}$).

Table 2A

Activity of N^1-Methylnicotinamide in the Urine of Rats.

Injected Compound	Group of Animals	N^1-Methylnicotinamide C.P.M. x 10^{-4}	% Excreted Dose	%
Nicotinamide-7-C^{14}	Control	13.04	17.7	100
	CS_2	30.5	34.8	235
Nicotinic Acid-7-C^{14}	Controls	7.35	10.2	100
	CS_2	14.02	20.6	191

The values given in the Table present activity of N^1-Methylnicotinamide excreted in the daily urine of rats 24 hours after intraperitoneal injection of 20 μC of nicotinamide (carbonyl-C^{14}) and nicotinic acid (carboxyl-C^{14}).

Table 3

The Level of the Nicotinamide-Adenine Dinucleotides in the Rat Tissues.*

Liver

Authors	NAD	NADH	NADP	NADPH
Authors own data	447 ±40	199 ±38	96 ±15	409 ±56
Glock, et al. (28)	654	178	34	308
Lowry, et al. (27)	628	252	115	502
Bassham, et al. (7)	485	133	25	251
Sundarm, et al. (25)	496	119	41	269
Ville (26)	390	170	15	350
Pande, et al. (29)	300	82	79	254

Brain

Authors	NAD + NADP	NADH + NADPH
Authors own data	406 ±31	61 ±6
Glock, et al. (28)	200	144
Lowry, et al. (27)	329	118
Garcia-Bunuel et al. (30)	309	58
Lowry, et al. (6)	251	119

Blood

Authors	NAD + NADP	NADH + NADPH
Authors own data	112 ±11	55 ±9
Glock, et al. (28)	90	58
Lowry, et al. (27)	124	99
Cartier, (31)	122	110

* The levels of nucleotides in blood presented in µmoles/ml of total blood, in the brain and in the liver in µmoles/g of wet tissues. ±S.D.

Table 4

Changes in the Nicotinamide-Adenine Nucleotides Levels in Blood, Liver and Muscles of Rats in the Course of Exposure to CS_2.*

Duration of Exposure (Months)	Blood Control	Blood CS_2	Liver Control	Liver CS_2	Muscles Control	Muscles CS_2
			NAD + NADP			
1	111±11	104±14	528±51	563±50	535±48	529±57
2	108± 4	109± 9	589±81	603±59	542±28	525±50
3	115±13	96±11	555±35	642±40	573±24	530±32
5	119±10	100± 4	525±53	627±57	582±35	527±35
			$NADH_2$ + $NADPH_2$			
1	48± 9	47±13	597±150	514±98	98±12	86±12
2	59±11	47±13	591±116	603±91	93±10	93±18
3	56± 5	43± 6	596±66	669±56	92±18	98±13
5	56± 7	46± 3	605±104	730±179	60± 6	70±11

* The level of the nicotinamide nucleotides in blood presented in µmoles per 1 ml of whole blood, in the liver and in muscles in µmoles per 1 g of wet tissue ±S.D. Mean values from 7-10 animals are given.

Table 5

The Level of the Nicotinamide-Adenine Dinucleotides in the Liver of Rats After 5 Months of Exposure to CS_2*

Groups of Animals	Number of Rats	Weight of Liver (g±S.D.)	NAD	NADP	$NADH_2$	$NADPH_2$ 88
CS_2	7	7.0±1.3	506±48**	95±18	228±84	502±117
Control	9	6.8±0.7	439±50	86±14	185±35	409±59

*** The level of the nicotinamide neucleotides presented in µmoles per 1 g of wet tissues ± S.D.**

**** Difference statistically significant as compared with control: $p < 0.05$.**

Table 6

The Levels of Nicotinamide-Adenine Dinucleotides in Brain, Liver, and Muscles of Rats After Long-Term Exposure to CS_2

Group	Brain		Liver		Muscles	
	NAD+NADP	NADH+NADPH	NAD+NADP	NADH+NADPH	NAD+NADP	NADH+NADPH
CS_2	385±23	62±5	623±26	456±62**	310±44.2**	74±15**
Control	396±10	58±3	629±62	523±27	613±40.4	109±23.3

* The levels of nucleotides presented in µmoles per 1 g of wet tissues ± S.D.

** Difference statistically significant as compared with control: $p < 0.05$.
Mean values from 10 - 14 animals.

Table 7

Intracellular Distribution of Nicotinamide Adenine Dinucleotides in the Liver of Rats Exposed to CS_2*

Cellular Fraction	Group	Total Protein	NAD+NADP	$NADH_2$+$NADPH_2$
Homogenate	CS_2 Control	201±9 204±8	623±26 629±62	456±62** 523±27
Nuclear Fraction and Debris	CS_2 Control	36±5 34±8	43±7 49±7	50±15** 67±15
Mitochondrial Fraction	CS_2 Control	48±3 48±3	115±18 124±20	203±37** 254±24
Microsomal Fraction	CS_2 Control	33±3 31±3	49±4 49±11	29±4 27±8
Soluble Fraction	CS_2 Control	87±7 88±7	410±17 415±54	221±25 199±18

* The levels of proteins in mg and nucleotides in µmoles are expressed per 1 g wet liver tissue ± S.D. Mean values from seven animals.

** Difference statistically significant in relation to control: $p < 0.05$.

Table 8

Nicotinamide Deamidase Activity in the Liver of Rats Intoxicated with CS_2*

Group of Animals	Number of	Activity	%
Control	9	16.6±2.1	100
CS_2	10	27±1.7**	163

* Enzyme activity expressed in µmoles of nicotinic acid formed from nicotinamide during one hour incubation at 37° calculated for 1 mg of protein of liver homogenate ± S.D. The incubation medium was composed of: 1 ml of 10% liver homogenate in 0.033 M phosphate buffer, pH 8.6 containing 200 µmoles of ^{14}C-nicotinamide of total activity 1 µC.

** Difference statistically significant, $p < 0.01$.

Table 9

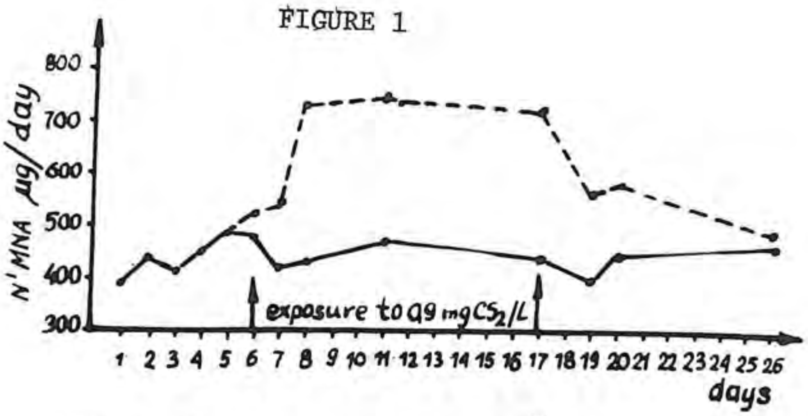
Change in the Nicotinamide-Adenine Nucleotides Levels in Muscles of Rats in the Course of Exposure to CS_2*

Duration of Exposure (months)	Number of Animals	NAD+NADP		$NADH_2+NADPH_2$	
		Control	CS_2	Control	CS_2
1	6	535±48	529±57	98.5±21	85±12
2	8	526±53	527±50	131±14	133±26
5	6	533±30	562±72	94±10	92.5±11
7	6	577±82	569±58	94±19	89±16
10	7	574±29	472±38**	118±16	97±21
12	5	573±36	400±52**	119±19	87±14**

* The levels of nicotinamide nucleotides in muscles presented in µmoles per 1 g of wet tissue ± S.D.

** Difference statistically significant as compared with control: $p < 0.05$.

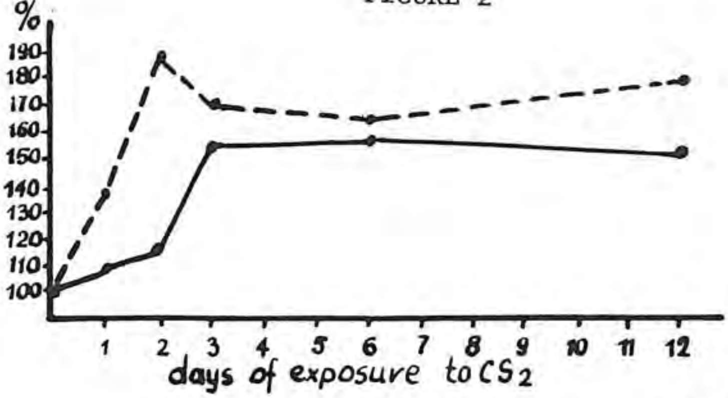
FIGURE 1



The daily excretion of N^1-methylnicotinamide in the urine of rats

——— control group ; ----- exposed to CS_2.

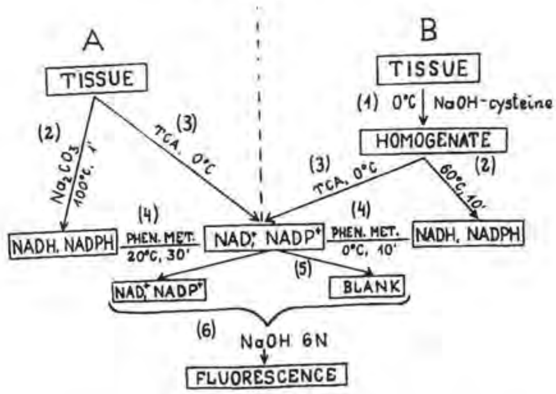
FIGURE 2



Relationship of CS_2 concentration and N^1-methylnicotinamide daily excretion in the urine of rats exposed to CS_2.

——— 0.9 mg CS_2/l ; ------ 2.0 mg CS_2/l.

FIGURE 3

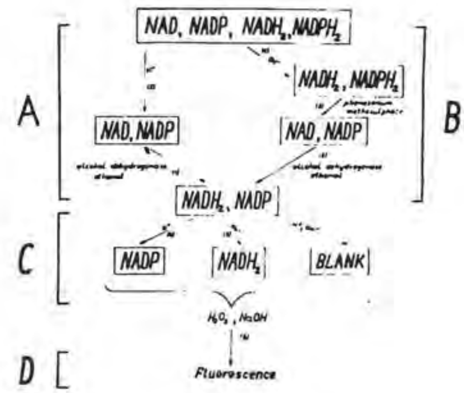


Scheme of the procedures for determination of the nicotinamide-adenine dinucleotides.

A- Procedure for fresh liver tissue/Sokal et al,1969/
B- Procedure for frozen brain tissue.
1- Tissue homogenization in cold NaOH-cysteine solution
2- Alkaline extraction of reduced nucleotides
3- Acidic extraction of oxidized nucleotides
4- Oxidation of reduced nucleotides with phenazonium methosulphide
5- Alkaline destruction of nucleotides
6- Development and measurment of fluorescence

Abreviations: TCA-trichloroacetic acid,
Phen.met- phenazine methosulphate.

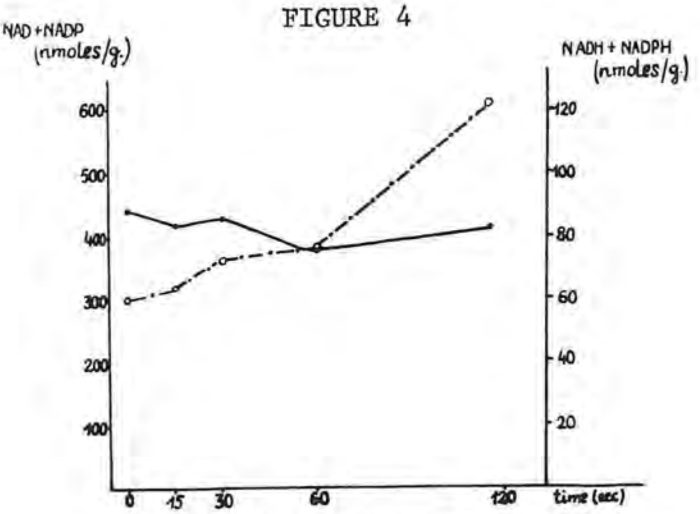
FIGURE 3a.



Scheme of the procedure.

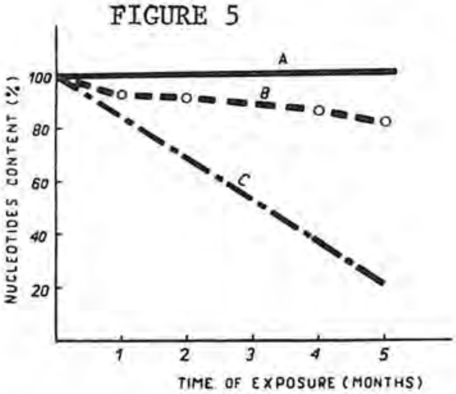
A - acidic extraction and enzymic reduction;
B - alkaline extraction, oxidation with phenazon methosulphate and enzymic reduction;
C - separation of $NADH_2$ and NADP;
D - development and measurement of fluorescence

FIGURE 4



The effect of duration of ischemia upon the levels of nicotinamide-adenine dinucleotides in the rat brain tissue
(—— NAD + NADP; —·—·— NADH + NADPH)

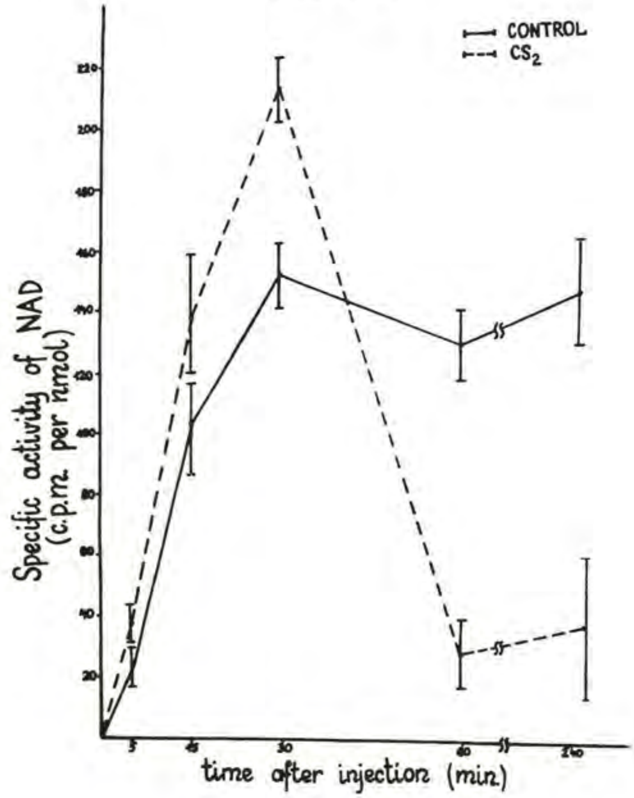
FIGURE 5



Changes in the level of the nicotinamide-adenine nucleotides in the whole body of rats in the course of 5 months exposure to CS_2.

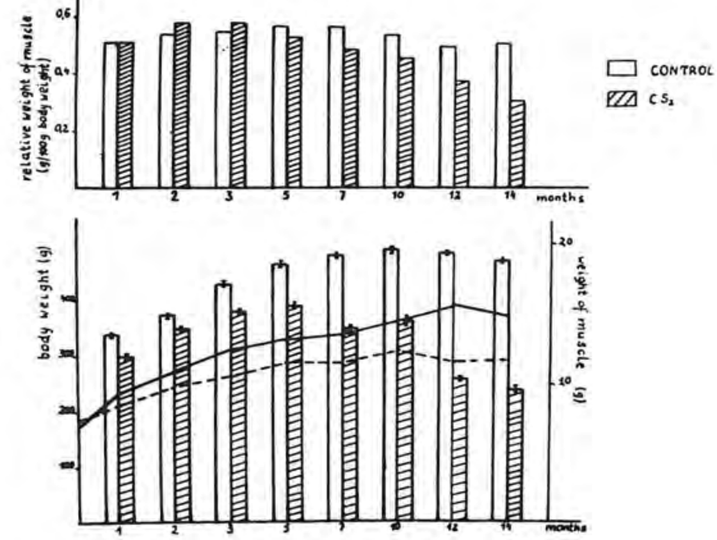
A - control; B - experimental data in rats exposed to CS_2; C - theoretical drop resulting from increased elimination of nicotinamide metabolites.

FIGURE 6



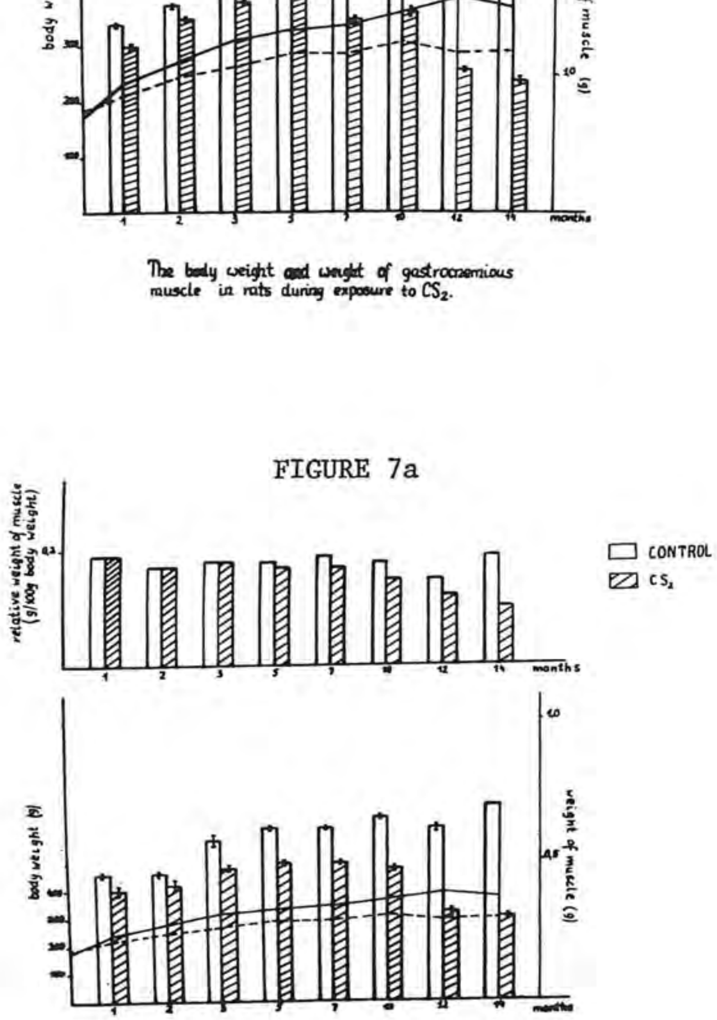
Dynamics of incorporation of nicotinic acid into NAD molecule in the liver of rats after intraperitoneal injection of 2 μmoles of ^{14}C-nicotinic acid (total activity 20 μC)

FIGURE 7



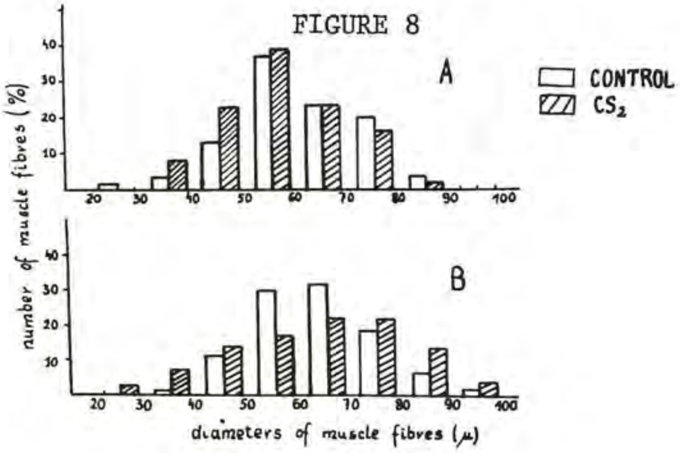
The body weight and weight of gastrocnemious muscle in rats during exposure to CS_2.

FIGURE 7a



The body weight and weight of tibialis anterior muscle in rats during exposure to CS_2.

FIGURE 8



Histogram of the diameters of muscle fibres from the gastrocnemious muscle of the control and exposed rats

A - 3 months of exposure to CS_2
B - 5 months of exposure to CS_2

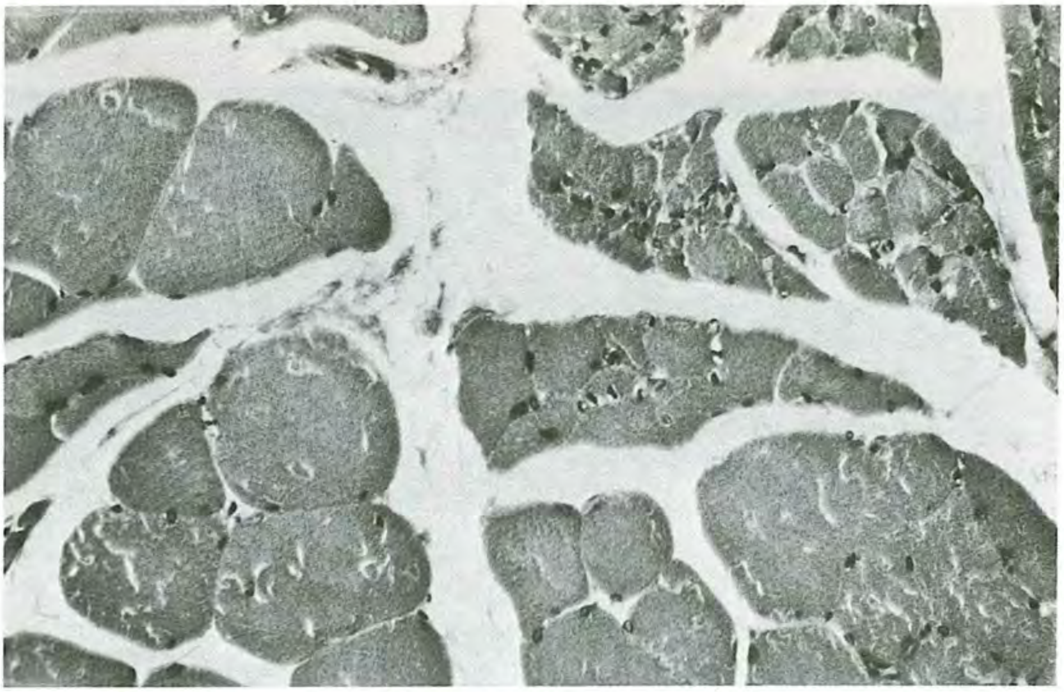


Figure 9. A bundle of muscle fibers undergoing atrophy is surrounded groups of normal and hypertrophic fibers. Gastrocnemius of the calf, 7 months of CS_2 exposure. Hematoxylin and eosin 300x.

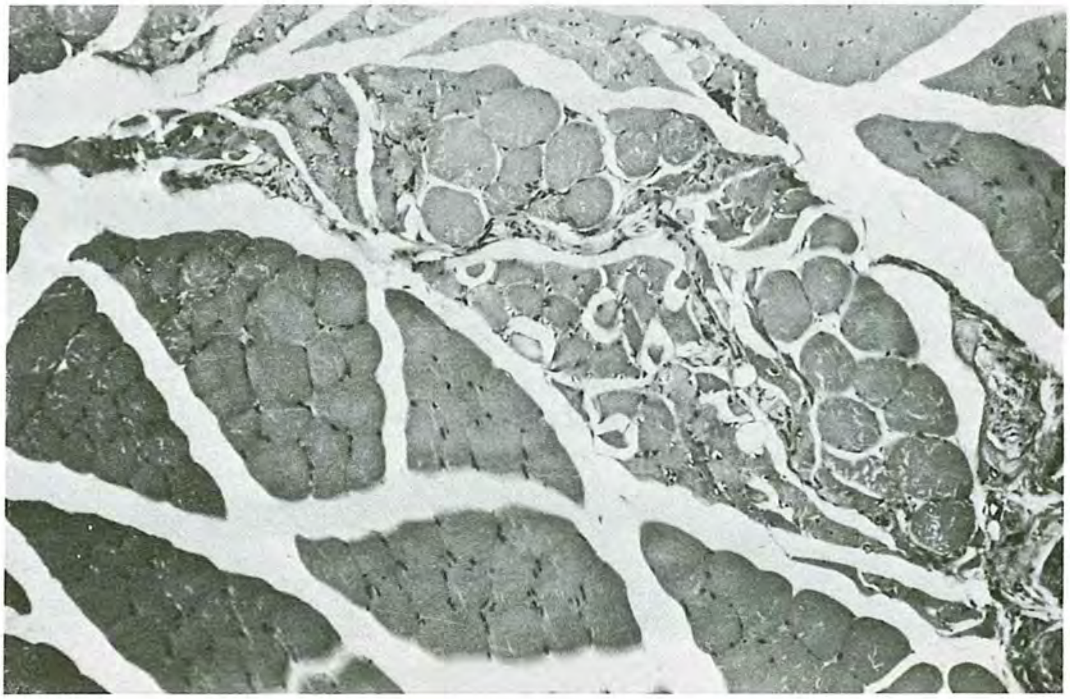


Figure 10. Hypertrophic muscle fibers between the atrophic groups. Gastrocnemius of the calf, 11 months of CS_2 exposure. Hematoxylin and eosin 120x.

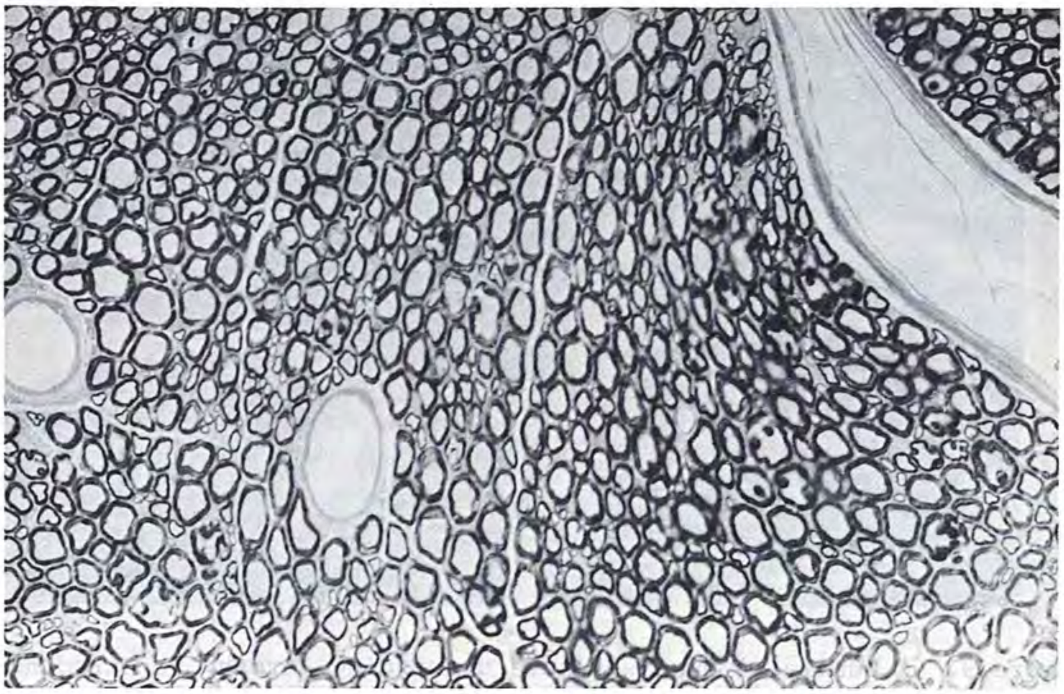


Figure 11. Ischiadic nerve of the control rat, transverse section. OsO_4 impregnation 300x.

Figure 12. Ischiadic nerve of the rat after 8 months of exposure to CS_2, OsO_4 impregnation.
Note the various diameter of nervous fibers and the amount of intertitium 300x.

INVESTIGATIONS ON BINDING AND RELEASE OF MERCURY IN RATS

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The rising interest in the toxicology and biochemistry of mercury and of mercury compounds has been connected with the ever growing use of these chemicals in many branches of industry all over the world and also in the agriculture, where mercurials are widely used as pesticides. The industrial sewage contaminates rivers and lakes and, subsequently, mercury is being captured and concentrated in fish. In this way the "abnormal" exposure of people to mercury increases considerably and the problem becomes important from the point of view of biological protection of the human being.

As far as it is recognized, the most severe exposure of people to mercury takes place in industry. Although typical chronic intoxications in the modern industry are rare nowadays, some uncertainty still remains with regard to the possible less evident or more delayed biological effects of exposure. This seems not excluded since mercury shows a high ability to be concentrated and stored in the kidneys over long periods of time.

In this respect, of a great value would be methods of estimating the actual exposure and the body burden of mercury in humans. Mercury may be found in the biological fluids such as blood and saliva, and also in the urine. The urinary mercury may be determined comparatively easily, however, there seems to be no close correlation of its level neither with regard to the biological damage nor with the degree of exposure or total body burden. It is generally accepted that mercury is being released from the body in an irregular way. This belief is based on several observations taken both from the examinations of human beings and from the studies on animals. These irregularities may be ascertained by: a) a lack of a regular dose: excretion relationship, b) irregular excretion curves in the same subject when followed in subsequent time intervals. Also, in spite of different chemicals checked for the ability of mobilizing mercury stored in the organism, none of these chemicals has hitherto been applied practically for assessment of the body burden.

Thus, the aim of the project has been to learn more about the binding and releasing of mercury in and by the body. More or less successful studies were devoted to the following problems which will be discussed in more detail here: 1) the dose-excretion relationship, 2) the biochemical background of binding and releasing of mercury, 3) chemicals accelerating excretion of mercury.

All the studies reported here were performed on rats (Wistar, female). Mercury was applied in single doses as mercuric chloride (i.v.); in some experiments also phenyl-mercury acetate was used, and the compounds were in most experiments labeled with ^{203}Hg. The measurements were based on gamma counting. The biochemical methods used will be mentioned further.

1. The dose: excretion relationship*

Since the early preliminary report of Trojanowska and Nofer (1961) confirmed later by Cember (1962) it has become evident for us that there was no common denominator for the kinetic behavior of mercuric chloride (or nitrate) given to rats in different single doses of the order of 0.1 - 1.5 mg Hg/kg. The urinary excretion could have been presented by two- or three-phase kinetic process, but the share of first (rapid) phase was much higher with the larger mercury doses. As it was found in the later experiments of Balazs et al. (1963), the toxic effects of mercury could be recorded by biochemical and morphological methods of urine analysis with doses exceeding about 0.4 mg/kg. This led us to suspect that the damage of the kidney may be the cause of the varying kinetics of mercury excretion.

Personally, we performed experiments in which different mercury doses in the range of 0.1 - 1.2 mg/kg were applied to rats (i.v.) and both sides of the phenomenon were followed in parallel. The excretion of mercury was measured in the urine and feces, and later the data on distribution were collected. At the same time the urine was examined for proteins**, aminoacids*** and for the kidney tubulae cells****, which were accepted as indices of damage.

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* For details: see Wiśniewska and Trojanowska (1968) and Piotrowski et al. (1970).

** Proteins - with tannic acid (Mejbaum-Katzenellenbogen, 1955).

*** Aminoacids, a compilation of methods of Yemm and Cocking (1955) and Angielski et al. (1959).

****The number of epithelial cells of renal tubulae in daily volume of urine, determined microscopically after Balazs et al. (1963); staining after Predteczenskij (1953).

The obtained results are shown in Figures 1 and 2 with respect to excretion and retention of mercury. It is evident that with increasing dose - at least over the range of 0.2 mg/kg, the 3-day output of urinary mercury rose considerably, whereas fecal excretion of mercury was not affected. The retention of mercury in the kidney appeared to be highly dependent upon the dose, while the remaining carcass showed no evident changes in the mercury retention, except for some increased retention in the liver noted after the highest dose.

The time-course of the signs of injury obtained from the urine analysis is shown in Figure 3. It may be seen that the changes observed did not persist for a long period and after three days they almost disappeared. The duration of injury seems to be longer with the higher mercury doses (Figure 4). An unequivocal interpretation is possible only with respect to the extreme doses: 0.1 mg/kg, where no signs of damage were noted, and with those over 0.8 mg/kg, where damage was evident.

From the two intermediate doses we concluded that some signs of damage occurred already with the dose of 0.4 mg/kg.

In another series of experiments the damage was assessed solely by the biochemical methods of kidney analysis. The most useful one appeared to be the estimation of inhibition of the oxoglutarate dehydrogenase.* Significant inhibition was found with the dose of 0.6 mg/kg. Thus, we believe that evident toxic effects may be proved in rats with doses of mercuric chloride of the order 0.4 - 0.6 mg Hg/kg. It should be noted that this is the range, which if exceeded, will be manifested by increased urinary output in the first period of mercury excretion.

As a result of this study we have accepted that kidney damage and the mercury output in urine are two processes closely related to each other. Some suggestions with respect to the background of this relation will be made later.

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* Oxoglutarate dehydrogenase, manometric technique according to Umbreit et al. (1964).

2. The biochemical background of binding mercury in the body*.

The ability of mercury to combine with different active groups of proteins has been known for a fairly long time and many practical uses have been made of this property. Therefore, it could have been expected that any new investigations in this field would have only confirmed the non-specific binding of mercury on many different groups of proteins. However, there had been some observations made by Surtshine and Yagi (1958) and Yagi and White (1958), which pointed to some differences in binding of mercury in the kidney by various proteins, and there was apparently observed some relation between the kind of binding and the toxicity.

In our investigations we took advantage of the relatively new technique of molecular sieving (gel filtration). introduced into the biochemical research a few years ago. Rats were given $^{203}HgCl_2$ in single doses ranging from 0.1 to over 1 mg Hg/kg and different tissues of the individual animals were analyzed for the distribution of the ^{203}Hg tracer among compounds of various molecular weight. The above technique, despite its simplicity, is rather laborious and therefore it cannot be readily applied to the study of the phenomena in large groups of animals. Therefore, taking into account some variations in the individual animals, only rough quantitative evaluation is possible. Nevertheless, even with this restriction in drawing the conclusions, the obtained results seem to be worthy of interest.

Now, let us discuss the findings, using the schematic diagram obtained by chromatography of the kidney homogenate on the Sephadex gel G-75 (Figure 5). It is evident that mercury is not uniformly distributed among the different protein fractions. There are two different groups of complexes from which that of lower molecular weight seems especially interesting for the reasons which will be further discussed. The first group of mercury complexes is not uniform. This is evident from experiments in which the chromatography of kidney homogenate was performed on the Sephadex gel G-200, more suitable for separation of high molecular weight proteins (Figure 6).

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*For details see: Jakubowski et al.(1970); Wiśniewska et al. (1970); Piotrowski and Bolanowska (1970).

It is interesting that a similar distribution pattern could be found also in the homogenate of liver which represents the second greatest reservoir of systemic mercury (Figure 7).

The complex of lower molecular weight (about 11,000) has proved to play an essential role in the mercury retention in the kidney. The share of this fraction of mercury decreased with increasing doses of mercury and increased with time after application of mercury (Table 1). From this observation it follows that; a) the actual level of the endogenous binding substance may represent a limiting factor in binding the mercury in the kidney in this form, and b) that when once chelated in this form the mercury shows a high stability in the kidney.

The compound in question proved to be identical with Hg-metallothionein. Metallothionein was discovered by Kägi and Vallee (1960, 1961) in the renal cortex of horses as the compound responsible for an enormous concentration of cadmium in the kidney. The proofs of identity obtained in our studies were based on the comparison of the molecular weight, spectral properties and the behavior of this fraction against mercury and cadmium in different pH media (Figures 8, 9, 10). Recently, we were also able to prove that the similar fraction occurring in the liver was also identical with Hg-metallothionein. (Wiśniewska et al., in preparation).

It seems worthy to mention that Hg-metallothionein was absent in the blood (Figure 11).

Phenyl-mercury acetate was known to differ from mercuric chloride with respect to distribution and excretion. Analysis performed using the technique of gel filtration revealed that in this case mercury was also distributed among similar protein fractions as mercuric chloride. This may be seen for the kidney (Figure 12), liver (Figure 13) and blood (Figure 14). However, the dynamics of binding mercury with metallothionein differed considerably. The Hg-metallothionein complex had been initially formed with low efficiency and only on the second day. Later the binding efficiency approached that of mercuric chloride. We believe that this difference is connected with the chemical structure and that the disappearance of these differences with time is connected with decomposition of the phenyl-Hg bond. It should be emphasized that the observations made in this experiment have thrown much light on the background of the different distribution and excretion patterns of both compared mercury compounds, when observed in the relatively short time periods after their application to animals.

It ought to be emphasized that the metallothionein complex of mercury showed a high stability in the kidney and that it was responsible for the low excretion rate of mercury in the urine. Whenever this kind of systemic chelating was less efficient, the urinary output of mercury rose, as was the case with high mercury doses and also with phenyl-mercury acetate in the first period after application. A further illustration of this view will be given later.

3. The relation between binding and toxicity*.

As it has been shown in the foregoing discussion, the signs of injury occurring after application of a single, sufficiently high dose of mercury, tended to disappear within a few days. On the other hand, it has also been shown that in later periods the mercury accumulated in the kidney was present predominantly in the form of a complex with a metallothionein. From both these observations the conclusion may be drawn that such a kind of binding may act as a true process of detoxication. It seemed logical to assume that mercury firmly bound with metallothionein would not exert a toxic action against other proteins among which the SH dependent enzymes could play an essential role.

To get further support for this hypothesis, separate experiments were performed in which toxic effects of two different metals, viz. mercury and cadmium were followed and parallelly their binding with metallothionein was assessed. The experiments were performed both in vivo and in vitro at the corresponding levels of tissue concentration of the metal, and as a measure of toxic effect, inhibition of the SH dependent enzyme, viz. oxoglutarate dehydrogenase, was used.

Both metals have been known as inhibitors of this enzyme in vitro. The distribution of these metals among different protein fractions of the critical organs, liver and kidney, respectively, is shown in Figure 15. In both cases there was a sufficient excess of the metal not combined with metallothionein and therefore able to react with the active sites of high molecular weight proteins (enzymes). In vivo, however, only mercury inhibited the enzyme, whereas cadmium was almost ineffective. This corresponded with a distinct difference in the distribution pattern of both metals among the different protein fractions, as is shown in Figure 16. In the case of mercury there was again some metal present which was not chelated by metallothionein, practically all cadmium was present as a Cd-metallothionein complex, and there was no excess left to exert toxic action against other proteins. In our view this experiment confirmed our hypothesis on the binding of metals with metallothionein as a true process of detoxication.

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*See Wiśniewska-Knypl and Jablonska (1970).

Following the hypothesis of metallothionein as a true detoxication agent naturally we made attempts to use it directly as an antidote in acute mercury poisoning. These attempts, which unfortunately failed in obtaining positive results, were performed in the following way: Metallothionein was obtained from the equine renal cortex in preparative amounts, using the method of Kagi and Vallee as a guide. The obtained preparation appeared reasonably purified and the identification tests were positive. This compound was applied in a single dose of 5 mg to rats, given previously mercuric chloride in a dose of 2 mg Hg/kg. Comparative observations of these animals and of the corresponding controls did not reveal any positive effect of this treatment.

The above negative experiment restrains us at present from undertaking any further attempts of direct use of exogenous metallothionein as an antidote. However, in our view it does not deny the postulated mechanism of the detoxicating action of the endogenous compound in mercury poisoning.

4. Urinary mercury

Little has been known about the chemical form of urinary mercury. Weiner et al. (1962) claimed that in dogs the only existing form was represented by the cysteine-mercury. More recently Clarkson and Magos have shown that the urinary mercury was composed of two different fractions differing in their response to protein precipitating agents. Moreover, Cember (1962) pointed out that a part of the urinary mercury could be found in the urine sediment which could be separated by simple centrifugation at low speed.

Our experiments had been started using paper chromatography; however, only scanty information could be obtained with this method. Therefore, in further experiments we again used gel filtration*. In other standard analyses, where the number of animals to be studied exceeded the possibility of this technique, we used simple dialysis against water. Additionally, in some of the experiments the mercury content of the urine sediment was determined.

Using gel filtration (Sephadex G-75) we obtained different types of chromatograms with no apparent dependence on either dose of mercury of the time after its injection (Figure 17). Three different types of compounds could be distinguished: 1) high molecular weight proteins, 2) complexes of intermediate molecular weight of the

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*For details see: Jakubowski et al. (1970); Piotrowski and Bolanowska (1970).

order 10.000, and 3) low molecular weight compounds. The high molecular weight mercury compounds occurred in all urine specimens. The second fraction occurred rarely. It resembled Hg-metallothionein with respect to the molecular weight, but no proof of its identity has been yet obtained. The third fraction, of low molecular weight mercury compounds, was studied more carefully, but no final identification could be achieved. This appeared difficult, because for unknown reasons, the share of this fraction varied in different series of experiments from 30-40% down to traces only. The latter were found usually when identification studies were to be started. The data which are available at present were obtained by rechromatographing of this fraction on the Sephadex gel G-25 and comparing with Hg-complexes of the known molecular weight (cysteine-Hg and glutathione-Hg). The results shown in Figure 18 suggest that the compound under investigation has not been identical with cysteine-Hg, as we had initially suspected from the report of Weiner et al. (1962). A dipeptide seems more likely to be the binding substance. Similar distribution patterns of mercury among different fractions were also found when applying phenyl-mercury-acetate.

The presence in the urine of high molecular weight mercury proteins seems easy to understand. These may be derived both from the glomerular filtration of serum in which mercury is present exclusively in complexes with higher molecular weight proteins and from the tubular secretion. The origin of the low molecular weight mercury compounds is obscure since usually this fraction is absent in the systemic fluids and tissues.

Special attention has been paid in our studies to the reasons for the observed great variability of the composition of the urinary fractions of mercury. Initially we suspected the influence of bacteria because the urine has not been protected by antibacterial preparations. This seemed also probable since Clarkson and Magos (1967) found that some kinds of bacteria were able to interact with the urinary mercury, leading to its volatilization.

In a longer series of experiments* we determined the two simple parameters of the urinary mercury, i.e. Hg in the urine sediment and the dialysing fraction in two groups out of which only in one group thymol has been added to the urine receivers. Additionally, in both groups the urine was kept overnight and the above determinations were repeated, and also the loss of Hg on standing was determined. The latter could be used as an index of bacterial action, if one followed the findings of Clarkson and Magos. The obtained data,

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*Preliminary report by Piotrowski, Bolanowska and Biedermann (1970).

shown in Table 2, revealed only slight and insignificant differences between the two groups. In accordance with our earlier studies no essential loss of mercury on standing overnight was found. There was also no distinct change in the mercury contained in the sediment, as well as in the fraction of the dialyzable mercury. With respect to the latter it has been found that its share decreased with time after mercury injection. This, however, does not represent a general rule, since in some other series of experiments we were able to find comparatively much higher content of the dialyzable mercury in later periods after mercury application.

In these studies the total number of bacteria was measured in parallel with the above determinations. From the first series it followed that the bacterial contamination of the urine in rats given mercuric chloride was much higher as compared with the control period, unless thymol was added to the receivers. Therefore, we checked this finding in a second series in which rats were given different single doses of mercuric chloride in the range of 0.2 to 1.2 mg/kg. The results of bacterial analysis presented in Table 3 seem to point to a rise of bacterial contamination with increasing doses of mercury; the reasons for this odd finding are obscure at present. In conclusion of the above experiment we had to accept that, instead of finding the influence of bacterial contamination on the urinary mercury, the reverse was unexpectedly discovered and the causes of the irregular composition of the urinary mercury fractions still remain to be clarified.

To summarize the studies on the urinary mercury: only first steps have been made in this problem and some more experience was gained with respect to the kind of findings that could have been obtained using some simple methods of analysis. Practically, the entire field is still open to further studies, including the identification of different mercury fractions and of their origin as well as causes of their irregular appearance in the urine.

4. Chemicals accelerating the release of mercury.

Several compounds had been tested by different authors for their ability to speed up the release of mercury. The underlaying assumptions were mostly those of chelating this metal, and the compounds used had predominantly active SH groups like cysteine, penicilamin and BAL. Those attempts had given only very weak or even doubtful effects, suggesting that the direct chelating might not be the most effective way. The only exception is represented by Unithiol, a Russian drug of which scanty data could be obtained.

Another group of mobilizing agents would concern substances acting probably in an indirect way, not elucidated in details. Here belong thioacetamide and maleic acid (Clarkson and Magos, 1967).

Our own studies have concerned mostly two chemicals, thioacetamide and Unithiol. Both of these compounds are very effective in mobilizing the systemic mercury but, apparently, they differ basically in their mechanism of action.

a) Thioacetamide*

Under the concept of mobilizing mercury this compound was studied first by Dutkiewicz et al. (1964) and further experiments of Trojanowska (1965) confirmed its effectiveness also with regard to mercury derived from phenyl-mercury-acetate.

Some data on the mobilizing effect, obtained in the present study, are shown in Figures 19, 20. The rise in excretion of mercury in the urine is obtained only after 2 to 3 days of treatment with TAA. The excess mercury in the urine is present not in the form of low molecular weight, dialyzable mercury compounds (Figure 21) as would be expected in the case of simple chelation. In some conditions it is possible to settle with all certainty a damage accompanying the increased mercury excretion (increased exfoliation of renal tubular cells), as is seen in Figure 22. This seems to be again a situation in which damage and increased mercury release are related closely.

Following the hypothesis of a damage brought about by a synergistic action of both thioacetamide and mercury as a cause of increased mercury release one would expect that the effectiveness would be low with low mercury doses (this has been proved) and with the application of TAA later when normally mercury is ineffective. The latter has not been proved in our results and further studies had to be done to bring some light in this question.

As mentioned formerly, in later periods mercury is contained in the kidney mostly in the form of complex with metallothionein. An analysis performed using gel filtration has shown that after application of TAA there is a distinct fall of mercury in this fraction (Figure 23). Using differential centrifugation it may be seen that TAA decreases the relative retention of mercury in the cytoplasm where Hg-metallothionein is located (Figure 24). At the same time the Hg-metallothionein complex does not appear in the urine (Figure 25) and the bulk of mercury is contained in the form of complexes with high molecular weight proteins.

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*For details see: Trojanowska et al. (in press); further report by Wiśniewska et al. (in preparation).

From the obtained results we believe that in the first step of action TAA releases mercury from the complex with metallothionein. This would give rise both to a damage and to increased urinary excretion of this element. Whether this release is brought about by simple destruction of the Hg-metallothionein complex or by an inhibition of biosynthesis of this simple protein is obscure at present. The latter possibility seems somewhat less probable since our experiments have shown no accompanying disturbances in the levels of proteins and RNA.

b) Unithiol*

This Soviet drug represents a modification of BAL in which the alcohol group is replaced by a sulphonic group. Its application for the mobilization of mercury was described first by Ashbel (1964).

Our experience with Unithiol is limited to single doses of mercuric chloride of 0.6 mg/kg. Some data on the excretion (Figure 26) and retention (Figure 27) show that it was almost as effective as thioacetamide. But two differences in the way of action were evident: it acts immediately without delay, and essential increase occurs also in fecal excretion. The former points to a direct action (chelation) as the mechanism involved, and this view could be confirmed by an essential increase of the dialyzable mercury in the urine (Figure 28). The analysis made using gel filtration has shown a decrease of mercury in kidneys in both fractions, high molecular weight proteins and metallothionein (Figure 29). The analysis of urine confirmed the presence of low molecular weight mercury compounds (Figure 30).

Despite the low toxicity of Unithiol, it is not yet certain if this drug can be used safely in human beings. Our preliminary experiments with higher mercury doses gave equivocal results. The mobilizing effect was apparently low and some increase of toxicity seemed to occur. This compound needs therefore further studies.

5. Final suggestions

Although our studies gave no detailed answer as to the causes of irregularity in excretion of mercury from the body, we believe that a key has been found. In our opinion the behavior of mercury in the body is governed by metallothionein.

Its level in the kidney represents probably a limiting factor for the retention of mercury in this organ. The excess, in the case of high doses, is relatively quickly excreted. The long term storage of mercury is conditioned upon the form in which it persists in the kidney and this is again a complex with metallothionein. Once complexed

*Trojanowska et al., in preparation.

with this protein, mercury seems to be less toxic as compared with other forms of binding and therefore a high retention of mercury in the kidney is possible without evident injury. Consequently, mobilizing of mercury by any factors must involve a change of the kind of binding with both increased rate of excretion and the possibility to cause injury.

Our actual data point also to the importance of metallothionein in explaining the metabolism and toxicity of another metal - cadmium. It seems therefore likely that this protein plays a more general role as a metal binding substance, and that it may be of some interest also with respect to other metals, especially those showing high affinity to kidney and liver.

It seems that further extensive studies should be devoted to this protein and its role in the detoxication processes. Therefore further studies along the same line will be continued in our laboratory, including analytical properties of metallothionein, its biosynthesis and distribution in the animal tissues.

ACKNOWLEDGEMENTS

Collaborating Senior Staff: Dr. Barbara Trojanowska, Dr. Justyna M. Wisniewska-Knypl, Dr. Wanda Bolanowska, Dr. M. Jakubowski (Department of Biochemistry) and Dr. Habil S. Szendizikowski (Department of Pathomorphology).

The author feels indebted to Professor J. Nofer, Director of the Institute, for the initiative in undertaking this study and his steady interest in its progress.

The assistance of Dr. Barbara Trojanowska in preparing this report is highly appreciated.

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Table 1.

PERCENT OF MERCURY IN KIDNEY PRESENT IN FRACTION II (SEPHADEX G-75), IN RELATION TO THE DOSE OF MERCURIC CHLORIDE AND TIME AFTER ADMINISTRATION

	Time after administration			
	Within 1st day		From 3 to 7 days	
Dose (mg Hg/kg)	Number of animals	%[a]	Number of animals	%[b]
0.1–0.2	5	63 ± 19	3	74 ± 5[b]
0.6–1.0	8	49 ± 15[b]	8	60 ± 9

[a] Mean ± SD.
[b] Significantly different from other values ($P < 0.05$).

Table 2

Data on Urinary Mercury, Dose 0.8 mg Hg/Kg

Urine	Mercury in sediment in %		Dialysable mercury in %		Mercury loss in % 24 hrs
	a	b	a	b	
with thymol series I	17 ± 6	18 ± 12	11 ± 10	9 ± 9	3 ± 5
without thymol series I	20 ± 7	27 ± 13	7 ± 6	5 ± 4	2 ± 4
with thymol series II	13 ± 8	—	9 ± 7	—	9 ± 8

Table 3

Count of Bacteria in Daily Urine After Different Doses of $HgCl_2$ (Three-days Observations After Administration)

Dose mg Hg/kg	Before administration	After administration
0.2	18 0 - 50	143 1 - 850
0.4	26 7 - 40	190 2 - 1625
0.8	11 3 - 21	280 4 - 925
1.2	9 0 - 40	1200 5 - 4580

Bacterial contaminations mln/day.

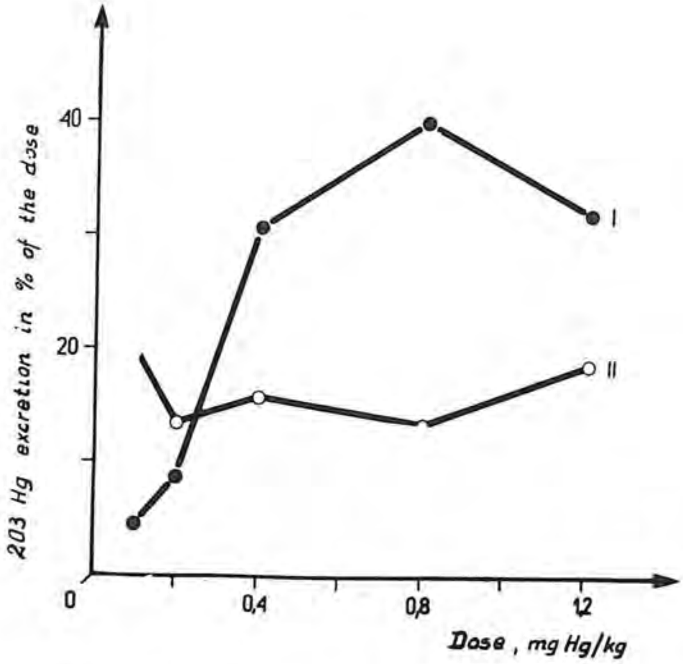

Figure 1. Three days cumulative excretion of Hg in urine and feces, in percent of the dose, as dependent on the dose $HgCl_2$.

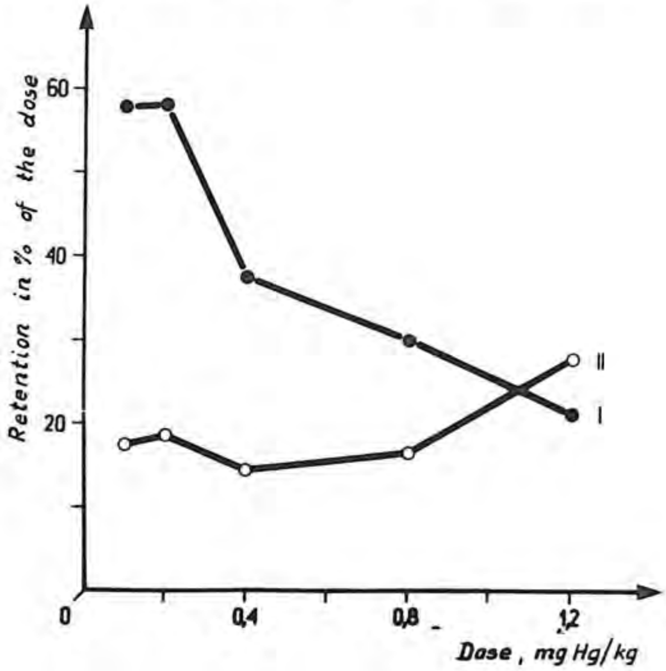

Figure 2. Retention of mercury in the kidneys and in the remaining carcass, 3 days after administration of $HgCl_2$, as dependent on the dose of $HgCl_2$.

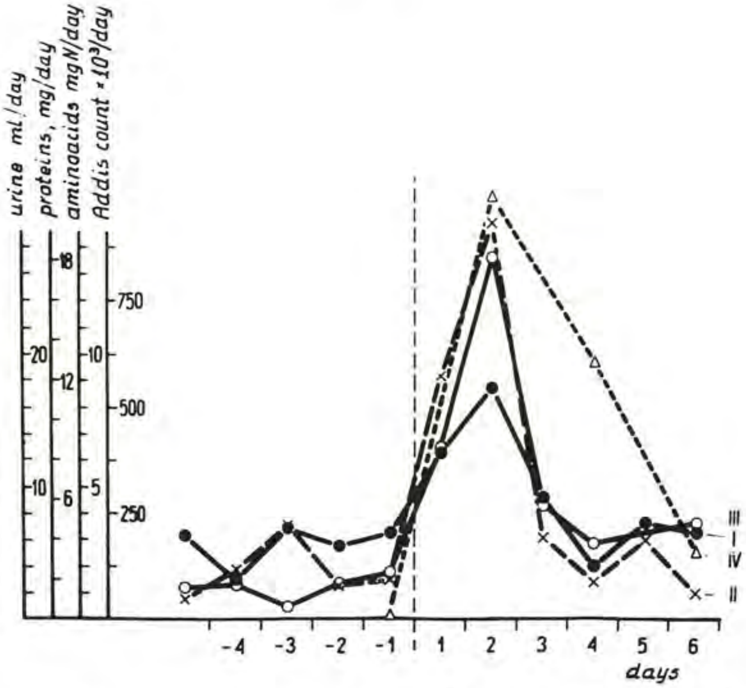

Figure 3. **The time course of kidney injury assessed by urine analysis: Dose - 0.8 mg Hg/kg.**

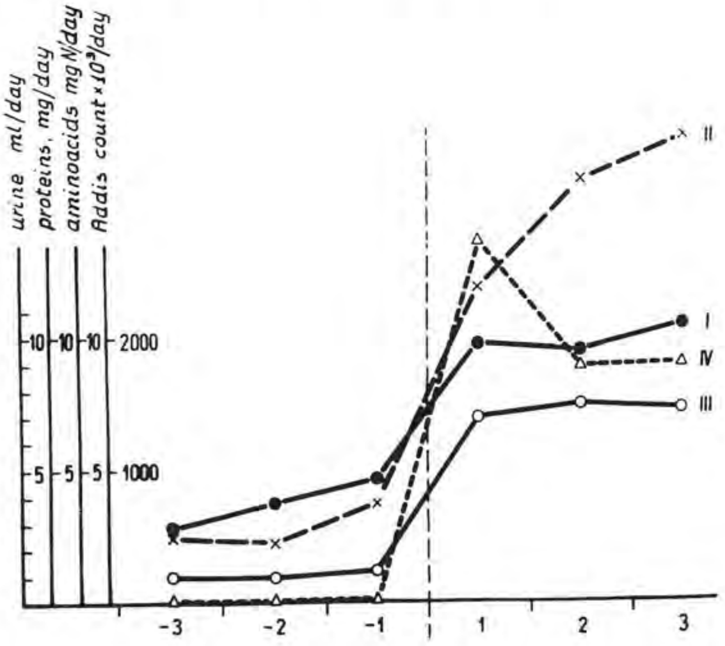

Figure 4. **The time course of kidney injury assessed by urine analysis: Dose 1.2 mg Hg/kg.**

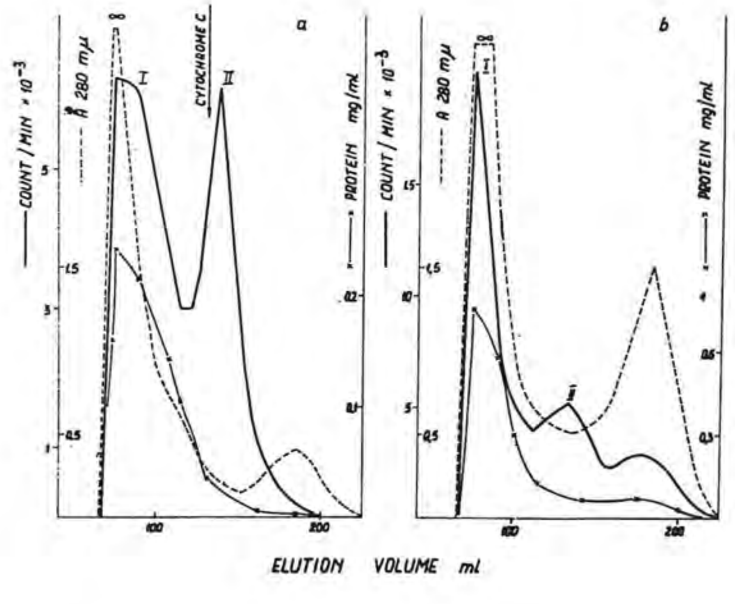

Figure 5. The chromatography of kidney homogenates: (a) Dose 0.1 mg Hg/kg, one hour after administration; (b) Dose, 3.0 mg Hg/kg five hours after administration. In both cases Sephadex G-75 was used. For details see Figure 1.

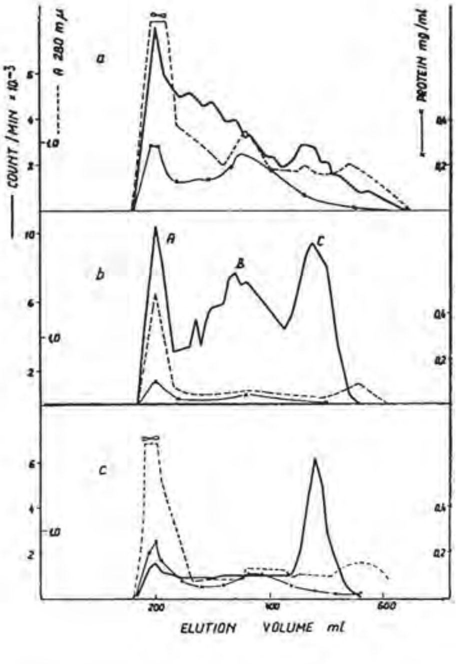

Figure 6. The chromatography of kidney homogenate on Sephadex G-200. For details see Figure 1. (a) Dose 3.0 mg Hg/kg, five hours after administration; (b) Dose 1.0 mg Hg/kg, one hour after administration; (c) Dose 0.1 mg Hg/kg, one hour after administration.

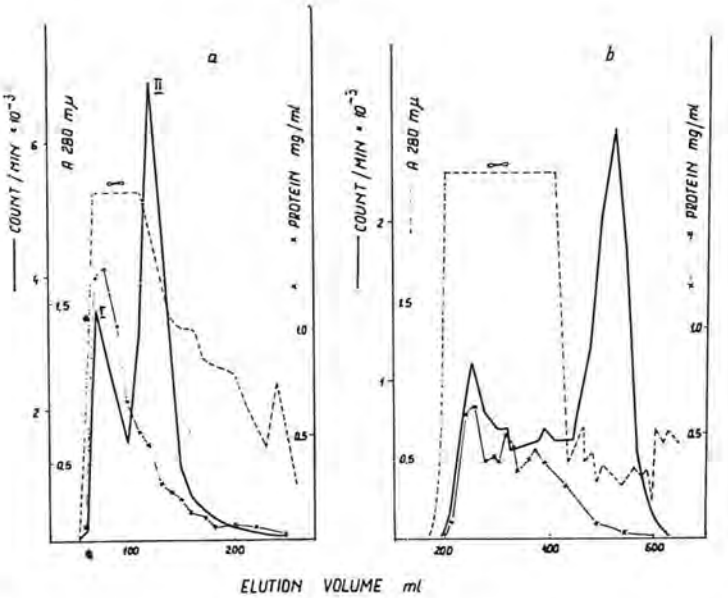

Figure 7. The chromatography of liver homogenate: Dose 0.2 mg Hg/kg one day after administration. (a) Sephadex G-75, column 1.9 X 53 cm, flow rate to 10 ml/hr, fractions of 5 ml each collected, (b) Sephadex G-200, column 41 X 4 cm, flow rate of 20 ml/hr, fractions of 10 ml each collected.

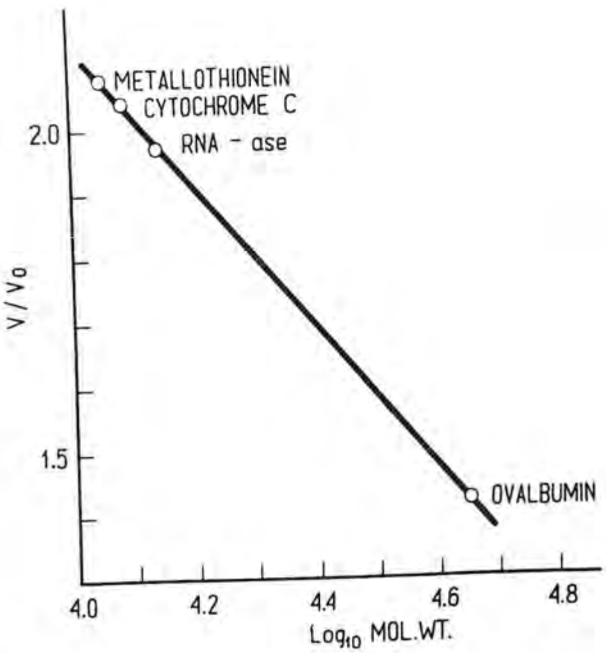

Figure 8. Determination of molecular weight of rat kidney metallothionein by gel filtration.

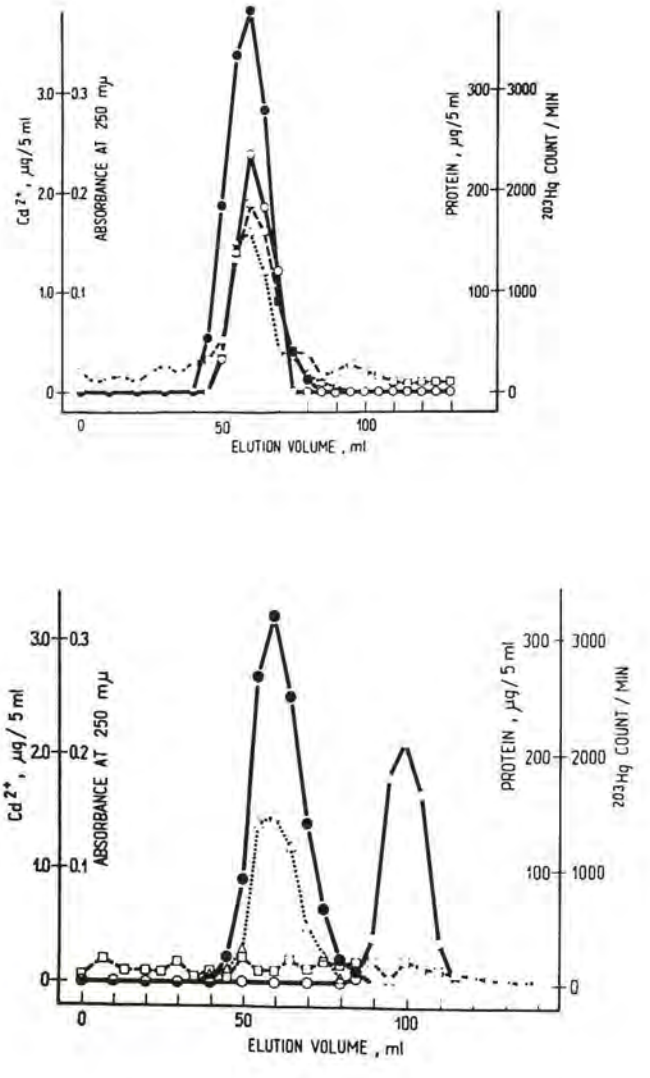

Figure 9. Chromatography of metallothionein on Sephadex G025, upper graph: neutral medium; lower graph: acidic medium.

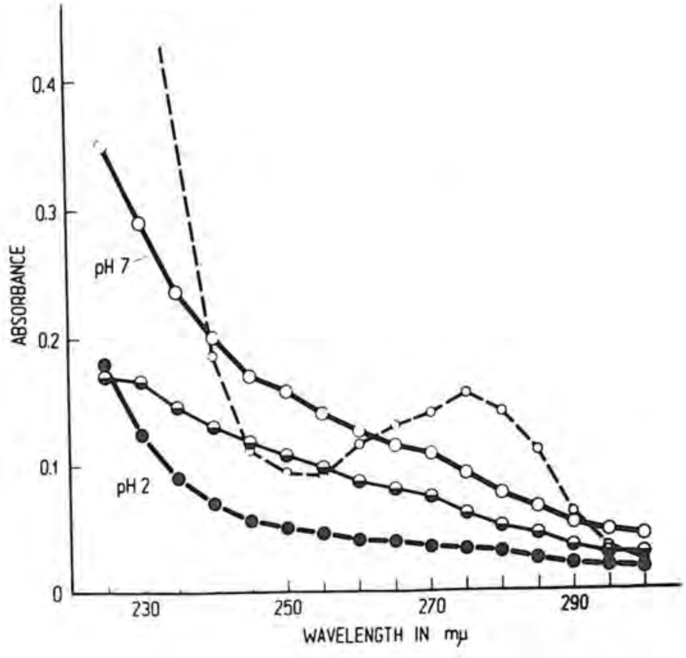

Figure 10. Absorption spectra of metallothionein in pH 2 and 7; intermediate line - difference spectrum; dotted line - ribonuclease (for comparison).

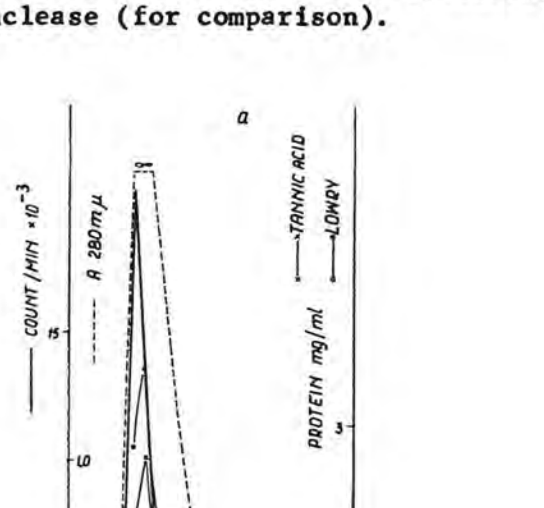

Figure 11. The chromatography of serum: Dose 1.0 mg Hg/kg, one hour after administration.

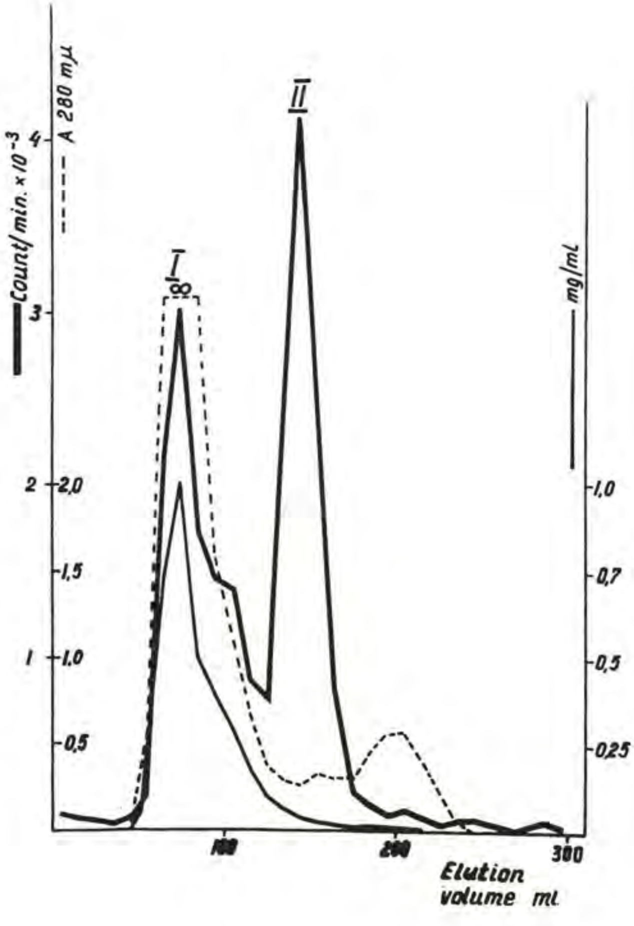

Figure 12. The chromatography of kidney, after administration of phenyl-mercury acetate (Dose 0.2 mg Hg/kg, time - 24 hours after administration, Sephadex G-75).

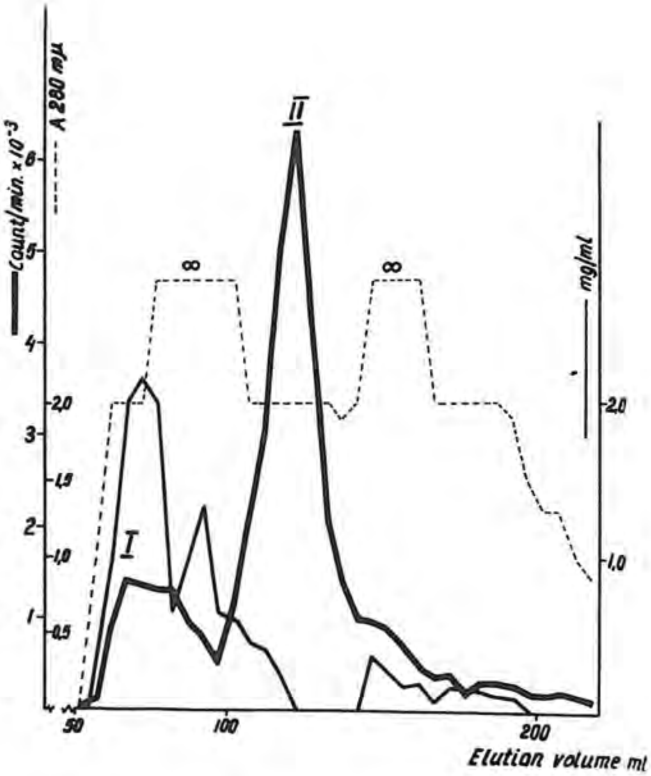

Figure 13. The chromatography of liver, after administration of phenyl-mercury acetate (Dose 1.0 mg Hg/kg, time - 24 hours Sephadex G-75).

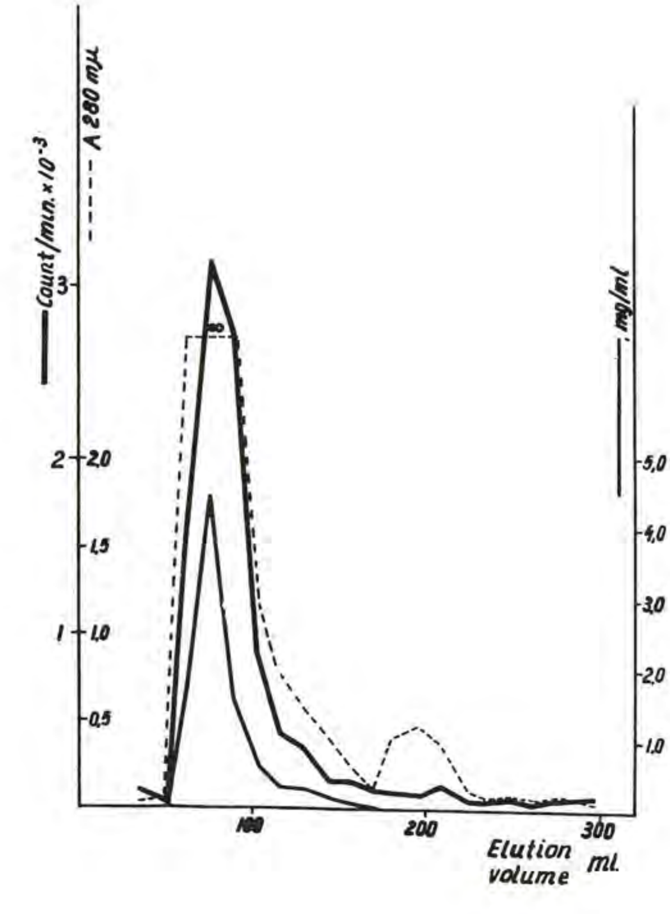

Figure 14. The chromatography of serum after administration of phenyl-mercury acetate (Dose 2.0 mg Hg/kg, time - one hour, Sephadex G-75).

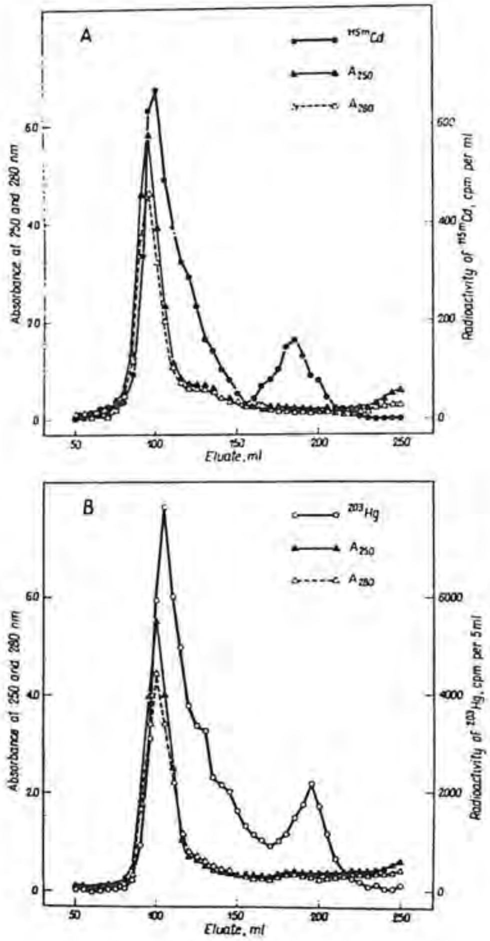

Figure 15. In vitro affinity of ^{115}Cd and ^{203}Hg to proteins of liver and kidney, respectively (Sephadex G-75).

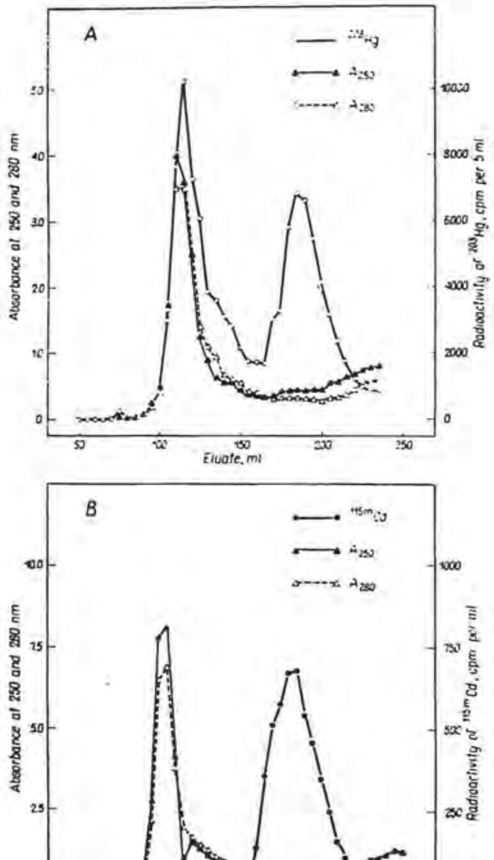

Figure 16. In vivo affinity of ^{115}Cd and ^{203}Hg to proteins of liver and kidney, respectively (Sephadex G-75).

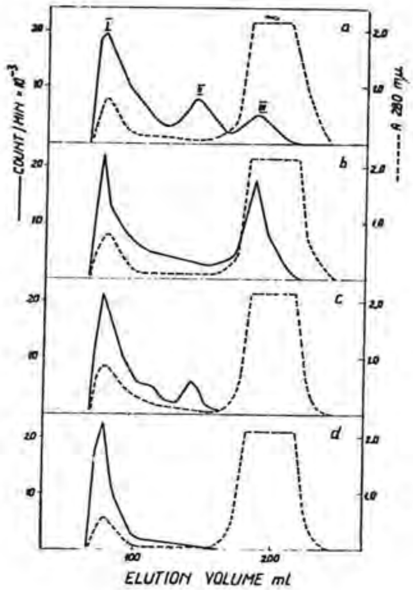

Figure 17. The chromatography of urine. Dose 1.0 mg Hg/kg, urine collected within first day (Sephadex G-75).

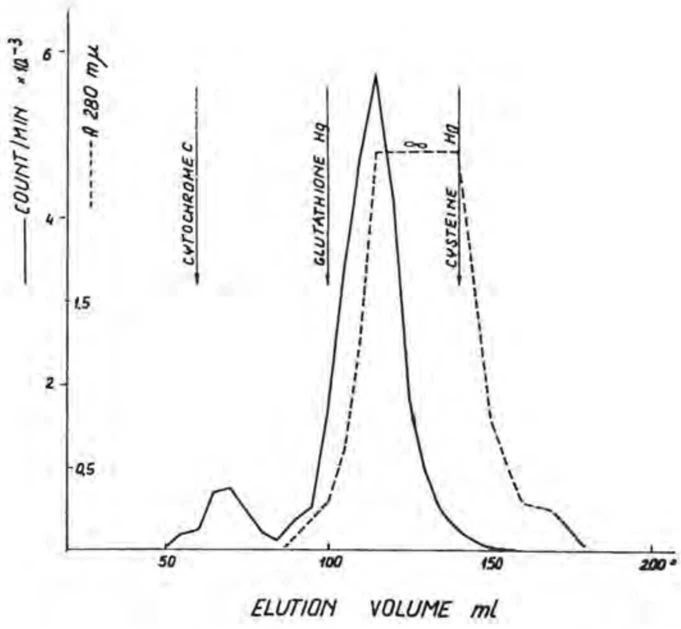

Figure 18. The chromatography of the urine dialyzate on Sephadex G-25 fine.

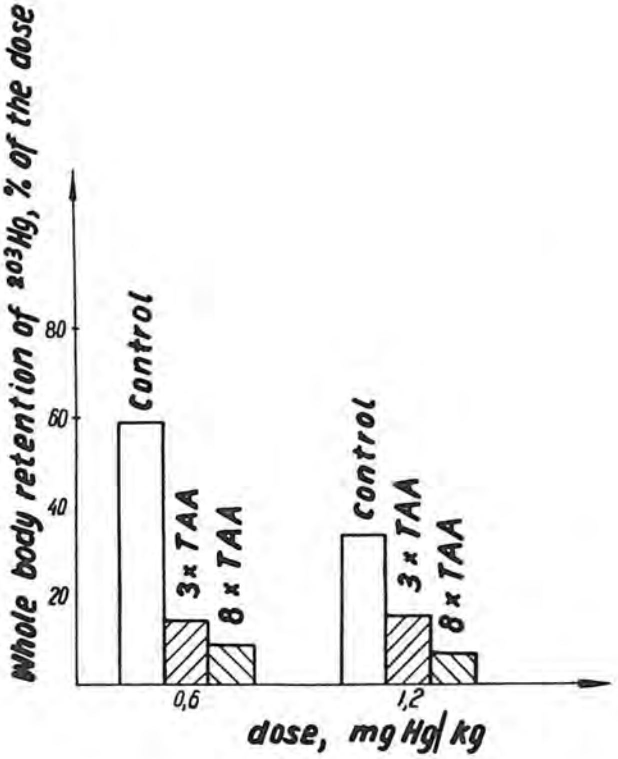

Figure 19. Whole body retention of ^{203}Hg in rats treated with TAA (Doses of Hg, 0.6 and 1.2 mg Hg/kg).

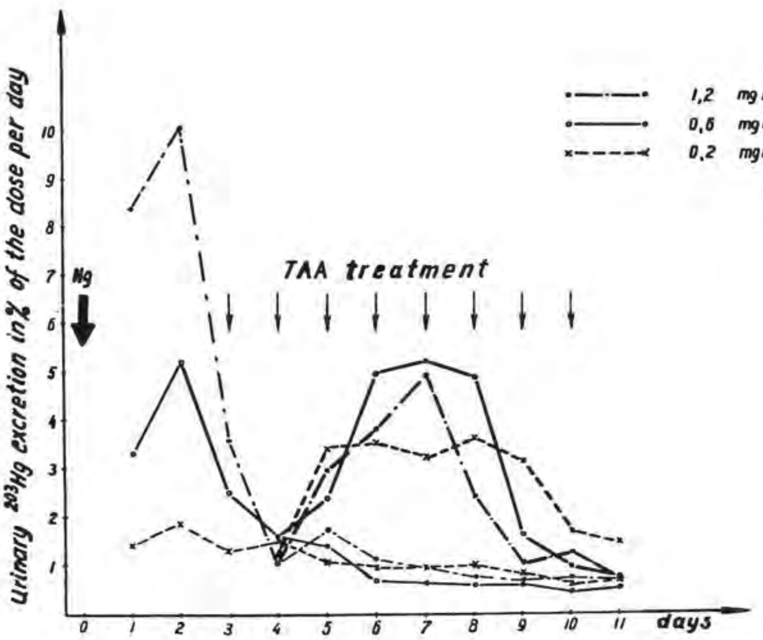

Figure 20. The time - course of urinary excretion of ^{203}Hg before and during TAA treatment.

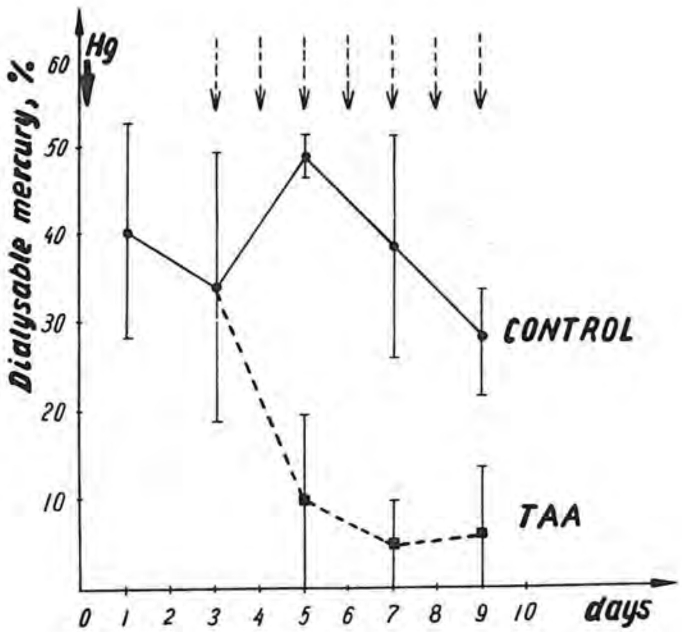

Figure 21. Dialysable mercury in urine in rats treated with TAA and the controls.

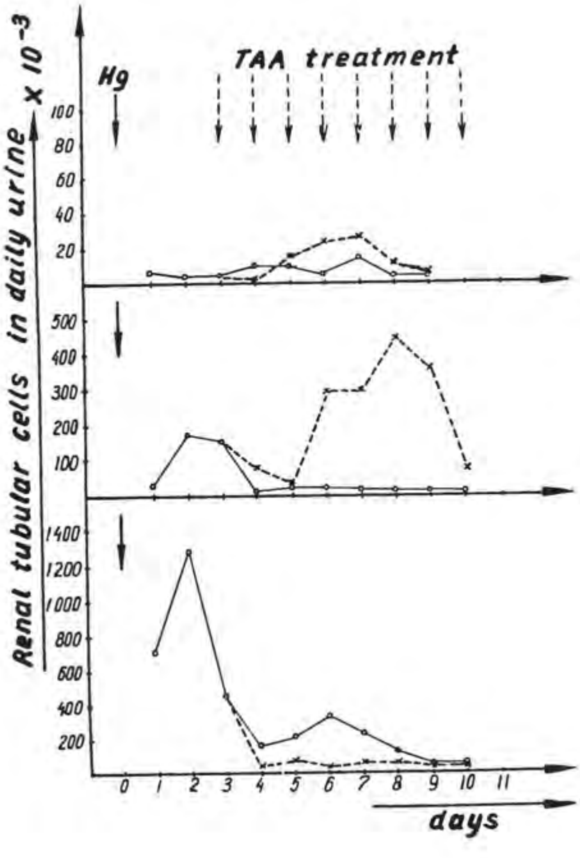

Figure 22. The exfoliation of renal tubular cells after administration of Hg (Doses 0.2; 0.6; and 1.2 mg Hg/kg) and within the period of TAA treatment.

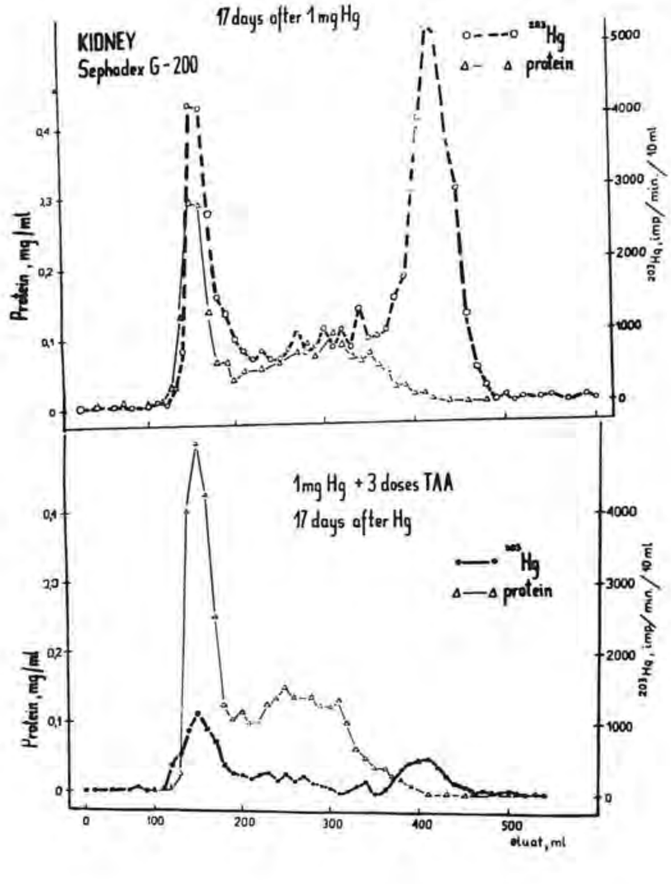

Figure 23. The chromatography of kidney 17 days after administration of Hg: upper graph control; lower graph - after TAA treatment.

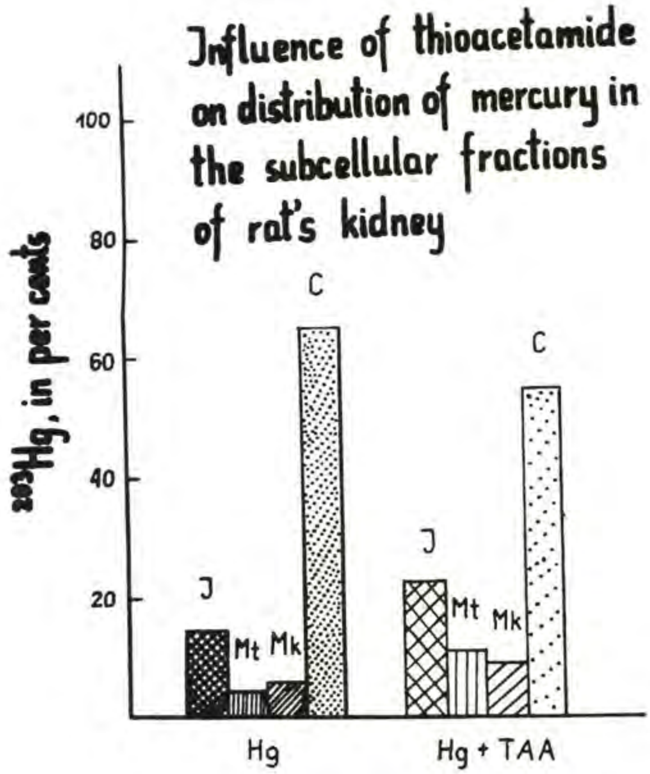

Figure 24. Influence of thioacetamide on distribution of mercury in the subcellular fractions of rat's kidney.

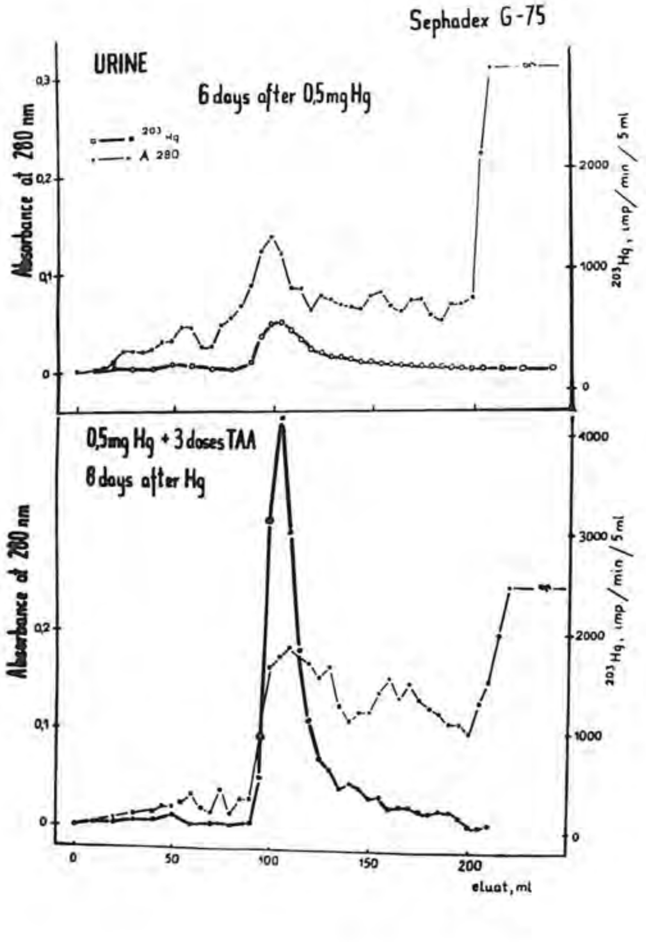

Figure 25. The chromatography of urine, six and eight days after administration of Hg in a dose of 0.5 mg Hg/kg. Upper graph: control; lower graph: after TAA treatment.

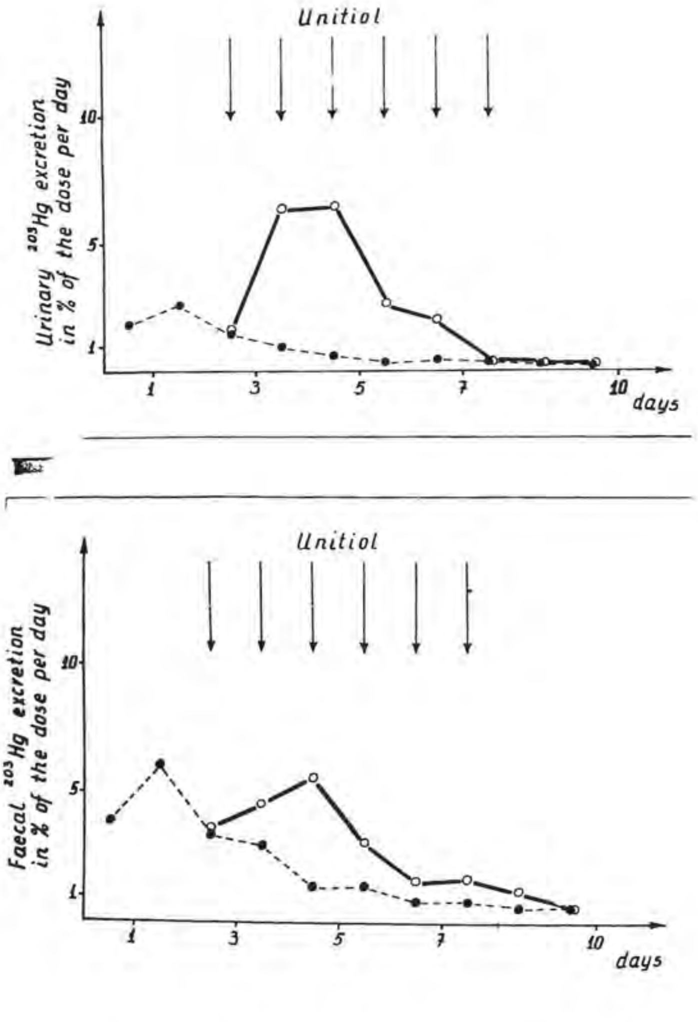

Figure 26. The time course of mercury excretion in urine and feces before and during the Unithiol treatment. (Dose 0.6 mg Hg/kg; Unithiol 10 mg/day).

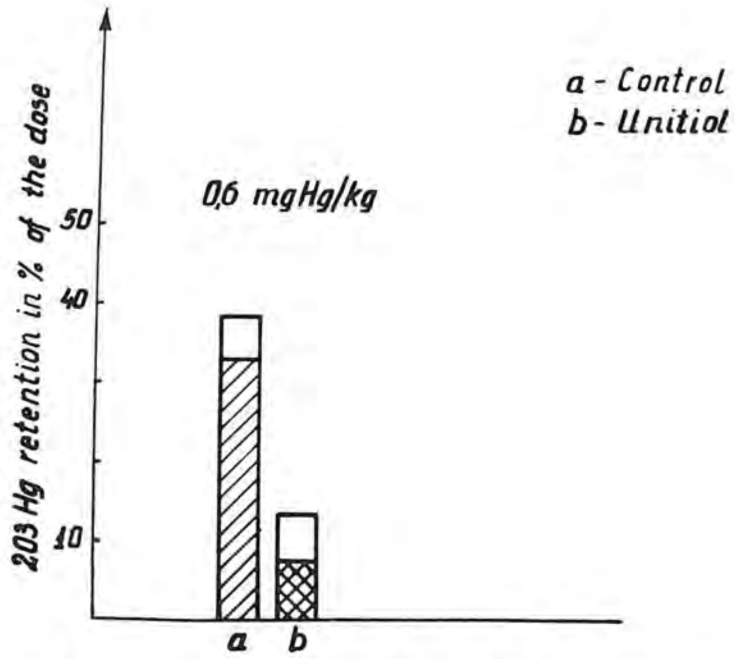

Figure 27. The whole body retention of ^{203}Hg 10 days after administration of mercuric chloride (shaded area - retention in the kidneys).

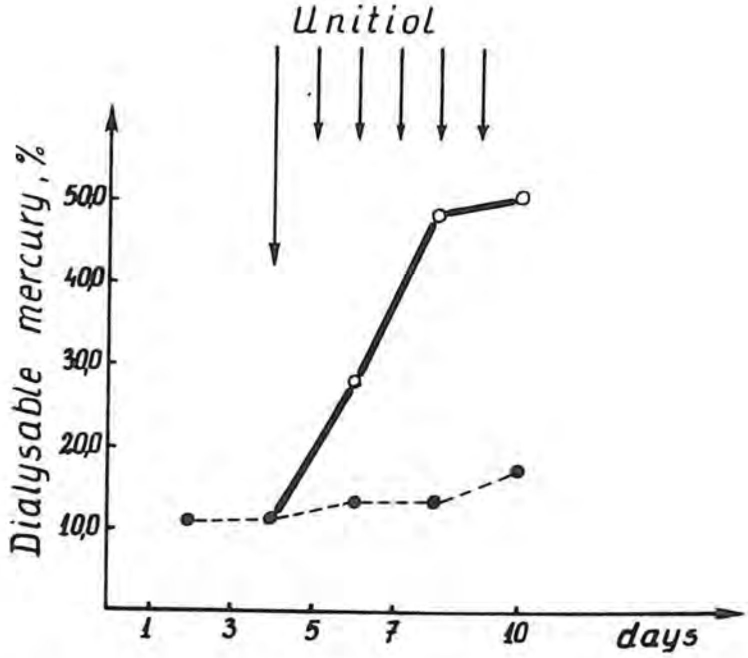

Figure 28. The dialyzable mercury in urine of rats treated with Unithiol and controls.

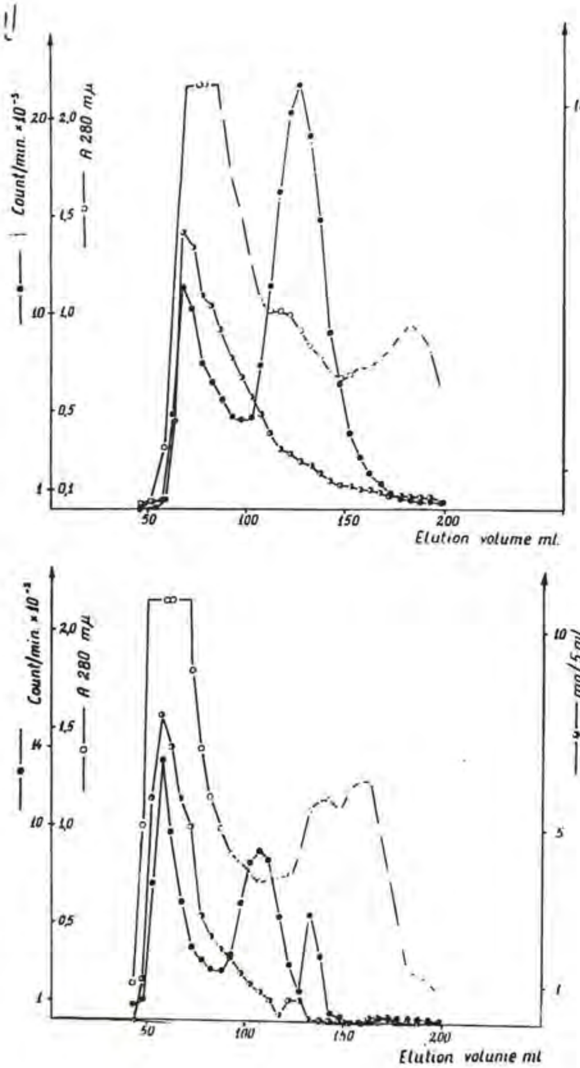

Figure 29. The chromatography of kidney on Sephadex G-75, dose 0.6 mg Hg/kg; time - four days. Upper graph: control; lower graph after one day's Unithiol treatment.

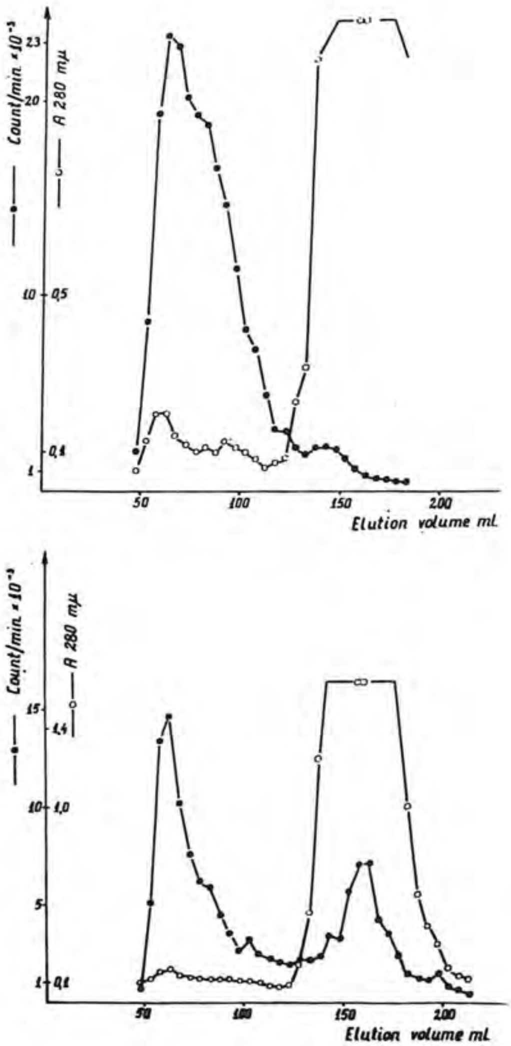

Figure 30. The chromatography of urine on Sephadex G-75. Upper graph: control; lower graph: after Unithiol treatment.

DISCUSSION AFTER DR. PIOTROWSKI'S PAPER:

Dr. Lainhart:

This variability and differences of patterns that you found in the excretion of mercury, is this within an animal or between animals? Would the same animal have the same excretory pattern over time?

Dr. Piotrowski:

I would like to underline that this technique which we applied, is not suitable to obtain series analysis with great amount of results. Therefore, the quantitative data have to be discussed with some reserve. If we obtain a group of results, usually from 5-7 animals, there may be many factors introducing the variability such as dose, time, individual differences between animals, and so on. Anyway, we were not able to find distinct differences between individual animals. This means that two chromatograms made of two animals at the same time did not differ in the pattern of Hg distribution; this applies also to the result from the first day and the third day in the same animal, which did not show any greater difference except in later experiments some fall in the share of the dialyzable mercury with time after administration. We obtained some data using the method of dialysis, which is much simpler. I couldn't answer in a clear way this question, only that we haven't noticed any difference between individual animals with respect to this question, but we noticed some differences in various series of our experiments. There was, for instance, a series of results showing a high share of the low weight compounds in urine and later we obtained another series of results with lower values. And as I said, we tried to elucidate this question, but without greater success.

Dr. Lainhart:

There is a great variability between humans with regard to toxicity and using your thesis that the toxicity is somehow related to the binding mechanism, does this mean that you and I may differ in our binding mechanism and therefore in our toxicity response to a particular dose of mercury?

Dr. Piotrowski:

As far as we are able to understand this question, I would say that with respect to the differences between different organs, species and individuals all this is just a hypothesis. All that could be explained is with regard to the amount of the binding substance in the respective tissues. But with respect to urine it's not so clear because the origin of the fraction is so obscure. For instance, there is no low molecular weight fraction of mercury in blood, kidney or liver. So it's not excluded that it is just present in kidney by cutting of higher complexes by some proteolytic processes. It is also possible because that compound of this group does exist in the systemic compartments, only the levels are very low, thus, making the detection very difficult. Then a concentration of these compounds could occur during the tubular resorption, reaching a level that may be as high as 50 times the blood level.

Dr. Urbanowicz:

I owe you my final response to yesterday's discussion. I see your point now. I was alloted 40 minutes for my presentation and the data have been so many that I concentrated on the positive results of our experiments. I could not speak about the negative results. Maybe the point was missed on my chart that it was related to red blood cells and I would stress that the activation of ALAD was found in red blood cells exclusively. We did the experiments also in liver and kidney and we found no changes in vivo in ALAD in the tissues. You stressed that you found no metallothionein binding of cadmium in red blood cells. I shall now check this problem in our experimental conditions.

Dr. Giec:

You have told us that the bacterial activity is increased with the roles of dose of mercury as I remember. I have one question; do you look for acidification of urine during your experiment because it is well known that the bacterial growth changes with pH of urine and may be that mercury influences pH of urine.

Dr. Piotrowski:

Just in this series of experiments. Also the effects of pH was included, but no differences could be found. I'd like to stress that in some of our former experiments on the kinetics of distribution of lead, we found something very surprising, that 30 minutes after intravenous injection of lead, about 60% of the whole dose was contained just for a short moment in kidney and liver. Later it disappeared from these organs and was shifted to other organs, mostly to the bones. But at some very short time, which had not been investigated by other authors, just 15 - 30 minutes, for some reason lead was concentrated just in liver and kidney. Of course we will try to find an approach to this question, binding of this metal by metallothionein up to now. We have made preparations of metallothionein from the renal cortex of horses and have checked for binding ability of different metals. After the spectral properties it seems to be possible a firm binding between lead for instance and metallothionein. But all this has to be done more in detail and I would not like to discuss it too extensively now.

STUDIES ON SWEAT GLAND FUNCTIONS

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Sweating and evaporation of water from the skin surface are the crucial factors of thermoregulation. That is why the sweat and sweat gland function are important fields of research of physiologists, physiotherapeutists, military doctors and industrial physicians.

No wonder that a lot of papers dealing with this subject written by authors representing different fields of science, using different methods for sweating stimulation and sweat collection, working at different sweating rate, either with or without water repletion, afforded information and opinions sometimes contradictory. Strange to say that the function of a deeply hidden organ having a very complexed structure, the kidney, is much better understood than that of the sweat gland which is very simple in structure and easily accessible.

To explain the mechanism of sweat gland functions we have studied in our previous investigations not only the composition of sweat but also the influence of different substances as ADH, glycocorticoids, mineralocorticoids, and diuretics on sweat secretion (3-11, 13-15.)

The experimental conditions in our study resembled the natural environment in which our mine rescue men have to work occasionally: 2 hours exposure to heat at rest with the temperature about 40°C and the relative humidity over 50%, with minimal air movement, without eating or drinking. In such conditions sweating was always profuse and enabled the direct collection of sweat into test tubes; they were safeguarded against evaporation. Body weight loss was regarded as an index of sweating rate. We think that this kind of thermal sweating disturbing body water and electrolyte balance and obviously involving body self-conservation mechanisms (thermoregulation, osmoregulation, circulation) can hardly be compared to that examined by most of the authors applying cholinergic stimulation on a limited skin area.

From our earlier investigations we concluded that sweat excretion is undoubtedly an active process. This is evident from the difference between the composition of the sweat and that of the substrate from which the sweat originates (Figure 1). The different total osmolarity and different concentration gradients of various components shown in Figure 1 are evidence of glandular work. There is, however, an important simplication, the time factor is not respected.

We think that not only the initial hypoosmolarity but also the step by step changing concentration of the final product are further evidence of the active functioning of the sweat glands (Figure 2). This is due to sodium chloride. During two hours exposure to heat the concentration of sodium and of chloride nearly doubles. Therefore, in our studies we used the rising Na and Cl concentrations as the second index of the gland's function.

The changing function during sweating concerns one more parameter, that is the sweating rate. In our experiments it always rose with the time of exposure. As a matter of fact this rising sweating rate means the increasing quantity of water supplied on the body's external surface for cooling despite the nonchanging environmental conditions in which the subject stays during that time. We think this work, rising from 0 to the top efficiency is the third evidence of glandular function.

It should be emphasized that many other components we have examined were excreted in the same concentrations which occur in the interstitial fluid and did not alter their concentrations with the rising sweating rate. This observation allows the conclusion that the rising NaCl concentration is not the result of condensation due to water reabsorption from the hypotonic sweat because in this case the concentrations of all the other constituents would rise in a similar manner. Special attention should be paid to potassium. It was examined also in the study on poor and good sweaters. Potassium concentration in sweat of the good ones was isotonic and constant; it averaged to 4 mEq/l. The particular values did not exceed 5.5 mEq/l. In the group of moderate sweating subjects five values (3%) exceeded 6 mEq/l but the mean was still maintained at the level of about 4 mEq/l. In the scarcely perspiring people, however, 20% of the determinations revealed values higher than 6 mEq/l, the mean being over 5 mEq/l in the initial phase of the experiment. It seems to indicate that slower sweat outflow in the poor sweaters is often associated with a higher potassium concentration.

In these experiments we collected the first sample of sweat after 30 minutes exposure to heat. The question arose how high is the potassium concentration in sweat in the preceding period because the sweating rate is very low at the beginning of the glandular function. Therefore in the succeeding experiment sweat was collected at 10 minute intervals and the early potassium concentration averaged 5.6 mEq/1 in a random group which was very similar to that found after 30 minutes in the group of poor sweaters.

The opposite behavior of potassium and sodium in sweat was claimed to reflect the adrenal activity. We wonder if high potassium and low sodium initial concentrations in thermal sweat can be regarded as equivalent compensation because of the quantitative differences. The potassium concentration usually fell by about 2 mEq (sometimes more) while that of sodium rose by about 30 mEq/1 or more. Neither could an ion exchange be responsible for this opposite behavior because the fall of the potassium concentration was accomplished in about 30 minutes while the sodium concentration continued to rise during 2 hours.

How to explain the elevated potassium concentration in the first sweat samples? Some authors believe that high potassium values in sweat may be due to the desquamated epithelial cells. In order to avoid this contamination we carried out the subsequent experiments centrifuging each sweat sample before the determination in the flame photometer; the results were comparable to the earlier ones (17). Neither could this phenomenon be explained by the greater water reabsorption in the initial phase of sweating because in this case it could not be limited to potassium alone and sodium content could not be so low as it is. Looking for another explanation we arrived at the conclusion that the higher potassium concentration, which is of very short duration and limited to the first sweat samples, may be due to the enrichment of interstitial fluid in potassium during its passage across the epithelial cells into the glandular lumen. Favorable conditions for such a process exist because if the transport of the extracellular fluid across the glandular cells is slow, as it is in the initial phase of sweating, there is a greater possibility for ion exchange. It need not be a hormonal effect but simply may be due to the very high concentration gradient. This high gradient between the extracellular and intracellular fluids may be maintained on the cell surface due to the cellular membrane and sodium pump. The intracellular structures enabling the fluid passage may not be equally sufficient, especially if the passage is slow. On the other hand the cell cannot be continuously depleted of its electrolyte and it struggles to maintain the potassium content. It is easier when the fluid passage is faster.

With the rule of joining the function and the structure in mind we extended our study to light and electromicroscopy (12). We examined the skin bioptic specimens taken from the volunteers just before and instantaneously after two hours thermal sweating. The histologic appearance of the gland in the light microscope as well as its ultrastructure in rest and at the top of its activity were compared.

The s.c. light cells in the gland are claimed to be "sodium secreting cells" as well as the way of fluid transport. They are surrounded by the free intercellular space accessible to the interstitial fluid from outside the gland. The intercellular space is firmly closed and separated from the glandular lumen by s.c. attachment zones. In the working gland the cells are erected and therefore higher than in the state of rest.

A constant phenomenon we have observed in the clear cells after sweating, was the appearance of many open circular spaces (Figure 3) with the distinct one layer membrane and some inner structures resembling microvilli. They look like transversal sections of intercellular contorted canaliculi. They may be also vesicles of extracellular fluid pinocytized inside the cell and transported to the secretory pole.

In any case, we supposed that these holes of circular or longitudinal shape arranged with a certain order correspond to the way of transport of the fluid to the gland's lumen. This is just the place where, as we have supposed, the fluid can be enriched in potassium from the intracellular stores especially when the process of sweating slowly starts.

Having learned that the interstitial fluid, which is the substrate for sweat production, must pass through the glandular cells we have to ask how much the different composition of both the intracellular and the extracellular fluids influence the sweat composition. The sodium concentration is always lower in sweat than in the interstitial fluid and this is believed to be the effect of its reabsorption in the terminal part of the gland. It is most effective when sweat is secreted slowly. The limited capacity of reabsorption results in the appearance of less hypotonic sweat when the rate of secretion rises. The NaCl reabsorption and elaboration of osmotic gradient take place at the very end of the glandular duct. There seems to be no more place for water reabsorption and isotonic equilibration.

This explanation is compatible with the phenomenon of quite constant concentration of potassium, calcium, magnesium and some organic constituents during the intensive thermal sweating. The concentrations of these constituents are equal to those in the interstitial fluid. The potassium concentration of about 10 mEq/l at the beginning of sweating is several times higher than the basic concentration. The explanation of the occurrence of such high values by the many-fold water reabsorption seems unlikely. Neither is it acceptable on the ground that other constituents such as calcium, magnesium, and urea do not change their concentrations. So the only explanation seems to be that the first portion of sweat is highly "contaminated" with the intracellular fluid. Such an explanation makes the low sodium and high potassium contents in sweat understandable at the initial phase of sweating. The intracellular milieu, however, is safeguarded and cannot be permanently altered. So, the afflux of the fluid from the extracellular compartment and its transport to the secretory pole of the cell quickly increases. At that time, the proper cell composition in the small glandular compartment may be easily restored from the increasing extracellular fluid transfer while the sweat composition approximates more and more that of the interstitial fluid. The cutaneous stores of the latter are quickly repleted from the intravascular compartment.

All these fluid and sodium fluxes are very rapid as we can see in the following experiments: 10μ Ci of ^{24}Na was injected intracutaneously to some subjects sweating after a thermal stimulus. The injection was executed very carefully with a long needle piercing a channel subcutaneously and finally inserted in the skin itself so superficially that the end could be seen. Thus, the radioactive solution was injected visibly under the skin. This technique was used in order to avoid any contamination of the skin surface by the radioactive material. The samples of the sweat appearing on the injected area were collected on filter paper strips at the 5 minute intervals and the radioactivity was determined on them. As evident from the Figure 4 the ^{24}Na injected into the interstitial compartment was forwarded at once to the sweat. It also disappeared quickly from the area surrounding the gland which indicated a high speed back penetration. The experiment could be repeated several times, each time furnishing a new portion of radioactive sodium into the skin with the same effect.

In another experiment 100 μ Cl ^{24}Na was injected intravenously to the subjects just sweating in a hot chamber. Round filter paper strips of 5 cm diameter were lightly pressed to the sweating skin on the back or on the arm for 1 minute and instantaneously protected against evaporation. Then they were immediately carried over to the laboratory where they were weighed, the radioactivity was measured and expressed in impulses per 1 ml of sweat per 1 minute. As evident from the Figure 5, the radioactive sodium reached the skin very quickly and appeared in sweat not much later than in the case of intradermal injection. Its concentration in sweat rises in a very short time, the peak occurring before 10 minutes after the injection. From that time it maintains on the high level; this probably represents an equilibrium achieved between the afflux of radioactive material from blood plasma and rising sodium concentration in sweat on one hand, and its distribution through the other fluid compartments as well as the continuous loss through the skin on the other hand.

The pattern of potassium excretion in sweat is different from that presented by ^{24}Na. It should be mentioned that the dose of ^{42}K corresponded to that of ^{24}Na. In this short lasting experiment, when the radioactive potassium was injected intravenously during thermally induced sweating, it appeared quickly in sweat. It also reached the peak in a similar time but afterward its concentration fell much more rapidly than that of ^{24}Na (Figure 5). I think we can tell from this experiment that potassium reaches the skin level as easily as sodium. Its more rapid disappearance may be due to the fact that glandular cells depleted of potassium in the first phase of sweating, capture it more rapidly from the substrate. In this case, the normal distribution of the injected potassium load throughout all the body fluids and tissues is reinforced by the repletion of the potassium deficient secretory cells. Therefore, the descending arm of this tracing falls quickly while that of sodium does not. The difference between the tracings is striking.

In subjects injected ^{42}K 5 hours before the sweating stimulation, it was absorbed by the body cells to such extent that only traces of activity could be found in sweat. This was not the case with sodium. It can be assumed that ^{24}Na injected i.v. 5 hours before the entrance to the hot chamber was equilibrated among the body compartments to some extent. So the further radiosodium clearance from the intravascular to the extravascular compartments would be insignificant at that time.

According to these changed conditions of the experiment the pattern of ^{24}Na excretion in sweat (Figure 6) takes a different shape from that in Figure 5. There is not a quick rise of concentration at the beginning. This figure represents the percent of radioactivity of sweat as related to that in plasma, at the beginning it was about 30%. As we remember the total sodium concentration in sweat in the first phase of sweating is also about 30% of that in blood plasma, i.e., circa 50 mEq/1. With the continued sweating it rose in our previous experiments to an average of about 80 mEq/1 (determined by flamephotometer) which is about 60% of the plasma sodium content; and the activity due to Na^{24} also rose to about 60% after one hour. We believe this is evidence that both methods afford fairly comparable results.

The well established influence of some adrenal hormones on the kidneys was often compared to their possible effects on sweat glands (1, 2, 16, 18). Now we shall consider an opposite condition, that is the case of hyperthyroidism which evidently enhances enormously sweating even in a patient resting at a normal temperature. The modern thyroid treatment provides an opportunity to conduct a clinical experiment. Its essence is the comparison of both sweat excretion and composition in the same subjects before and after the successful treatment. Theoretically a reverse experiment is also possible, that is the comparison of both parameters in patients with hypothyroidism improved after the substitution of the thyroid hormone.

A group of 21 cases of hyperthyroidism were studied. Their disease was characterized besides the clinical state by the following parameters: the mean ^{131}I uptake was 83%, the mean cholesterol level was 162 mg% and the BMR averaged to +43%. After the patients have reached the euthyroid state the serum cholesterol concentration rose to an average of 240 mg% and the BMR fell to the mean of +17% besides the clinical signs of improvement (Table 1).

It was surprising for us to find that the hyperthyroid patients, who sweated easily at the normal room temperature and were expected to sweat far more profusely in the hot chamber lost a little less body weight during the first exposure than after the recovery (Figure 7).

In Figure 8, we can see the sweat sodium concentration in three groups of subjects: (1) healthy, (2) hyperthyroid, and (3) the same patients in recovery. The group of healthy subjects examined simultaneously was small (12 persons) but the findings obtained correspond exactly to those in our previous experiments. The hyperthyroid patients demonstrated lower sweat sodium concentration than the healthy subjects. After the successful treatment, sodium concentration in sweat of the same subjects reached exactly the normal level.

The chloride behavior in sweat resembled exactly that of sodium.

On the contrary the potassium concentration in sweat was always higher in the phase of hyperthyroid than in the euthyroid (Figure 9).

The conclusion from these experiments could be that the excess of the thyroid hormone does not enhance the rate of thermal sweating, but on the contrary it rather diminishes the final response of the gland to the thermal stimulus and forces it to work out a higher osmolarity gradient than in the normal.

The group of patients with hypothyrodism was, as yet, too small for interpretation and their corresponding values cannot be regarded as convincing. In any case, both the sodium and the chloride concentrations were always lower after hormone substitution than in hypothyroidism.

Based on the clinical experiments, we examined the local influence of topically applied pure thyroxine in the healthy people. It is known that one of the four main biological actions of the thyroxine is an effect on water and ion distribution. Therefore, the supposition that thyroxine might act directly upon the glandular secretory cells seemed acceptable.

The first step was to determine if we could introduce the hormone into the skin by electrophoresis. Triiodothyronine labelled with ^{131}I was introduced into the skin by iontophoresis, 4 μg of the compound with the radioactivity of 5 μ Ci was applied each time under the plus electrode. Then the skin was carefully cleaned with pure water and dried. The radioactivity persisted in the place of application. A sham procedure was carried out on the other arm which consisted of application of the same solution, but without electric current. After washing very low activity was found on that place (Figure 10.) This pilot experiment seemed to indicate that triiodothyronine was introduced into the skin.

The direct influence of thyroxine on the sweat gland function was examined in the following way: four equal squares were designed on the symmetrical spots, two on arms and two on forearms. The 1-thyroxine electrophoresis was performed in a cross-wise manner: either on the left arm and right forearm or vice versa. In the other two squares normal saline electrophoresis was performed. The subjects pretreated in this manner entered the hot chamber for one hour and sweat samples were collected at 10 minute intervals.

Iontophoresis is commonly used for stimulation of topical sweating by cholinergic drugs. In the same way, we were able to demonstrate the inhibiting effect of atropine. As evident from Figure 11, the iontophoresis of 1 mg of atropine sulfate into the skin resulted in the almost complete suppression of sweating in this area while on the opposite arm the sweating rate was normal. When 0.5 mg of atropine was applied, the suppression of the sweating rate did not exceed 45% of that on the other arm and was quickly extinguished. The effect of 0.1 mg of atropine was doubtful. As soon as the sweat appeared on the atropinized area its composition was similar to that on the other side.

It should be emphasized that in these experiments thermal sweat and thermal sweating were examined while iontophoresis was applied not for sweating stimulation but in order to change the natural milieu in the small area of skin so that the glands in that area could be immersed in the interstitial fluid with an excess of thyroxine.

No difference resembling that between hyperthyroid and euthyroid patients was found either in the sweating rate or in the sweat composition on both opposite sides (Figures 12 and 13).

The results of studies upon the mechanism of action of thyroxine are confusing, and it is difficult to decide which of the many supposed ones is the most likely. Some effect of the hormone appear in a few seconds while the others require many hours (19). One of them is the enrichment of mitochondria in respiratory and phosphorylative units. This phenomenon is considered to be the result of the induction of enzymatic protein synthesis.

Maybe our experiments with introduction of the l-thyroxine into the skin just before exposure to heat were not adequate in time and should be repeated with a longer time interval. Another explanation should also be taken into consideration, namely, that the high ability of hyperthyroid patients to sweat in rest might not be due to the direct influence of the thyroid hormone but to the higher sympathetic excitation. In the last case, it would not be surprising that the enhanced glandular activity in rest is altered in an unexpected way under the heat stimulus.

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TABLE 1

SOME LABORATORY FINDINGS IN 21 PATIENTS (MEAN VALUES) BEFORE AND AFTER TREATMENT

	Hyperthyroid	Euthyroid	t	p
^{131}I Uptake	83%			
BMR	43.5%	17.3%	7	0.001
Cholesterol	162.8 mg%	240.5 mg%	4.9	0.001

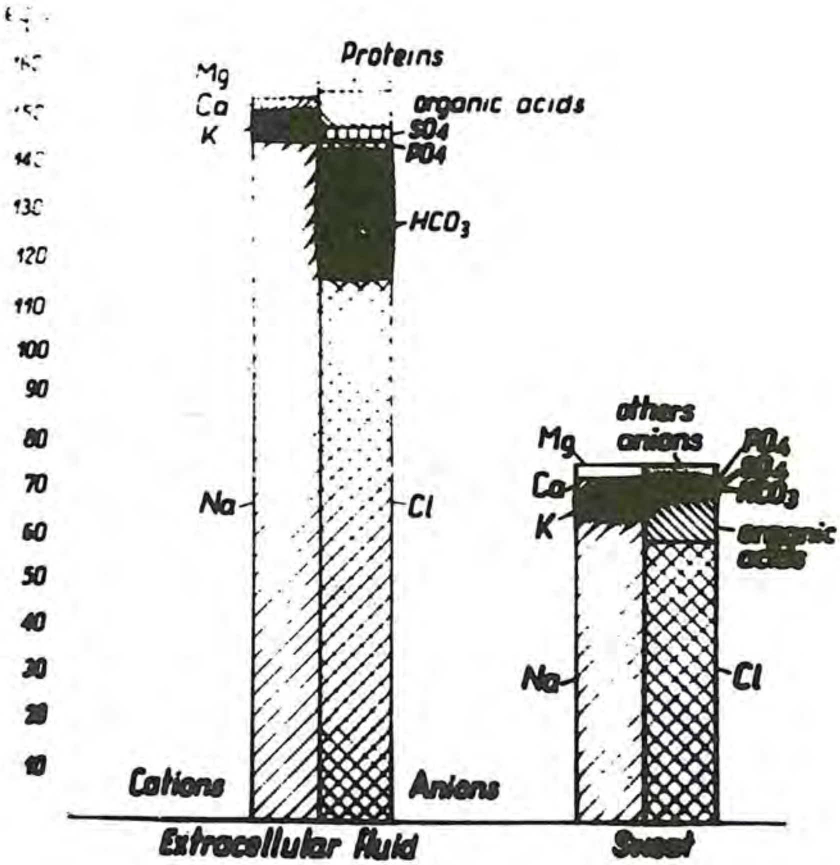

Figure 1. Extracellular fluid (according to Weisberg) and sweat composition.

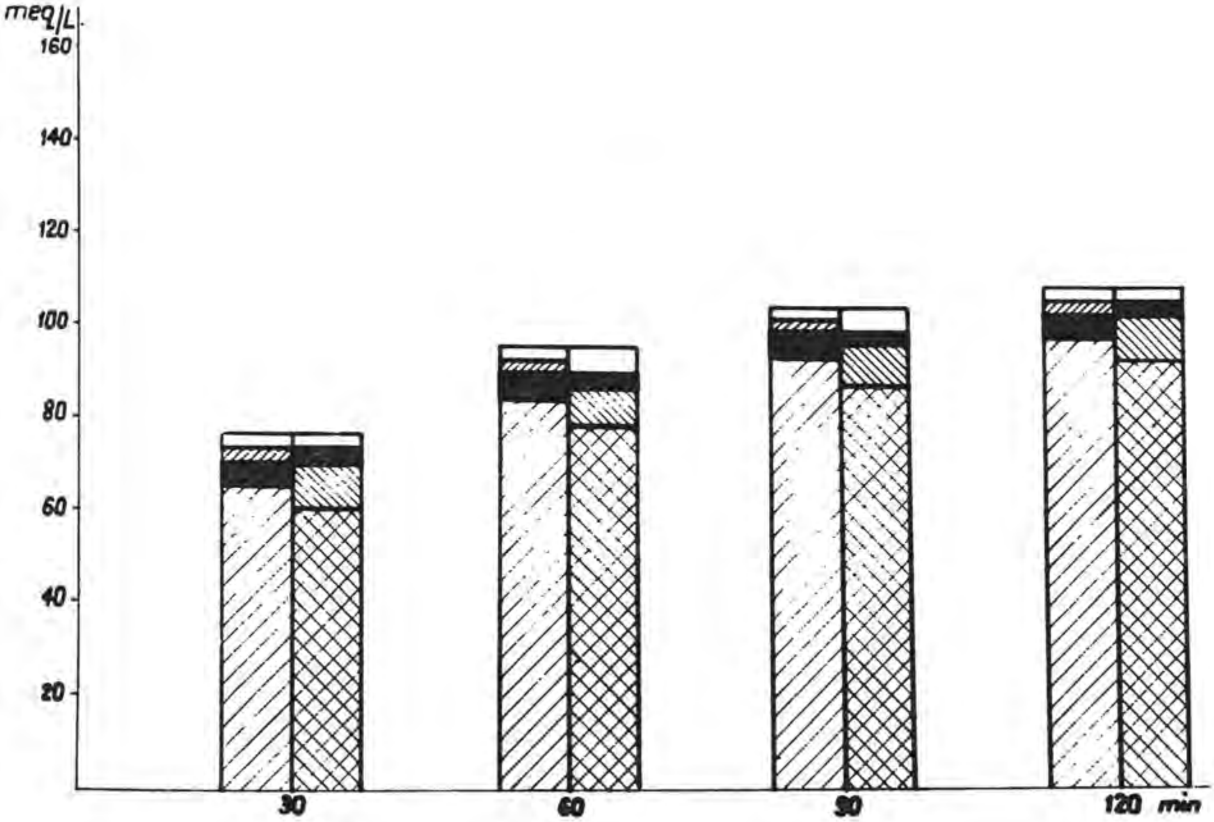

Figure 2. Composition and increasing osmolarity of continuously excreted thermal sweat.

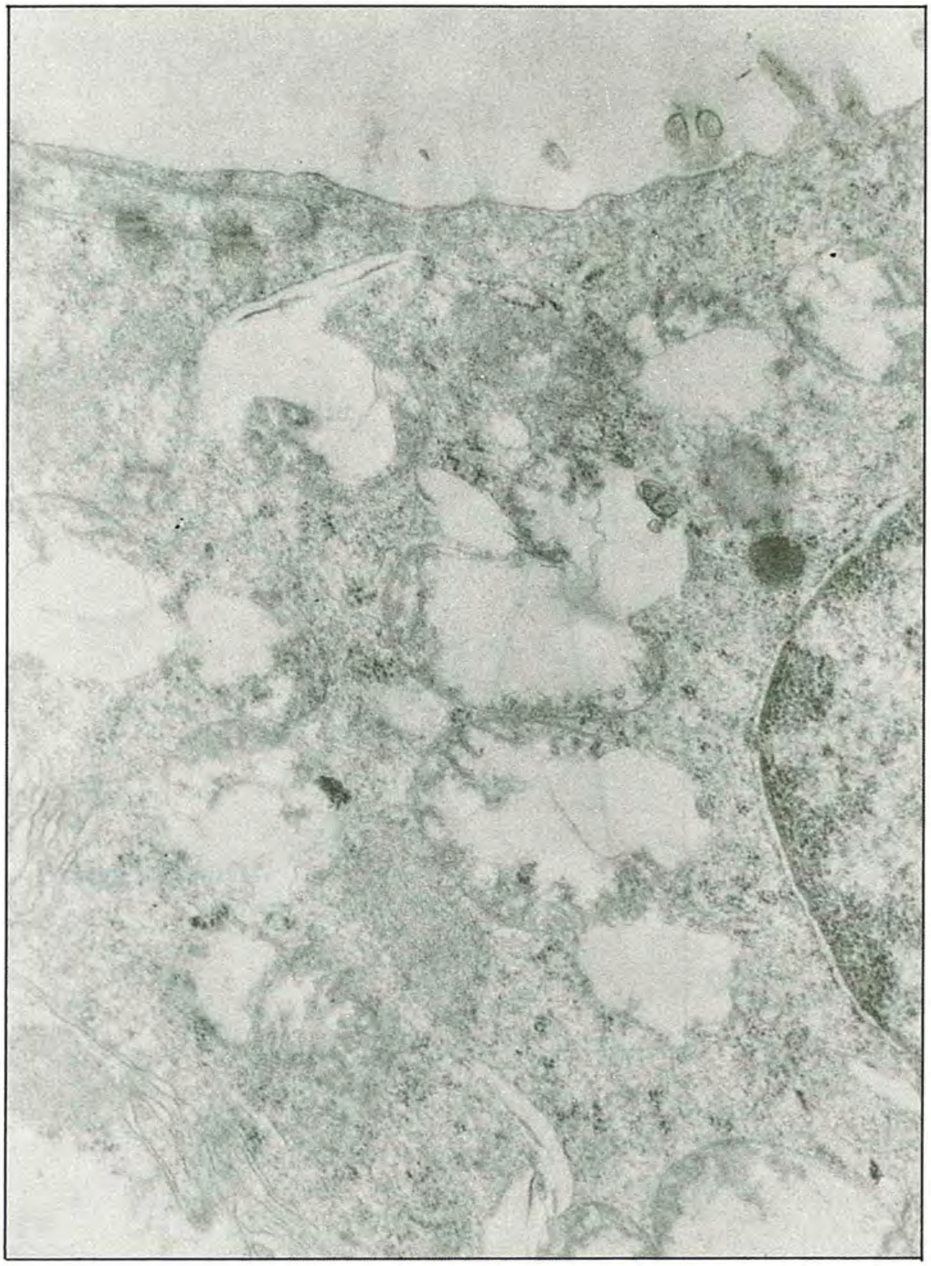

Figure 3. Numerous vesicles of various size arranged along the cell and approaching its apical border. Some of them seem to fuse their membranes and/or for a canaliculus (Mag. 28,000x).

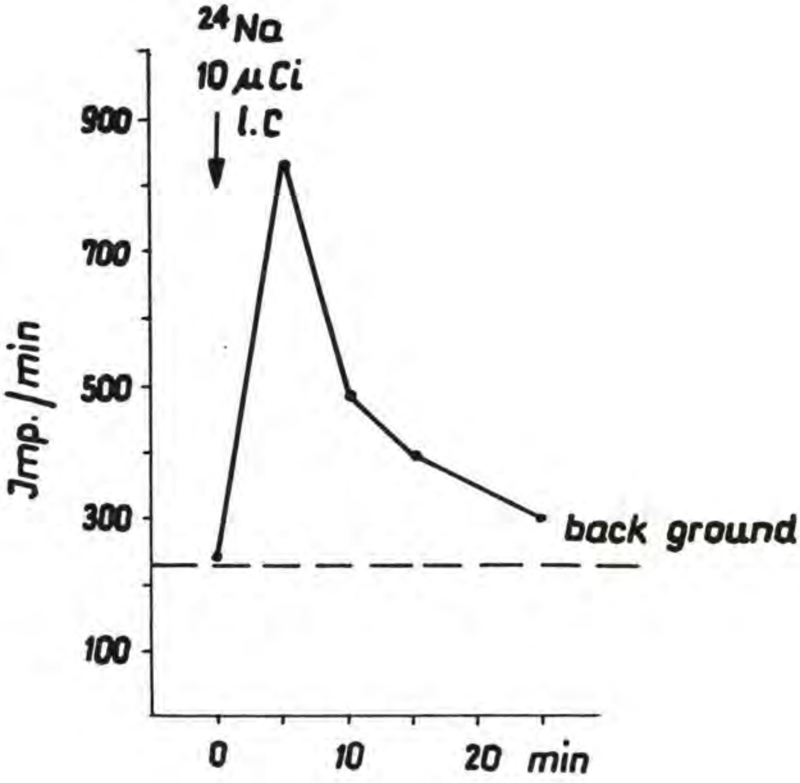

Figure 4. ^{24}Na appearing in thermal sweat after intracutaneous injection.

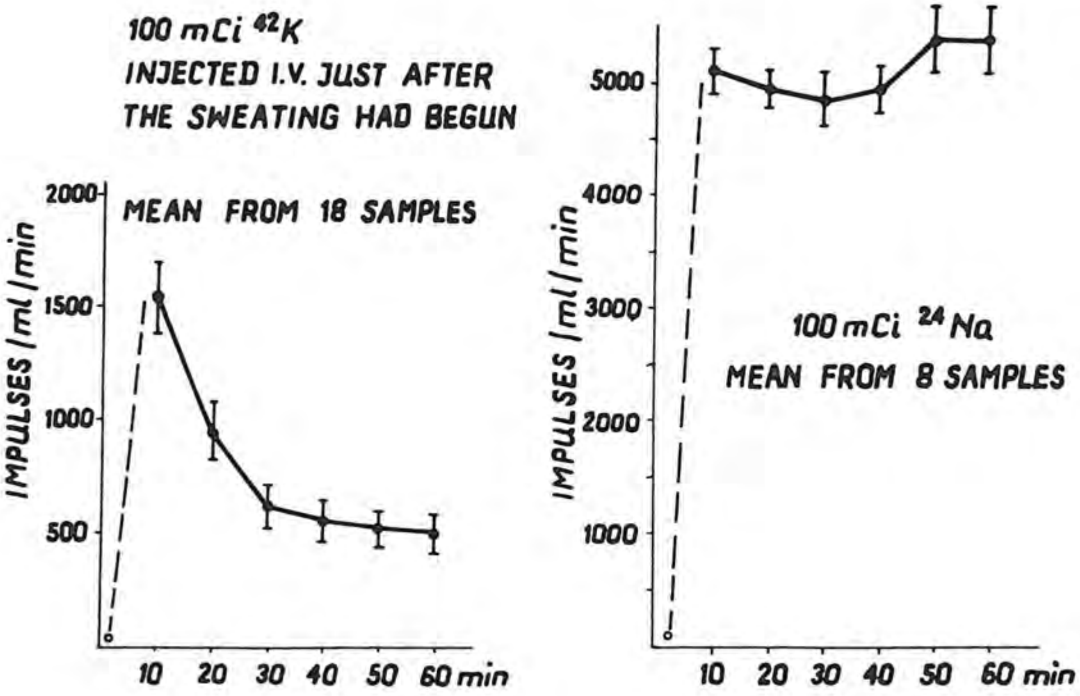

Figure 5. ^{24}Na and ^{42}K in sweat after intravenous injection.

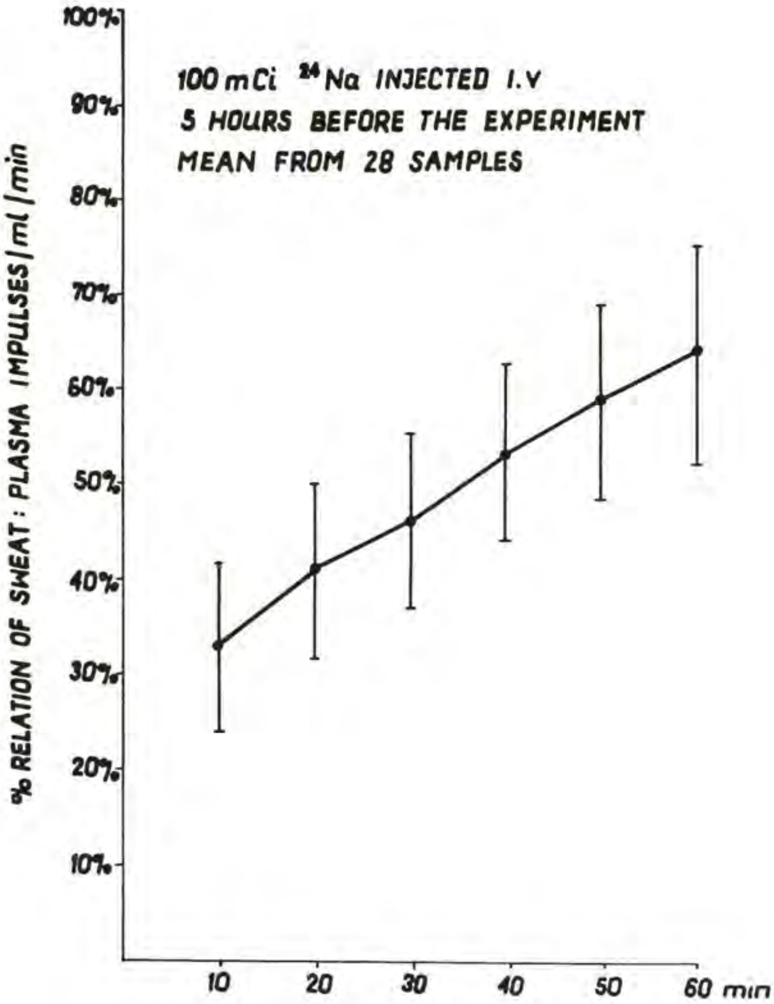

Figure 6. ^{24}Na injected intravenously five hours before entering the hot chamber.

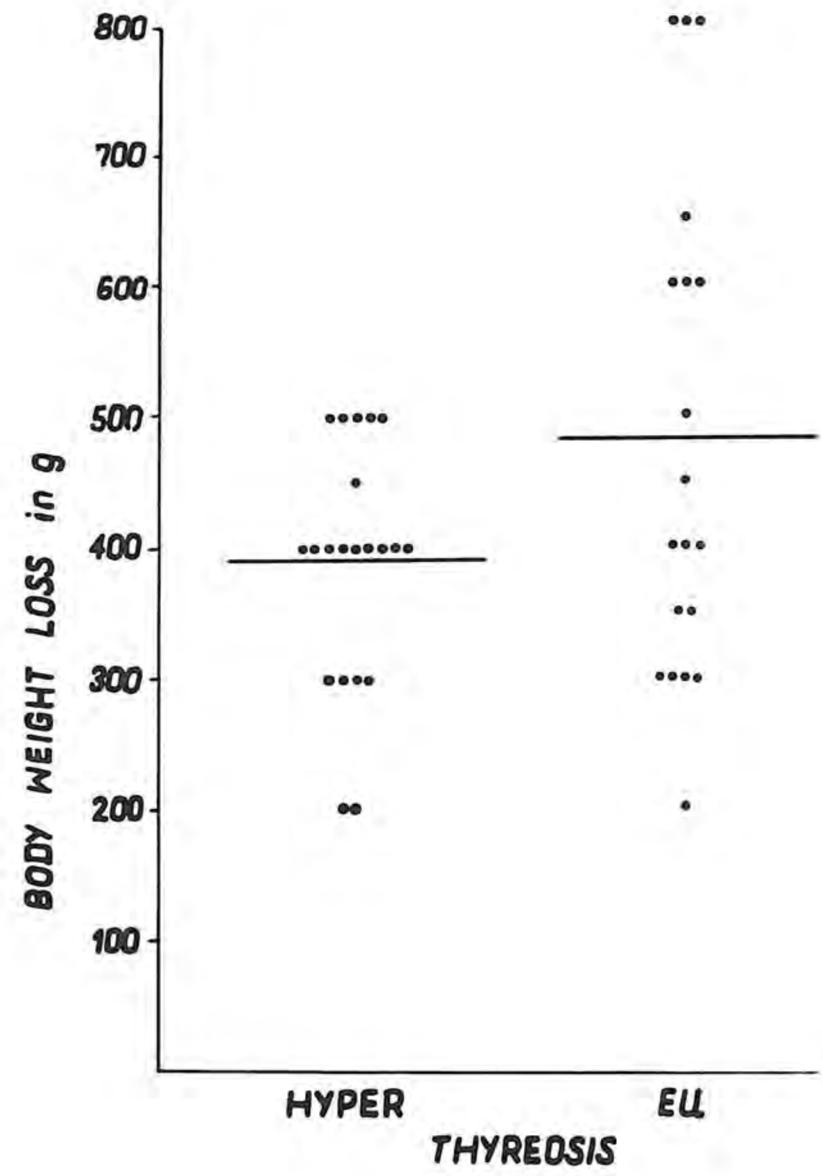

Figure 7. Body weight loss as an index of sweating rate in hyperthyroid and euthyroid subjects.

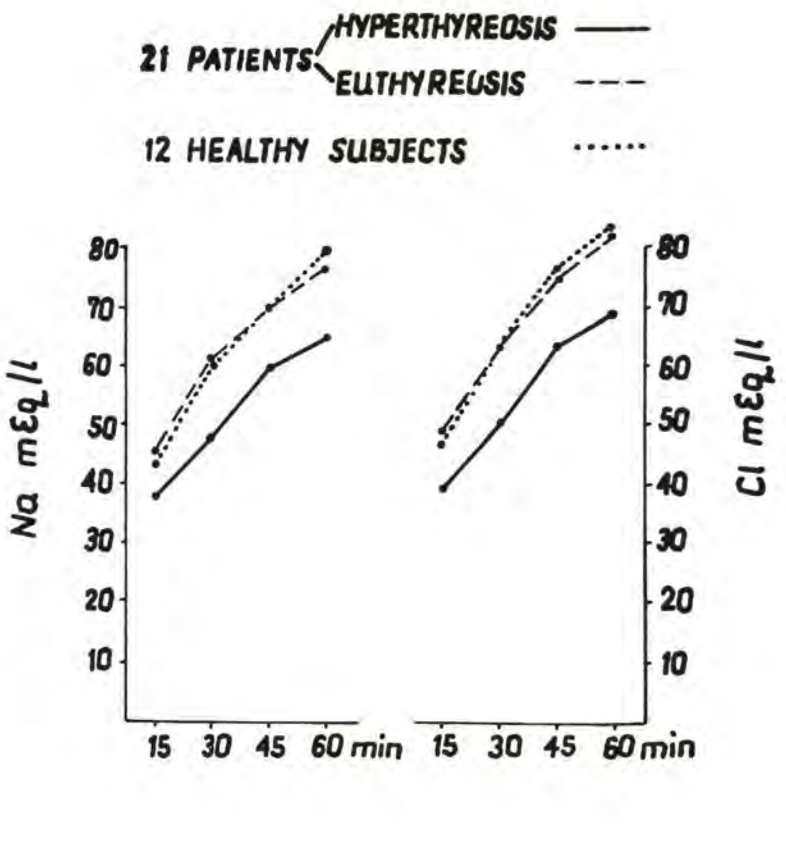

Figure 8. Sodium and chloride concentrations in thermal sweat of healthy subjects, of hyperthyroid patients, and of the same patients after recovery.

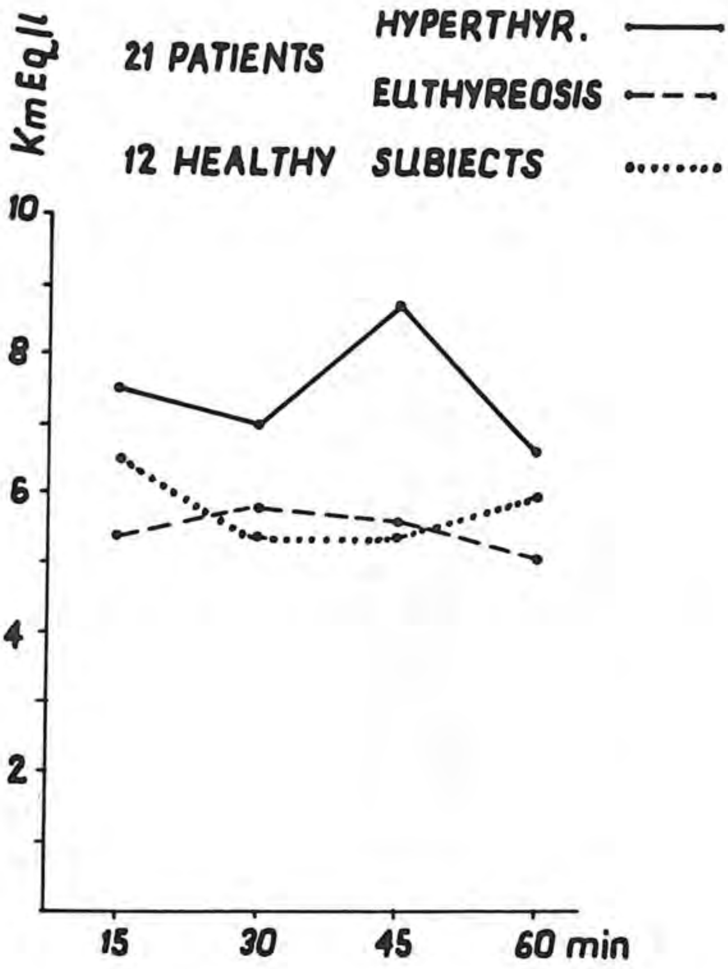

Figure 9. Potassium concentration in thermal sweat of healthy subjects, of hyperthyroid patients, and of the same patients after recovery.

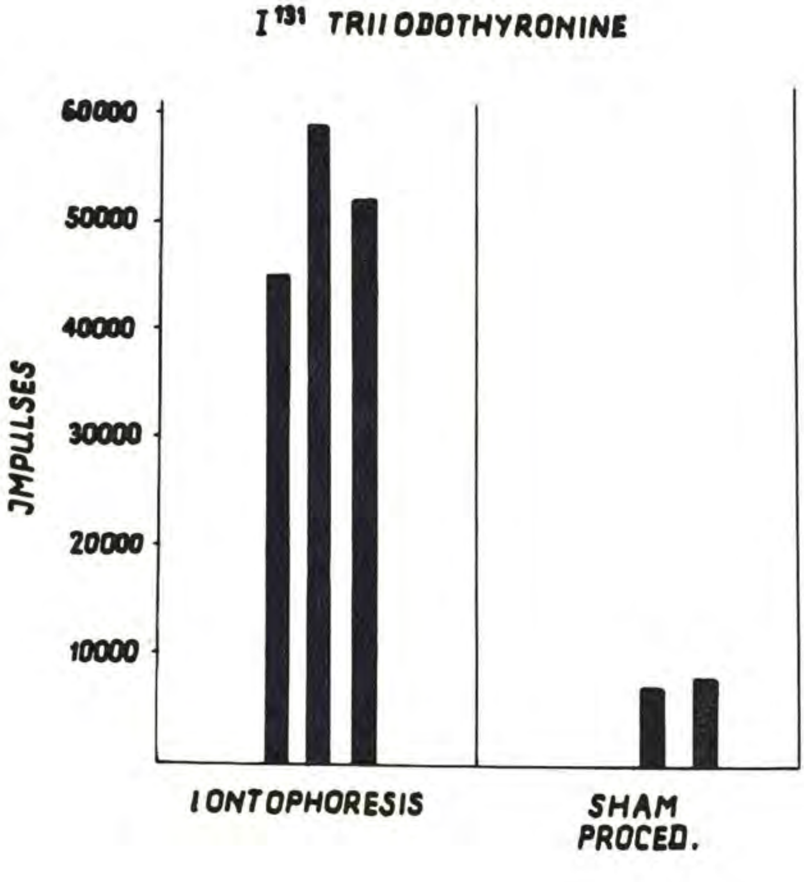

Figure 10. Persisting radioactivity on the place of labelled triiodothyronine iontophoresis and its lack on the place of the same procedure.

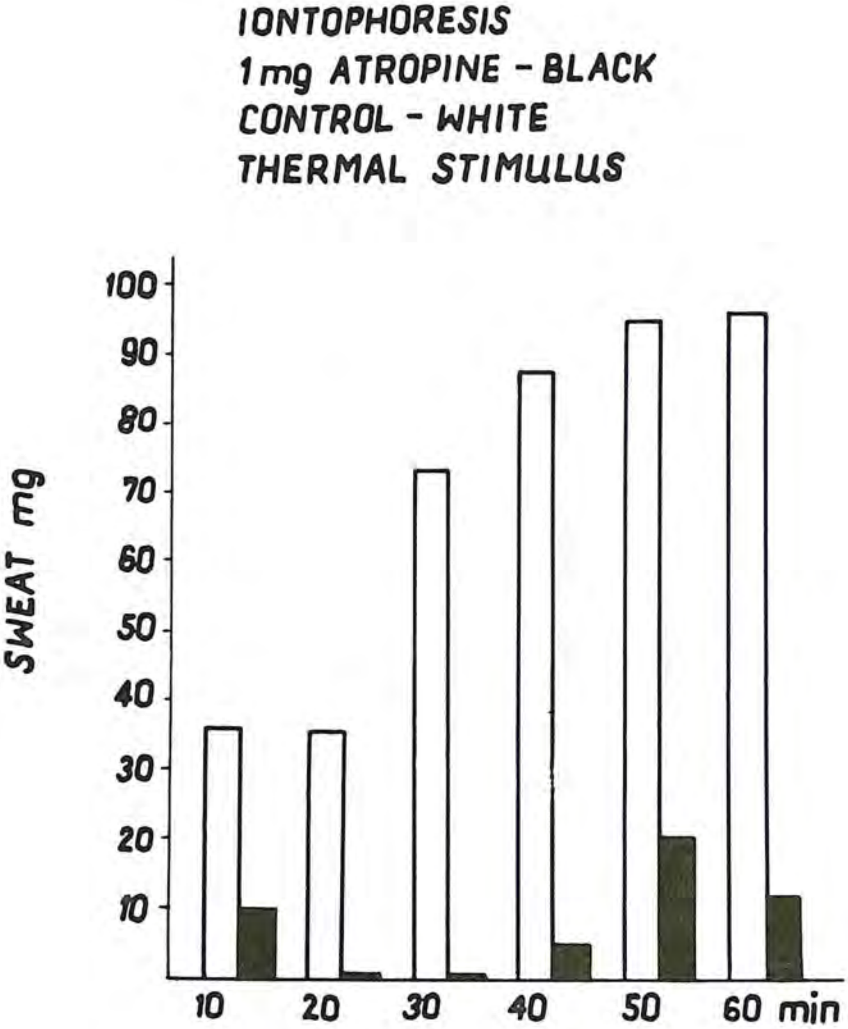

Figure 11. The effect of iontophoresis of 1 mg of atropine on sweating rate.

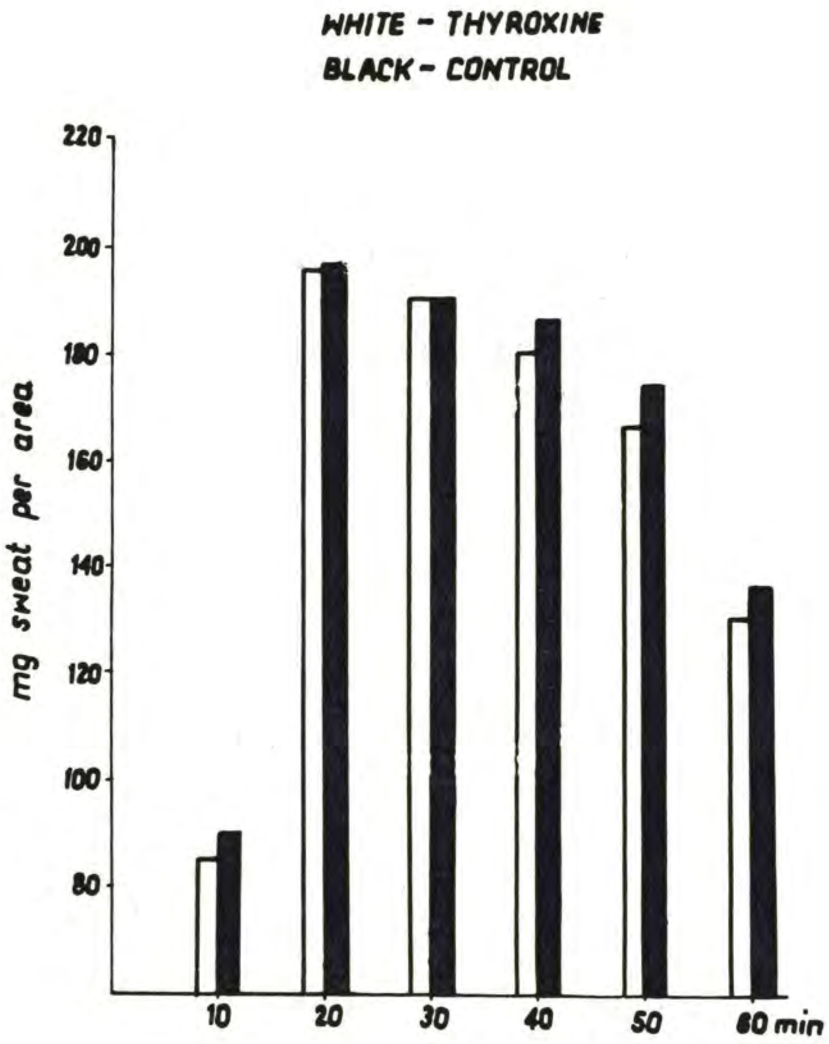

Figure 12. Similar sweating rate after intracutaneous introduction of 1-thyroxine and saline, respectively.

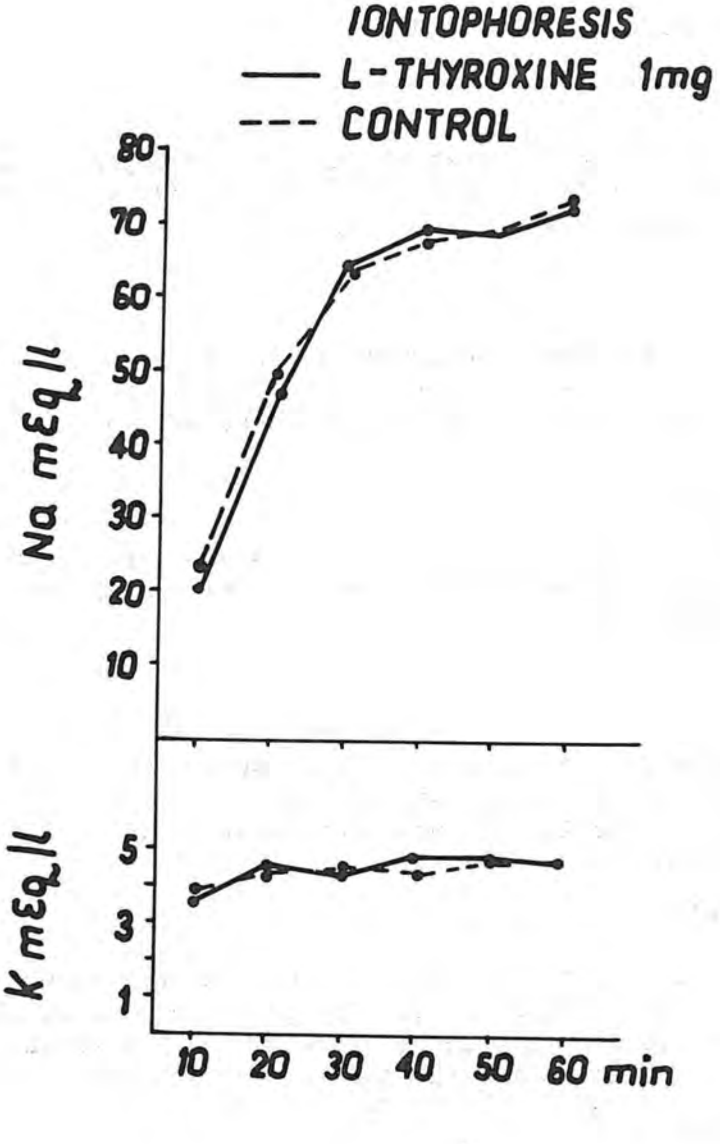

Figure 13. Similar sodium concentration in thermal sweat after intracutaneous introduction of l-thyroxine and saline, respectively.

DISCUSSION AFTER DR. GIEC'S PAPER:

Dr. Henschel:

Did you reduce skin temperature in the hyperthyroid and euthyroid and the controls? Differences in skin temperature will influence the response of the sweat gland cells.

Dr. Giec:

Yes, it could be done, but nevertheless, it is well known that the temperature of the skin of hyperthyroid patients is higher than in normal people. We have not done this measurement.

Dr. Henschel:

May the differences in the skin temperature explain the differences in the response of the controls and the euthyroid patients as compared to the hyperthyroid?

Dr. Giec:

I don't think so, because as we know from other experiments, the sweating rate increased parallelly to skin temperature. In our experiments in these hyperthyroid patients, the sweat rate were rather lowe
in these hyperthyroid patients, the sweat rates were rather low than in healthy people.

Dr. Henschel:

May this reflect the fact that the sweat glands themselves are used to being at a higher temperature. The point is that at the higher skin temperature you actually do not have as much of stimulus as you do in a normal skin where the skin temperature is lower. Therefore, there is really not as great a temperature differential as far as the gland is concerned.

Dr. Giec:

The difference of skin temperature in hyperthyroid patients before and after heating may be higher or less. You are right. We didn't measure this.

Dr. Henschel:

Didn't your sweat gland work show that the sweat gland functions partially as the kidney tubule in selective reabsorption. But the secretory part of the gland is quite different from the Bowman's Capsule. In some ways the sweat glands functioned as the kidney and some ways they are different?

Dr. Giec:

I think that there is no difference because we know that in kidney the reabsorption of water is one known phenomenon and that the reabsorption water is in sweat glands.

Dr. Cralley:

To what extent would very heavy exposure to toxic agent such as mercury, cadmium, lead, have any affect on the sweat rate, and the second point to what extent does the skin (the sweat) serve as an excretory organ for these metals?

Dr. Giec:

We use diuretics for research in sweat gland function and we could find no changes in sweat gland composition. But maybe the toxic doses of these metals may have influences. We use only normal doses of substances which are used in the treatment as diuretics.

THE EFFECTS OF CHRONIC VIBRATION AND NOISE EXPOSURE ON THE HEALTH OF WOODCUTTERS - A PRELIMINARY REPORT

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Our investigations cover longitudinal and cross-sectional observations on the health state of woodcutters working with motor-chain saws in order to determine the influence of chronic exposure to vibration and noise on the prevalence of pathological changes in these workers. These investigations are designed to give comprehensive information which will support or correct previous concepts on vibration disease and occupational hearing loss.

I. Theoretical and Methodological Assumptions of the Study

Our investigations are justified by the unsatisfactory state of knowledge of such an important problem of occupational medicine as changes in the health state of employees working with tools producing vibration shocks and noise. This concerns many hundreds of thousands of workers exposed in various branches of industry and other branches of the national economy. This exposure and its influence on the health of employees creates a concrete medico-social demand for our investigation. A demand for examining the motor-chain saw woodcutters constituting about a 15,000 person homogenous group of employees in our country is implied by the necessity for seeking methods and organization of working conditions to prevent the occurrence of vibration disease and occupational hearing loss.

Results published up to now in national and international literature concern single and partial observations based on a small number of characteristics studied. Those papers do not explain the prevalence of pathological changes in the whole working population or whether pathological changes found in the woodcutters are connected to and to what extent with working with the motor-chain-saw. Even if this relationship was observed, it has not been stated after what period of occupational work these changes occur and what is the relation between their extent and the period of occupational exposure. The relation between the exposure to various levels of vibration and noise, cooling ability of air and noted pathological changes is not clear either. Other than vibration and noise, pathogenic factors influencing the health state of the woodcutters were not taken into account. There was therefore a need for investigations comprising multi-variable observations, using various methods from a number of medical disciplines, among others, internal medicine, neurology, otolaryngology, ophthalmology, physiology, psychology, occupational medicine and medical statistics.

Performing such multi-disciplinary investigations on motor-chain saw woodcutters is possible in our country thanks to the existence of the social health service under which there are occupational scientific research institutes, industrial health services, and sanitary-epidemiologic stations, also thanks to the citizen's right to health care and to the work legislation allowing for carrying out mass preventive health examinations.

The aims of our investigation are the following:

1. Establishment of statistical relations between characteristics of working conditions with motor-chain saw and pathological changes in the woodcutter.

2. Evaluation of prevalence of vibration disease in motor-chain saw woodcutters.

3. Description of prevalence and intensity of metabolic, functional and morphologic symptoms accompanying vibration disease.

4. An attempt to determine the mechanism of the pathological changes during vibration disease.

5. Determination of the level of noise of the motor-chain saw causing occupational hearing loss.

In order to accomplish these aims a pilot study on 100 motor-chain saw woodcutters was performed before establishing a list of variables, selecting measurement methods and organizing the investigation. The woodcutters of the pilot group were submitted to a single general medical, laryngologic, X-ray, physiologic, and biochemical examinations in order to have an idea about the extent of exposure to vibration and noise and the type of pathological changes. Results of the pilot study also served for method verification, organization of the study and recording of the results.

II. Assumptions

The investigation comprises a group of longitudinal observations on 150-200 woodcutters and 40-50 forestry workers of similar age but not exposed to vibration and noise.

In this group, observations were performed on the occurrence and dynamics of metabolic, functional and morphological changes arising because of the chronic exposure to vibration, and noise of the motor-chain saw in woodcutters working during five years in the same conditions, and in the same forest area. Examinations are performed once a year during five years of work in selected occupational conditions. Therefore, every woodcutter and employee from the control group will be submitted to the same set of examinations done by the same methods. Woodcutters and the control group submitted to the longitudinal observation are subject to the internal, anthropometric, neurologic, ophthalmologic, otolaryngologic, radiologic, psychologic, physiologic, and biochemical examinations. These investigations are performed by a team of professional specialists who were trained in this field and work in the Voivodeship Occupational Dispensaries. Every motor-chain saw woodcutter and a control person comes once a year to the Voivodeship Dispensary and is examined according to the schedule during three subsequent days. Every person is conscious of the need for these investigations and comes voluntarily.

At the same time, the measurements of vibration and noise levels of motor-chain saws, the woodcutter's daily exposure to vibration and noise, cooling ability of air, and energy expenditure of the woodcutter at work will be performed twice in the five year period. These measurements are taken by a qualified team visiting certain work posts of motor saw woodcutters in the forest.

A group of cross-sectional observation comprises 2000-3000 motor-chain saw woodcutters with different length work histories in order to distinguish some subgroups of different years of experience with the motor-chain saw in different tree covers and different climate zones. A control group of about 200-300 persons was concluded. A cross-sectional (single) observation on this group is to give information on the prevalence and type of pathologic, functional and morphologic changes in woodcutters.

All woodcutters of the cross-sectional group will be subjected to clinical, anthropometric, neurologic, otolaryngologic, and radiologic examinations and some chosen psychologic, physiologic, and biochemical tests. These examinations will be carried out by the specialist teams instructed in this field. They will be organized in 3-4 units of Voivodeship Occupational Dispensaries and Occupational Medicine Institutes. The professional teams of chosen units will use the same organization and the same sets of characteristics and research methods in order to get comparable results. Every woodcutter will come to an examining unit for three days and will be subsequently submitted to particular examinations.

Among many possible variables those which seem to be important to the achievement of the study aims were chosen. A division into independent and dependent variables was made:

- Among independent variables, ten characteristics relating to exposure to vibration and noise, and to the characteristics of vibration and noise of the saw; ten characteristics relating to the socio-occupational status of the woodcutter;

- Among 88 dependent variables there are such pathological changes which constitute vibration disease and occupational hearing loss, and those which characterize general health state of the persons examined. Within the independent and dependent variables a grouping was performed according to the method of gaining them and according to their direct and indirect importance in elucidating the relationship between the exposure to vibration and noise, and pathological changes.

Among 110 variables, because of their relevance in explaining the relationship between the exposure to vibration and noise and the symptoms of vibration disease and hearing loss, the following indices were designated as the direct ones:

> among independent variables: age, period of work, working time and exposure to vibration and noise of motor-chain saw during the day and year, level of vibration (velocity, acceleration, amplitude), level of noise (frequency, intensity) at felling and cutting and exposure to cooling ability of air according to the season, energy expenditure at work, usage of personal protection, smoking, and alcohol; and

> among dependent variables: muscle tension, muscle force, coordination of movements, state of peripheral vessels, state of upper extremities, disturbance of senses, disturbances of sensitivity to pain and vibration, otoscopy, bone and air conduction, state of hearing according to acuity and audiometric examinations, labyrinth excitability, state of eye fundus vessels, vision acuity, state of wrist joints, state of elbow joints, state of shoulder joints, state of lumbar vertebral column, single reaction time, vision-movement coordination, coordination of hand movements, ECG, oscillogram, plethysmogram, state of vessels after pressure, body weight, arm and forearm circumferences, shoulder breadth, skinfold thickness, levels of protein, sugar, alkaline phosphatase, phosphorus, calcium, aldolase, transaminase, hemoglobin, hematocrit, white blood cell smear, sedimentation rate, total fat level, cholesterol and esters levels in blood and urobilinogen and urobilin levels in urine.

The structure of variables can be presented as follows:

- Object of Investigation
  - Independent Variables
    - Characteristics of Working Conditions
    - Socio-Occupational Characteristics
  - Dependent Variables
    - Vibration Disease
    - Occupational Hearing Loss
    - General Health State

Information on the above characteristics is written on specially designed questionnaires and records using instructions prepared for a particular specialist's examinations. The whole set of information on variables comprises 17 questionnaires and records which have been prepared in such a way that the information on the variables can be analyzed using mechanographic computation technique. The necessary statistical and mathematical measures will be used in the analysis of variables.

In order to carry out these investigations four research groups were created:

- longitudinal observation of motor-chain saw woodcutters;
- cross-sectional observation of motor-chain saw woodcutter group;
- analysis, hypotheses, and publication group; and
- study control and verification group.

Work distribution of particular groups during the five year period of the contract from 1 August 1968 to 31 July 1973, is the following:

- longitudinal study group's work during the five years period:
- cross-sectional study group's work during two years, namely, the 3rd and 4th years of the contract; and
- analysis, hypotheses, and publication group, the control and verification group's work during the 2nd, 3rd, 4th, and 5th years of the contract.

Organizational links within the groups and between the groups are based on person-activity networks adjusted to particular organizational cycles of the study.

The report comprises a list of the most important methodological notes and results which were achieved during the first two years of the five-year study. According to the organizational cycle it was planned among others; examination of a pilot group, double examination of the motor-chain saw woodcutters, and control persons of the longitudinal observation group.

III. Preliminary Results of the Pilot Study

One hundred motor-chain saw woodcutters exposed to vibration and noise of motor-chain saws were examined. The age of examined persons ranged from 20 to 60 years and on the average it was 34 years. The working period with the motor-chain saw groups ranged from one month to nine years and was on the average 3.4 years.

The woodcutters worked with two types of saws which produced noise and vibration on similar levels.

The woodcutters reported pain in upper extremities, higher nervous irritability, headaches, and sleeping disturbances. It was found that the occurrence of these disturbances had statistically proved connection with the working period with motor-chain saw.

Internal medical examination showed existence of pathological changes in the cardiovascular system, bone and joint system, respiratory system, nervous system, vision system, and changes in the skin.

Otolaryngologic examination with audiometric tests found traumatic hearing loss caused by noise in 75 percent of woodcutters. A significant relation was found between hearing loss and the working period with motor-chain saws and between hearing loss and the age of the woodcutters.

X-ray examination showed that 69 percent of woodcutters had pathological changes in bones. These changes concerned bone structure and were determined as osteoporotic, atrophic, and cystic, particularly in wrist bones and forearm epiphyses and vertebral bones. A signficant relation was found between the working period with motor-chain saws and pathological changes in vertebral bones and upper extremities bones and the relation between the age of the woodcutter and changes in bones. Changes in wrist bones depended however only on the working period with motor-chain saw.

Electrocardiogram curves showed in 50 percent of woodcutters conduction disturbances and coronary ischaemia. A statistically significant relation was found between the working period with motor-chain saws, conductivity disturbances, and ischaemia.

These relationships between age, the working period, and pathological changes in woodcutters which were found and calculated were also reported in the professional literature.

IV. Preliminary Report of the First Examination of the Longitudinal Observation Group of Woodcutters and the Control Group

The age of 305 examined motor-chain saw woodcutters and 50 control persons (forest workers) ranged from 20 to 60 years and on the average it was 36 years. The working period with motor-chain saw groups ranged from 1 to 18 years. Sixty-five percent of all woodcutters worked with motor-chain saws less than 5 years, 25.6 percent from 6 to 10 years, and only 9.4 percent over 10 years.

Among control groups, 62.8 percent worked in the forest over 10 years, 16.3 percent from 6 to 10 years, and 20.9 percent less than 5 years.

Eighty-five percent of motor-chain saw woodcutters and control persons apart from their regular job activities worked on their own farms too. The examined persons have primary general education, moreover, the woodcutters have professional training to work with the saw.

Sixty-five percent of employed woodcutters are stabilized in their occupation. The remaining 35 percent leave their work after different working periods.

Smokers constituted 76 percent of woodcutters and 78 percent of control persons.

Certain characteristics of working conditions of motor-chain saw woodcutters and control persons are the same. They work in the forest in lowland areas where among the prevailing trees are pine, oak, birch, and hornbeam; spruce, larch, and poplar are less frequent. The climate has a continental character, average yearly temperature ranging from 6 to 9 degrees centigrade. The lowest temperature at which work is allowed is of minus 20 degrees centigrade.

Woodcutters and control persons work about 300 days in a year, however, felling and cutting of wood take place mainly in the autumn-winter season. Daily working time ranges from 8 to 10 hours. Effective daily working time of the woodcutter ranges from 55 percent to 91 percent, on the average 70 percent.

Effective daily working time with motor-chain saw ranges from 43 to 50 percent, on the average 31 percent of total daily working time.

Energy expenditure of the woodcutter assessed in 20 examined persons is on the average 4.8 Kcal/min net while felling, 3.9 Kcal/min net while cutting, and 6.2 Kcal/min net while felling and moving to the next tree.

Exposure time of the woodcutter to vibration and noise of motor-chain saws checked in 30 examined persons amounts to an average of 149 minutes daily, and 334 hours in a year. Exposure to these factors has an unsteady character because on the average, the woodcutter is exposed every 33 minutes to vibration and noise for about 17 minutes. This cycle is repeated irregularily regarding different interval times between sawing spells. On the average, the woodcutter is exposed to a 17 minute vibration and noise period about 8 times during the 8 hour working day. General values of parameters of vibration of the saws examined are the following:

Parameters	BK-3	Partner R-11
Velocity cm/sec	5.0 - 12.5	4.5 - 14.0
Acceleration cm/sec^2	5,600 - 18,000	10,000 - 28,000
Amplitude mm	0.06 - 0.48	0.22 - 0.27

Analysis of vibration of both motor-chain saws shows that oscillations of frequencies 63, 125, and 31.5 Hz prevail. It was found that vibration velocity of the saw is high in frequency bands ranging from 63 to 2000 Hz, particularly in the band 125 Hz.

Noise levels of the examined saws are the following:

Saws	Noise Intensity Hearing Correction		In dB According Curves
	A	B	C
BK-3	106 - 108	106- 109	107 - 110
Partner R-11	107 - 108	105 - 106	107 - 108

Spectral analysis shows that the highest noise intensity exists in frequencies 500, 1000, 2000, and 4000 Hz.

Analysis of the health state of 247 examined motor-chain saw woodcutters exposed to vibration and noise of the saw and 43 forestry workers not working with the saw shows that 141 (57 percent) of woodcutters and 22 (51 percent) control persons have various pathological changes.

Forty-seven (33 percent) out of these 141 woodcutters showed symptoms of the vibration syndrome in the form of vascular, osseous, and nervous changes. Statistically significant relationship was found between the symptoms of vibration syndrome and the working period. However, this relationship was not found between the symptoms of vibration syndrome and the age of woodcutters. Furthermore, 40 examined (28 percent) out of 141 woodcutters have changes in wrist, forearm, and vertebral column bones. These is a significant relationship between osseous changes and the working period, but no relationship was found between these changes and the age of the woodcutters. Other pathological changes found in the examined woodcutters show significant relationship with age and no significant relationship with the working period.

Among 43 control persons seven (16 percent) showed symptoms similar to vibration syndrome and five (12 percent) pathological symptoms in bones similar to changes in woodcutters. These two types of changes do not, however, show any relationship with age and the working period of the control person. Findings of similar changes in control persons required further details in investigations in order to find their cause.

Evaluation of pathological changes diagnosed by internist, neurologic, otolaryngologic, ophthalmologic, radiologic, psychologic, physiologic, and biochemical examinations showed that in woodcutters pathological changes of cardiovascular system, nervous system, hearing and vision systems, and in bones are significantly more frequent than in control persons. These changes simultaneously aggravate the working period of the woodcutter in the range of one to 15 years and more working with the motor-chain saws.

More important findings of the examinations of woodcutters from the pilot group, the first examination of woodcutters, and control persons of the longitudinal observation group are the following:

> changes in upper extremity bones, vertebral bones and hearing losses depend on the working period with the motor-chain saws.

pathological changes in cardiovascular and nervous systems, hearing and vision systems, and bones are significantly more frequent in woodcutters than in control persons and depend on the working period with the motor-chain saws.

On the basis of the two year period of investigation a confrontation could be made between the methodological assumptions and experiences and preliminary results achieved. Methodological and organizational experience acquired enable us to state that the study aim can be achieved within the five year observation.

Discussion After Dr. Rafalski's Paper:

Dr. Lainhart:

I was interested that you were including in your investigation the educational-social differences. We examined some workers in the community where there are coal miners and other workers and the small differences between education-social backgrounds were apparently important. We don't know why, but they have the same role to play in the differences, for example, symptoms and signs of disease. Completely aside from such obvious things as pneumoconiosis and coal miners. So I was glad that you included those factors in your analysis.

Dr. Henschel:

First a comment. In some animal work in Poland with high frequency and intensity vibrations in the rats, very short exposures produce changes in the production of acetylcholine and acetylcholinesterase in the nervous tissue. I was told the frequency level is similar to the frequency of vibration that the woodcutters are exposed to. Did you find any differences between the men who are using the lower frequency vibration saws and the higher vibration saws? Were you able to break your group down to see whether there are any differences in the responses of these two groups?

Dr. Rafalski:

According to Dr. Lainhart's comment we think that there is the influence of social-economical condition on the increase of pathological symptoms. The education plays a role in using protections. Maybe the more educated workers used them properly. They protect themselves every day. We always include in our epidemiological study the social-economical condition.

Dr. Lainhart:

Is there any attempt at getting at the type of nutrition in the two groups? Are there social differences, with regard to the kind and amount of food eaten? You said that they lived in the same general environments in the woods or, I suppose, small villages, but do they in essence have the same kind of nutrition background?

Dr. Rafalski:

The answer is yes. As I said about 85-90 percent of the woodcutters are not pure workers. We call them peasant workers. They have small farms and usually men become a forestry worker or do other work, for instance, in the industrial plants. In that area we are talking about, there is the similar pattern of diets and nutritional status of the persons. We are considering to include diet surveys but it is easier to state the nutritional status than to examine the diets. As you know, it consumes a lot of time to investigate diets. We need one week observations at different seasons in a year. So it is a time consuming examination in addition for each family an investigator must be there to see what they eat, how they eat, what quantity they eat, etc. So this problem is very complicated to study. At present we include the observation of nutritional status of woodcutters and control persons, so we can get some idea of food intake.

Dr. Lainhart:

But would the financial or the percapita income or family income be the same in the two groups between the woodcutters and the controls? In terms of the amount of Zlotys that they have to buy the things they need: household, furniture, food. In the whole social aspects, are they the same?

Dr. Rafalski:

Yes, but woodcutters earn more than the control group, because their work is more dangerous and specialized. Simultaneously they have smaller land. So, maybe the income percapita is quite similar.

Dr. Rafalski: (answer to Dr. Henschel's question)

They use two saws, but both saws are similar in producing the level vibration and the level of noise. One is Swedish and another is Polish, and the Polish one was constructed according to the pattern of Swedish. Well, let us say the Polish one is a little bit better, maybe less dangerous, but it should be proved. According to the detailed answer to Dr. Henschel's question we shall look for exposure to two saws. Talking of the usage of the saws I should like to say that about 70 to 80 percent of the woodcutters use Polish saws and 20-30 percent use the Swedish one.

Dr. Potkonjak:

I understand your study is just in progress and it seems to me far from the end; therefore I understand you are in the same situation as is with my project, and it is not possible to give any definitive conclusion, but anyway, I would like to ask you the question not about a conclusion but about your impression. It is stated that low blood pressure is background for the development of the effects of the chronic vibration. In our examination we did not prove this statement, what is your impression?

Dr. Rafalski:

I am not sure that I can give you the right answer because I have no data with me about the blood pressure in woodcutters. We measure among the physiological test the blood pressure before the effort and after the effort.

Dr. Potkonjak:

May I ask you a question: Did you include finger plethysmography as a method of examination?

Dr. Rafalski:

The answer is yes. We included it.

Dr. Potkonjak:

I am asking that because I had the opportunity to follow-up a group of woodcutters and we noticed that in a number of workers, who in the first year of the examination had no complaints, had abnormal plethysmography curves, only one year later these workers showed the other signs of the vibration syndrome. Therefore, we are inclined to conclude that plethysmography method is the best one for discovering very early changes.

Dr. Rafalski:

Yes, you are right. We have similar experiences which we got from our pilot study.

Dr. Giec:

You have told us that some of your investigated subjects have the sign of coronary disease. How do you explain these changes, because as I know this kind of work does not give predisposition to the coronary vessel changes.

Dr. Rafalski:

We have some data on rats. We examined rats exposed to the different kind of vibration (low and high frequency vibration) and we found some biochemical and morphological changes in vessel collagen. The impression is that the vibration might have an influence on vessels. In that observation we are talking about, we examine the electrocardiogram of woodcutters. We found some disturbances in conductivity and non-specific ST-T changes. We think the prevalence of those disturbances is too high. We shall look into our results whether the disturbances have been involved by the vibration or by the age.

THE STUDY ON EFFECTS (BIOCHEMICAL, MORBIDITY, AND MORTALITY) OF RADIOACTIVE CONSTITUENTS OF URANIUM ORE ON HEALTH OF MINERS

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GENERAL STUDIES

The project "The Study on Effects (Biochemical, Morbidity and Mortality) of Radioactive Constituents of Uranium Ore on Health of Miners" has performed in the last six years. In this period many problems were studied and here we would like to summarize the obtained results.

1. Morbidity and mortality of miners in the uranium and control mines.

All miners in the uranium and control mines were medically examined every year. In the medical examination we included the working anamnesis, smoking habits, the clinical examination, tests of pulmonary function, the ophtalmologic examination (each second year) and the roentgenography of lungs.

We examined a total of 1369 miners: 765 uranium miners and 604 miners from control mines. The duration of exposure is presented in Table 1. The average exposure duration of uranium miners is 5.3 years and the average age is 38.4 years (Table 2). In the control group the average exposure was 9.1 years and the average age 39 years. The analysis of smoking habits showed that 45.8% in the exposed group and 50.4% in the control group were smokers (Table 3).

The results of the morbidity examination and the analysis of the blood cells together with the results of the renal examination are presented in Table 4. These results suggest that there is an increased inflammation of the upper respiratory tract in the exposed group (exposed 55.8% and control 41.8%). It is also evident that smokers show an increased number of respiratory troubles (Table 5).

In this period one uranium miner died from microcylindriccellular carcinoma of bronchus after 12 years of exposure. We evaluated the average yearly exposure of this man as 0.8 of maximum allowable concentration (MAC: 3.10^{-10} Ci/liter) of radon and daughters in the working atmosphere. We established the polonium concentration in the lungs as 0.12 pCi per gram of fresh tissue, and the excretion of Po in the urine as 1.34 pCi/l.

The ophtalmologic examination was performed on 630 miners of the exposed group and 305 in the control group. Special attention was paid to the determination of the visual field. The results showed no changes in the visual field of the miners. Also, no differences in ophtalmologic disturbances were found in the exposed and control groups which could suggest any influence of the ionizing radiation on this organ.

2. Study of anaplasia and metaplasia of cellular elements in the sputum of exposed miners.

We sampled the sputum of the exposed uranium miners to perform a cytomorphologic analysis of malignant cells of the respiratory tract. This study has been in progress from 1964 to today. As a control group we chose miners from zinc and lead mines. The sputum samples were colored by the Papanicolau method and the results of the examination were systematized by the Saccomano method. In Table 6 we present the results obtained on exposed miners, and in Table 7 the results on the control group. The presented results are from 1964 to 1968, because the results obtained in 1969 are still being evaluated.

If we analyze the obtained results from the exposed and control groups we are not able to establish any significant difference. Some shifts in classes I-II/3 could be explained by differences in the status of bronchitis, taking in consideration the seasons and the exposure to dust in connection with the intensity of the mine operations.

In the examined material the coniocytes and neutrophil granulocytes are in majority. Very often lymphocytes and bacteria could be found. On the other hand, eosinophylic leucocytes were not found. Exfoliated cells of the respiratory tract cells were found in various numbers in connection with the type and intensity of the inflammation processes. No cases suspicious of malignancy were found.

In some cases we observed cells of class III in the sputum, but when new samplings were made they were not found. Such shifts to the right are in accordance with the clinically established acute bronchitis. As valid results of the examined sputum, we accepted the status of a quiet inflammatory phase.

During this work we established great differences in the cytomorphologic examination in connection with the season. As an illustration we present the results of the examinations of 27 miners in Table 8. The first examination was performed in June and the second one in November 1968. In both cases the same persons were examined. It is evident that in November we found 11% as Class III. In the next examination in 1969, we did not find any case in this class.

3. Study on influence of radon on the respiratory function.

We performed a routine spirometric analysis and also an examination of the respiratory function in the miners (uranium and control) before and after work. The aim of this study is to establish if the work in the atmosphere of radon has any influence on the respiratory function. We performed a parallel study on patients inhaling radon at Niška Banja.

This study showed that in 75% of cases in uranium miners (Gorenja Vas, Zletovska Reka) an increase in Vital Capacity (VC) and Forced Expiratory Volume (FEV_1) after the work started. On the other hand, such differences did not exist in the control group. Using statistical analysis we did not establish any statistically significant changes in the respiratory function (see report from 1965). On the other hand, in patients from Niška Banja we found a statistically significant increase of changes of the respiratory function after 1 hour of radon inhalation. A detailed statistical evaluation of the material in general did not show statistically significant changes in the VC value and the FEV_1 after radon inhalation in exposed miners as well as in patients from Niška Banja.

4. The lung clearance after radon inhalation.

In order to get exact data about the elimination of radon from the lung in relation to the status of the function of the respiratory tract after inhalation, we studied the lung clearance in healthy persons and patients with respiratory injuries. We examined four healthy persons and 28 patients with lung emphysema, chronic bronchitis, and bronchial asthma. We divided the patients into three groups: low, medium and high degree of respiratory insufficiency. The examined persons spent 40 minutes in the atmosphere of radon and daughters in a concentration of 2×10^{-8} Ci/l of radon.

Breath samples were taken every 30 minutes during 4 hours of exposure and the measurement of radon and daughters was performed after the achievement of an equilibrium. The obtained results are presented in Tables 9, 10, 11, and 12.

The analysis of the results was performed according to Harley, taking into consideration only the value of K constant for the fourth phase of expiration. From the tables it is evident that K_4 for healthy persons is 0.21 $\pm$0.02, for patients from the first group 0.39 $\pm$ 0.06, and for those from the third group 0.40 $\pm$ 0.12.

The results show the existence of a disturbance in the fourth phase of expiration in the examined patients having an injury of the respiratory function. These results point out the fact that miners with changes in the respiratory tract retain radon and daughters in the lung for a longer period of time. Obviously, further studies are necessary for the evaluation of disturbances in phases I, II and III.

5. Study in chromosome aberrations in lymphocytes of the peripheral blood.

In this project great attention was paid to chromosome aberrations in persons professionally exposed to ionizing radiations:

a. in a group of uranium miners;

b. in radiologists and roentgen technicians;

c. workers on dial painting (using Ra-226);

d. a control group.

The uranium miners (41) were exposed 5-15 years with an average duration of exposure of seven years. The average age of the exposed group was 35 years.

The average concentration of radon in Kalna was 1.75×10^{-10} to 19.67×10^{-10} Ci/1, in Gorenja Vas 3.85×10^{-10} Ci/1 to 14.85×10^{-10} Ci/1, and in Zletovska Reka 3.61×10^{-10} to 19.97×10^{-10} Ci/1.

We examined 16 radiologists and 16 roentgen technicians between 31-55 years of age (average 42 years). The duration of exposure varied from 5 to 25 years (average 10 years and 8 months). The examined persons underwent a clinical, hematologic, and dermatologic examination together with an analysis of chromosome aberrations. In two persons anhydrosis of the palm skin and distrophic changes of nails were found. Clinical signs of palms radiodermatitis of first degree were evident in six persons. The standard hematologic examination showed lymphocytosis in four persons. In 18 persons no clinical signs of radiation injury were found. The dosimetric control of the exposure to ionizing radiation has been performed for 10 years. The yearly exposure doses did not reach the maximum allowable doses. All persons were exposed to X-rays.

A group of eight women between 31 and 41 years of age worked on dial painting with an average exposure duration of 7 years and 7 months. The concentration of Po-210 and Ra-226 in the urine was high only in two persons (Po-210 to 75 pCi/liter and Ra-226 to 68 pCl/l). In other women the concentrations were so low that it was impossible to detect them. The control group consisted of seven persons who were not professionally exposed to the ionizing radiation, their age being 26-42 years.

The results of this study showed that in uranium miners the presence of cells of type B (chromatid gap, isochromatid gap) was 1.6%, of cells Cu (chromosomal fragments and ruptures) 0.4% and of cells Cs (abnormal monocentric chromosomes) 0.07%. Dicentric chromosomes and chromosome rings were lacking.

Taking into consideration the results obtained in the control group (B cells 1.5%, Cu cells 0.5%) it is possible to conclude that the frequency of chromosome aberrations in the examined exposed uranium miners is not greater than in the control group.

In radiologists and technicians with an exposure of more than 16 years, the frequency was higher (B cells 3.9%, Cu 1.9% and Cs 3.2%) than in the control group.

In dial painters a relatively small number of cells were examined, but here also there is an evident increase of chromosomal aberrations in relation to the control group. In this group the most frequent types of chromosomal aberrations were chromatid and isochromatid gaps, chromatid rupture, acentric fragment, translocation, and deletion.

6. Examination of binuclear lymphocytes

The frequency of binuclear lymphocytes was studied in 154 uranium miners and 44 workers of a control group. In the exposed group binuclear lymphocytes were found in 16 cases (10.3%) and in the control group in 17 cases (18.6%).

These results showed that a higher frequency of binuclear lymphocytes was evident in miners of lead and zinc mines exposed to a low concentration of radon. It would be necessary to find out how the combination of radon with heavy metals (lead, mercury, zinc) influences the frequency of binuclear lymphocytes in the peripheral blood.

RADIOTOXICOLOGICAL STUDIES

During six years of this project, we introduced the methods for determination of uranium, Radium-226 and Polonium 210 in biological material. In this period we examined 901 urine samples for uranium and 856 urine samples for Polonium 210.

The results of these determinations showed that uranium miners in Yugoslavia eliminate up to 30 micrograms of uranium per liter of urine (average value 10 μ/1), 3.8 pCi of Ra-226, and 4.5 pCi of Po-210 per liter as average values. In 70% of cases the elimination of Po-210 was under 1 pCi/1.

1. Studies on body burden and excretion of Po-210 in the urine of miners

Studies in radioactive contamination and excretion of Po-210 in miners showed that the contamination in uranium mines depends on many factors. The body burden of Po-210 in uranium miners is about 2.25×10^{-8} Ci/1 due to the exposure to radon and daughters. By food and drink they introduce daily about 7.25 pCi Po-210, by smoking a further 1.5 pCi, and due to inhalation in the mine 10.13 pCi/day.

Our studies showed that 31.3% of miners excreted from 0.6 to 1.0 pCi/1 Po-210 by the urine. Only 10.1% of miners excreted more than 2.5 pCi. These results showed that uranium miners excreted 2-8 times more Po in the urine than non-exposed persons (0.3 pCi/1). We established also that smoking of 20 cigarettes a day will increase the Po excretion in urine by 0.23 pCi/1.

We also determined the Po in the urine before and after weekends. The results showed a statistically significant decrease of Po excretion after the weekend. That means the weekend has an important effect on the Po elmination from the organism.

Our studies showed there is no correlation between the concentration of radon in the working atmosphere and the excretion of Po-210 in the urine of exposed miners (correlation co-efficient $r = 0.308$).

We established that polonium concentrations in the urine were about five times greater than expected (values) due to radon and daughters in the mine. The elimination of 1.5 pCi of Po in the urine can be tolerated in miners exposed to a radon concentration up to $3x10^{-10}$ pCi/1. We took into consideration quantities of Po-210 introduced by inhalation, smoking, and by ingestion of food and beverages. More data will be sent during 1970 after the evaluation of all obtained results.

2. Studies on deposition of Pb-210 and Po-210.

We studied the deposition of Pb-210 and Po-210 after inhalation of radon in experiments with rats. In the exposed animals the highest quantity of Pb-210 was found in the skeleton (0.198 $\pm$ 0.36 pCi/gr) and of Po-210 in the kidneys (0.553 pCi/gr). The exposure was 900 hours by an average daily activity of 0.4×10^{-8} pCi/1 of radon.

A detailed report was prepared for the Symposium in Richland (Washington), 15-17 May 1967, and for the European Radiological Symposium in Czechoslovakia in 1967.

3. Studies on Po-210 excretion after exposure to radon.

We studied the excretion of Po-210 in the urine and feces after exposure to radon on rats and rabbits. The animals were exposed for 30 days to an average daily radon concentration of 10^{-8} pCi/1 in the air for six hours a day. The urine and feces were sampled every 24 hours and the Po-210 determined from the beginning of exposure until 40 days later.

The results showed an increase in excretion of Po in the urine and feces of the exposed animals. The average daily excretion of Po in the urine of rats was 1.9 pCi/1 and in feces 7.26 pCi/gr. In the control group these values were 0.07 pCi for urine, and 1.34 pCi for feces.

The statistical evaluation by the t-test showed a statistically significant difference in excretion of the exposed and control group in urine and feces. Similar results were obtained in 12 rabbits. The relation between the excretion of Po in feces and urine was 3.84:1 which is in accordance with other authors (Berke and Dispasqua, 1961).

4. Study on Po-210 excretion after intratracheal application of Po-210 and SiO_2.

We studied the excretion of Po-210 after the application of Po-210 together with the quartz dust in rats. After an intratracheal application we followed the excretion for 40 days.

The results showed an increase in the feces excretion which is about 300 times greater than in urine in relation with the excretion after the inhalation of pure polonium. This fact suggests a different distribution of Po-210 when it is applied by inhalation with SiO_2 than by inhalation of pure radon.

Details were given in Panov's doctor's thesis (1970)

5. Study on the influence of radon, daughters, and quartz dust on the respiratory system of animals.

We performed many experiments studying the influence of radon, daughters, and quartz dust on the respiratory system of experimental animals (rats). These results were reported at the Symposium in Richland (May 1967) at the Fifth International Symposium on Radiology in Czechoslovakia, 1967 at First European Congress on Radiological Protection in Menton, France, October 1968.

The results showed that the presence of radon and daughters did not change the development of the experimental silicosis. We did not confirm the results of other authors who stated that radon and daughters increase fibrotic processes in the lungs of experimental animals.

These studies have been continued in order to perform a long-term exposure (2 years) of the experimental animals to radon, daughters, and quartz dust. We hope to draw certain conclusions about the effects of radon, daughters, and quartz dust. This work has partially been financed by IAEA in Vienna and the Yugoslav Scientific Fund.

STUDIES ON CONTAMINATION OF THE BIOSPHERE IN URANIUM AND CONTROL MINES

In the study period we performed measurements of the concentration of radon in the working atmosphere, determinations of short-lived daughters, as well as measurements of concentrations of uranium, Radium-226 and Po-210 in the dust.

1. Determination of radon in the air.

We measured radon by balloon scintillators and recalculated the results on MAC which is 3×10^{-10} Ci/1 for a 42 hour working week. The results of these measurements are presented in Tables 13, 14, and 15. These results showed an exposure of uranium miners of 7.86×10^{-10} Ci/1 radon as an average yearly value in Kalna, 7.36×10^{-10} Ci/1 for Gorenja Vas, and 7.43×10^{-10} Ci/1 for Zletovska Reka. In the control mine "Rudnik" the average value was 2.2×10^{-11} Ci/1.

2. Determination of short-lived daughters of radon.

We measured the short-lived radon daughters applying the method of Kusnetz (1956) and recalculated them in working levels (WL). The first measurements in Kalna and Gorenja Vas as well as the measurements of radon were performed together with Mr. Duncan Holaday in October 1964. The results are presented in Table 16. It is obvious that the contamination of the working environment at that time was much higher in Gorenja Vas, due to the lack of artificial ventilation in this mine.

We performed parallel measurements with membrane and "Rose" filters. The results are presented in Table 17. These results show that the measurements with membrane filters are slightly higher than with "Rose" filters due to the self-absorption of the latter. In 1968 we performed similar measurements which are presented in Tables 18, 19, and 20.

These results show that the concentrations are over maximal allowable concentrations (MAC) values. Only in operation H_1/H_2 is the situation opposite. In the future measurements of radioactivity will be performed applying the French and Kusnetz methods.

3. Determination of concentration of uranium Ra-226 and Po-210 in the dust.

In order to establish the content of the long-lived radon daughters and other radionuclides in the dusts of uranium mines, we introduced a method of determination of uranium, Ra-226 and Po-210 in the same sample. The method was developed by our French scientists.

Samples were taken continuously for 10 days on chosen spots with "Delbag" filters. In the dust were parallelly determined uranium, Ra-226 and Po-210. These studies showed that the concentration of Po-210 in the dust was about 100 times higher than Ra-226, which indicates that these two nuclides are not in an equilibrium. Po-210 is also increased due to the presence of radon daughters.

We are continuing this **study** in order to find out the relation of these radionuclides in the ore and dust, and to discuss safety regulations for these radionuclides in the occupational exposure.

4. Study on dustness in uranium and control mines

We performed a study to determine the number and size of dust particles. Samples were taken from various mines by thermoprecipitators and by gravimetric determinations of the dustness. The results showed that the greatest concentration of the dust exists in Gorenja Vas (32.7 mg/m^3), in Zletovska Reka it is only 8.6 mg/m^3 and in Kalna 6.7 mg/m^3.

The study on size distribution showed that about 50% of particles are of the size under 0.3 microns, and 80% up to 1 micron.

The number of particles was $1817/cm^3$ for Gorenja Vas, 1225 for Zletovska Reka, and 973 for Kalna. The SiO_2 concentration showed 60% of free silica.

The results suggest a high degree of dustness which could influence injuries of the respiratory system of exposed miners.

5. Study on Po-210 content of food and cigarettes

In order to evaluate the radioactive contamination of miners to Po-210 by food and smoking, we studied the concentration of Po-210 in food and cigarettes.

The results of our studies showed that the miners in the Kalna region were introducing into the body daily 16.6 pCi of Po-210 by food and 1.5 pCi by smoking. The highest content of Po-210 was in the flour (1.98 ± 0.28 pCi/100 gr) and in cigarettes (0.38 ± 0.13 pCi/gr), and tobacco 0.48 ± 0.18 pCi/gr.

From these results it is obvious that we have to keep in mind these factors when evaluating the radioactive contamination of miners.

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Note: Papers published on Research Agreement 02-002-3

Table 1

Time of Exposure in Mine

		Duration of exposure in years							
		0-3	4-6	7-9	10-12	13-15	16 and +	Unknown	Total
Number	Exposed group	340	174	155	39	21	24	12	765
	Control group	120	117	69	102	98	89	9	604
%	Exposed group	44.4	22.7	20.2	5.0	2.7	3.1	1.5	100%
	Control group	19.8	19.3	11.4	16.8	16.2	14.7	1.4	100%

Average duration of exposure - Exposed group 5.3 year
- Control group 9.1 year

Table 2

Age of Miners

		Age in Years								
		20-25	26-30	31-35	36-40	41-45	46-50	50 and +	Unknown	Total
Number	Exposed Group	17	84	208	158	111	97	66	24	765
	Control Group	- -	81	144	142	73	78	70	16	604
%	Exposed Group	2.2	10.9	27.1	20.6	14.5	12.6	8.6	3.1	100.0%
	Control Group	- -	13.4	23.8	23.5	12.0	12.9	11.5	2.6	100.0%

Average Age - Exposed Group = 38.4 year
- Control Group = 39.0 year

Table 3

Occupational Structure of Miners

		Occupation						Smoking			
		Miners	Aid of Miners	Drivers	Others	Unkown	Total	Smokers	Non Smokers	Unkown	Total
Number	Exposed Group	160	95	299	207	4	765	351	342	72	765
	Control Group	149	81	126	248	-	604	305	214	85	604
%	Exposed Group	20.9	12.4	39.0	27.0	0.5	100.0	45.8	44.7	9.4	100.0
	Control Group	24.6	13.4	20.8	41.0	-	100.0	50.4	35.4	14.0	100.0

Table 4

Respiratory Diseases, Changes in Blood Picture and Urine

Diseases	Number		Average Incidence in Percents	
	Exposed Group	Control Group	Exposed Group (765)	Control Group (604)
Pharyngitis chr.	427	253	55.8	41.8
Bronchitis chr.	40	22	5.2	3.6
Emphysema pulm.	125	122	16.3	20.4
Bronchitis + emphysema	31	57	4.0	9.4
Pneumoconiosis 0-I	112	138	14.6	22.8
Pneumoconiosis I	7	9	0.9	1.4
Pneumoconiosis I-II	4	0	0.5	-
Total	746	601	-	-
Anaemia	16	9	2.0	1.4
Leucocytosis	58	59	7.5	9.7
Leucopenia	23	11	3.0	1.8
Trombocytopenia	2	4	0.2	0.6
Lymphocytosis	30	18	3.9	2.9
Total	129	101	-	-
Albumen	5	1	0.6	0.1
Cylindres	1	0	0.1	-
Erytrocites	1	0	0.1	-
Total	7	1	-	-

Table 5

Respiratory Diseases

	Smokers	Non-Smokers	Total
Diseases, Total	305	214	519
Pharyngitis	157 51.4%	67 31.3%	224 43.1%
Bronchitis chr.	17 5.5%	4 1.8%	21 4.0%
Emphysema	75 24.5%	39 18.2%	114 21.9%
Bronchitis + Emphysema	37 12.1%	13 6.0%	50 9.6%
Pneumoconiosis 0-I	78 25.5%	45 21.0%	123 23.6%
Pneumoconiosis I	5 1.6%	2 0.9%	7 1.3%

Table 6

Sputum's Cells Classification of Experimental Group

Year	No. of Miners	Class I	Class II		
			Group 1	Group 2	Group 3
1964	161 100%	58 36%	65 40.3%	37 22.9%	1 1%
1965	222 100%	65 29.2%	69 31.8%	82 36.9%	6 2.7%
1966	140 100%	53 37.8%	60 42.8%	24 17%	3 2.1%
1967	132 100%	39 29.5%	73 55.3%	20 15.1%	
1968	125 100%	74 60%	35 28%	15 12%	1 1%
Total	780 100%	289 36.6%	302 38.2%	178 22.5%	11 1.4%

Table 7

Sputum's Cells Classification of the Control Group

Year	No. of Miners	Class I	Class II		
			Group 1	Group 2	Group 3
1965	177 100%	42 23.7%	64 36%	58 32.7%	13 7.4%
1966	170 100%	65 38.2%	61 35.8%	36 21%	8 5%
1967	156 100%	54 34.6%	72 46%	26 16.6%	4 2.5%
1968	69 100%	34 49.2%	20 29.0%	13 18.8%	2 3%
Total	572 100%	195 34%	217 38%	133 23%	27 5%

Table 8

Cytomorphologic Changes in Sputum in Dependence of Season

Year	Month	No. of Miners	Class I	Class II			Class III
				Group 1	Group 2	Group 3	
1968	June	27 100%	24 88.8%	1	1	1	
1968	November	27 100%	11 40%	10 37%	3 11%		3 11%

Table 9

Normal Values

No.	Examined Persons	imp/min/1		time/h		K_4
		A_1	A_2	t_1	t_2	
1	J.D.	93	69	2	4	0.19
2	Ž.S.	204	151	2	4	0.18
3	J.M.	130	81	2	4	0.23
4	P.D.	96	55	2	4	0.23
		72	38	3	24	- - -
Mean						0.21±0.02(SD)

Table 10

Patients Group 1

No.	Examined Patients	imp/min/1		time/h		K_4
		A_1	A_2	t_1	t_2	
1	A.V.	0.27	0.007	2	4	0.52
2	T.L.	0.27	0.10	2	4	0.37
3	R.J.	0.24	0.08	2	4	0.32
4	T.R.	0.21	0.10	2	4	0.28
5	P.Z.	0.13	0.06	2	4	0.29
6	R.S.	0.14	0.07	2	4	0.27
7	B.S.	0.18	0.07	2	4	0.35
8	D.P.	0.26	0.09	2	4	0.39
9	P.B.	0.27	0.11	2	4	0.32
10	V.V.	0.23	0.11	2	4	0.28
Mean						0.34±0.07

Table 11

Patients Group 2

No.	Examined Patients	imp/min/1		time/h		K_4
		A_1	A_2	t_1	t_2	
1	S.D.	0.22	0.07	2	4	0.48
2	M.I.	0.17	0.07	2	4	0.33
3	A.S.	0.18	0.07	2	4	0.35
4	P.C.	0.13	0.04	2	4	0.44
5	R.B.	0.18	0.07	2	4	0.35
6	M.R.	0.18	0.12	2	4	0.19
7	Dj.P.	0.24	0.09	2	4	0.36
8	J.M.	0.17	0.65	2	4	0.46
9	A.G.	0.17	0.07	2	4	0.33
Mean						0.39 ± 0.06

Table 12

Patients Group 3

No.	Examined Patients	imp/min/1		time/h		K_4
		A_1	A_2	t_1	t_2	
1	J.M.	0.24	0.09	2	4	0.36
2	T.J.	0.32	0.09	2	4	0.61
3	V.G.	0.22	0.09	2	4	0.33
4	I.D.	0.27	0.12	2	4	0.30
5	P.S.	0.24	0.08	2	4	0.32
6	M.R.	0.26	0.07	2	4	0.63
7	T.S.	0.15	0.05	2	4	0.40
8	J.D.	0.39	0.14	2	4	0.37
9	K.D.	0.24	0.08	2	4	0.32
Mean						0.40 ± 0.12

Table 13

Radon Concentrations in Uranium Mine Kalna
(1963 - 1966)

Month	1963 Median Month Values Ci/1	1964 Median Month Values Ci/1	1965 Median Month Values Ci/1	1966 Median Month Values Ci/1	Total Average Values Ci/1
January		2.77×10^{-10}	15.37×10^{-10}		
February		3.31×10^{-10}	12.73×10^{-10}		
March		3.14×10^{-10}	26.99×10^{-10}		
April		3.95×10^{-10}	23.61×10^{-10}	20.02×10^{-10}	
May	1.38×10^{-10}	2.46×10^{-10}			
June	1.58×10^{-10}	2.69×10^{-10}			
July	1.28×10^{-10}	1.72×10^{-10}			
August	1.85×10^{-10}	3.30×10^{-10}			
September	2.03×10^{-10}	2.86×10^{-10}			
October	2.02×10^{-10}	3.65×10^{-10}			
November	2.13×10^{-10}	30.99×10^{-10}	5.22×10^{-10}		
December		19.48×10^{-10}			
Median Year Value	1.75×10^{-10}	6.69×10^{-10}	19.67×10^{-10}	20.02×10^{-10}	
Min.	1.28×10^{-10}	2.46×10^{-10}	12.73×10^{-10}		7.86×10^{-10}
Max.	2.13×10^{-10}	30.99×10^{-10}	26.99×10^{-10}		

Table 14

Radon Concentrations in Uranium Mine Gorenja Vas
(1964 - 1968)

1964		1965		1966		1967		1968		Total Average Value Ci/l
Month	U 10^{-10} Ci/l	Month	U 10^{-10} Ci/l	Month	U 10^{-10} Ci/l	Month	U 10^{-10} Ci/l	Month	U 10^{-10} Ci/l	
July	9.09×10^{-10}	July Aug. Oct.	26.01×10^{-10} 10.77×10^{-10} 7.61×10^{-10}	June Nov.	2.89×10^{-10} 4.69×10^{-10}	July Sept. Nov.	4.13×10^{-10} 1.97×10^{-10} 5.44×10^{-10}	July Nov.	5.44×10^{-10} 1.87×10^{-10}	
Median Year Value	9.09×10^{-10}		14.85×10^{-10}		3.80×10^{-10}		3.84×10^{-10}		3.65×10^{-10}	
Min.			7.61×10^{-10}		2.89×10^{-10}		1.97×10^{-10}		1.87×10^{-10}	7.36×10^{-10}
Max.			26.01×10^{-10}		4.69×10^{-10}		5.44×10^{-10}		5.44×10^{-10}	

U - Uranium

Table 15

Radon Concentrations in Uranium Mine Zletovska Reka
(1965 - 1968)

1965		1966		1967		1968		Total Average Value Ci/l
Month	Ci/l	Month	Ci/l	Month	Ci/l	Month	Ci/l	
June	17.97×10^{-10}	July Nov.	3.57×10^{-10} 3.65×10^{-10}	June Nov.	5.40×10^{-10} 1.97×10^{-10}	June Nov.	22.68×10^{-10} 16.78×10^{-10}	
Median Year Value	17.97×10^{-10}		3.61×10^{-10}		3.68×10^{-10}		9.73×10^{-10}	
Min.			3.57×10^{-10}		1.97×10^{-10}		16.78×10^{-10}	7.43×10^{-10}
Max.			3.65×10^{-10}		5.40×10^{-10}		22.68×10^{-10}	

Table 16

Concentration of Short-Lived Products of Decay in Kalna (1964)

Operation	Radon pCi/l	Radon's Descendants xW.L.
C.H.	280	1.6
P.S. (tunnel)	10	In trace
R.	30	0.44
	Gorenja Vas	
1.	It was not measured	9.1
2.	It was not measured	8.4
3.	It was not measured	7.7

Table 17

Parallel Results of Measurements in Kalna (1964)

Operation	Radon pCi/l	JUNO jon. com.		DT 12	
		Membrane xW.L.	"Rose" xW.L.	Membrane xW.L.	"Rose" xW.L.
H387	2040	10.	8.55	12.5	9.5
	382	4.1	4.10	4.56	4.26
	700	5.0	4.10	5.72	4.57
H387	897	5.45	4.25	6.1	5.04
	858	0.35	5.0	6.35	6.0

Table 18

Concentrations of Short-Lived Products of Decay Gorenja Vas (1968)

Operation	Radon pCi/1	Gamma WR/h	xW.L.	W.L.M. (W.L.h.)
H27	24	30	0.08	14.56
H259	1020	1100	2.32	422.00
H2	577	150	2.41	438.00
H260	561	130	2.13	388.00
H249	320	50	1.65	300.00

Table 19

Concentrations of Short-Lived Products of Decay Zletovska Reka

Operation	Radon pCi/1	Gamma WR/h	xW.L.	W.L.M. (W.L.h.)
H76	15	30	0.02	3.64
H761	21	30	0.10	18.20
H14	740	70	4.66	850.00
H12	1820	1400 to 1700	16.00	290.00

Table 20

Concentration of Radon's Products
Gorenja Vas (1969)

Operation	Radon pCi/l	Gamma W.R./h	xW.L.	W.L.M. (W.L.h)	RaH	RaD pCi/l	RaL
H 269	78	28 to 30	0.825	150.00	34	10	7
H 271	94	30	1.07	195.00	54	13	9
H 2	78	30	1.16	212.00	19	10	9
H 267	159	60	1.935	353.00	80	20	8
H1/H2	404	1100	11.36	2.067	360	240	120

DISCUSSION AFTER DR. KILIBARDA'S PAPER:

Dr. Cralley:

Dr. Kilibarda, have you had a chance to look at the paper given at the London Conference on the subject of radon. It was reported that the exposure to radon alone and to radon with uranium ore (some radon concentrations) gave different degrees of biologic response.

Dr. Kilibarda:

Yes, I saw these reports today.

Dr. Cralley:

Mr. Holiday was at this meeting. He was very impressed with the set up of the study and the findings, and believed it's going to give valuable information. This may explain some of the differences in biological response not otherwise apparent.

Dr. Henschel:

How do your exposure levels, that you now have in your mines, compare to the permissible levels that are now in our TLV for uranium miners?

Dr. Kilibarda:

We were on many occasions in a situation to have higher concentrations above the maximal permissible level. So we could find different results in different mines and also on different working places. Comparing with French and American conditions, we had many times more concentration of radon daughters, sometimes three times more than MPL.

Dr. Henschel:

What is your opinion in connection with pulmonary diseases and incidence of cancer?

Dr. Kilibarda:

We had no evidence of cancer of the lungs, except one case. By analysis of the sputum findings up to now there is no evidence of the cancer which we can speak about. But I must say that our workers in the first years were not exposed continuously as in some other uranium mines, namely in the first year of exposure we had more experimental work in mines. Sometimes the work was stopped or the workers changed the places for some period of time. I also want to point out that we are able now to follow one big group of miners, which is now no longer exposed, because the first mine was closed seven years ago. We show some interesting results about the correlationship between Polonium in the atmosphere and Polonium in the body. I mean the situation in this case is quite different. We can say something about the body burden of Polonium for that group of workers, but we can't say the same about the correlationship between the body burden in continuously exposed groups, i.e., for the group of the workers who are still working in the uranium mine.

Dr. Djuric:

You established that determination of Polonium in the urine is not a good exposure test. Can you recommend at this moment a better test, for example, perhaps determination of radon in the breath or Polonium in the feces. What is at this moment relatively the best exposure test?

Dr. Kilibarda:

It is difficult to answer precisely, but probably excretion of Polonium 210 could be some kind of test.

Dr. Potkonjak:

I am interested in the problems of lung function. As your study has been continued, what is your opinion of studying the relationship of the lung function and absorption of radon? Rumors are heard, as far as I know from Switzerland, that the subject with impaired lung function is at an advantage because the absorption of toxic materials is lower; the lower ventilation, the lower the absorption of toxic materials. I believe it could be very interesting to try to see whether it was true.

Dr. Kilibarda:

You mean any toxic material or material which could induce cancer?

Dr. Potkonjak:

All material that enter the body by the respiratory tract.

Dr. Kilibarda:

As we said we established that the miners with changes in the respiratory tract retain radon and daughters in the lung for a longer period of time.

RESPIRATORY TRACT DISEASE OF COTTON WORKERS LIVING IN AREAS OF VARIOUS DEGREES OF AIR POLLUTION

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Introduction

It has been generally accepted that high prevalence of chronic nonspecific respiratory diseases (CNRD) - bronchitis, emphysema, byssinosis - in textile industries is due to the pathogenic effects of the dust arising from processing of cotton and/or flax. Recently, the microflora of cotton origin as a pathogenic factor in the development of those diseases has also been suggested. Nevertheless, some recent investigations (Lamers et al.) have lead to the conclusion that general air pollution may also play a synergistic role, especially in urban areas.

The main aim of this study was evaluation of the influence of air contaminants as well as of work conditions (cotton dust concentration and microflora of air, microclimat) on the prevalence of CNRD and sickness - absenteeism of textile workers, employed in two cotton mills, located in an urban and a rural district.

I. Plan of Study and Scope of Investigations

The study compares the medical records and sickness-absenteeism of workers employed in two similar cotton mills with the in-factory and general atmospheric air pollution patterns of the area over a two year period. One factory (a) was located in an urban area and the other one (b) in a rural one.

The studies encompassed preparatory departments of both mills, and the particular in-factory production halls were grouped together in accordance with the technological processes into IV comparable work-department-groups, presented in Table I.

The processing raw material in Mill A was cotton of an average quality from the Soviet Union, UAR, USA, Greece, Syria and Pakistan, and in the Mill B - cotton of an average and inferior quality from SSR, USA, and Pakistan.

According to the aim of the study, the work encompassed the following surveys: (a) atmospheric air pollution; (b) working environment; (c) medical studies of the factories population; and (d) sickness absenteeism.

II. Methods, Results and Comments

1. Atmospheric air pollutions survey.

Investigations of the atmosphere air pollution were performed in both areas during 3 whole years and comprised of:

- Monthly mean determination of SO_2 concentration, using contact method with lead oxide,
- Determination of 24 hr. - mean concentration of SO_2, using pararozaniline colorimetric method,
- Determination of dust, concentration in the air using sedimentation method.
- Determination of benzyrene concentration, by extraction of pitchy substances with benzene from dust samples, collected in glass-filters for 24 hours three times a week by Stopex standard pump.

For separation of extracted substances the chromatographic method was used. Identification of benzpyrene has been made with spectrophotometric method in ultraviolet.

Measurements of free fall of dust and determination of SO_2 concentration were conducted by means of the contact method in 30 measurement points in the town district and in 7 points in the rural area, during the three years of 1966 to 1969.

The mean monthly values of the dust fall in relation to the season of the year for two investigated areas are illustrated in Table II and Figure 1.

In Table III and Figure 2 have been respectively presented mean monthly concentrations of SO_2 for both investigated areas.

As it comes from the data presented in Table II and III and Figure 1 and 2, the air pollution in the town district was on the average twice higher than in the rural area, and that especially in the cold period of year. The concentrations of dust, and of SO_2 especially, were considerably higher in the cold period (November to March) than in the warm period (April to October.)

Similar distribution of pollution concentration with regards to localization as well as to seasonal fluctuation were obtained by measurements of benzpyrene in the atmospheric air, which were carried out during two years (1967 to 1968), Table IV, Figure 3.

Regularity in seasonal fluctuations corroborate the results for SO_2 concentration parallely conducted by colorimetric method in two measurement points in the town district during three years (Table V, and Figure 4.)

2. Working Environmental Survey

Studies of the working environment in work places of both plants comprised of:

- Permanent studies of microclimate components of temperature, humidity and air velocity, conducted throughout the whole year.

- Microflora pollution of cotton and culture diagnosis of microorganisms, isolated from the raw-material.

- Microorganisms count determination in the air of work-rooms and work-premises with identification of the kind of microorganism.

- Gravimetric determination of dust concentration, using M.S.A. electostatic dust sampler.

- Konimetric determination of dust concentration, using M.S.A. midget impinger.

- Determination of free silica content in the dust samples, using colorimetric method after Polezaew.

- Determination of a particle size distribution of dust by indirect measuring of diameter of 200-300 particles.

a. Microclimate

The investigations were carried out in a continual way during the whole year, recording the temperature of the air by means of the thermohygrographs at 1 to 3 points in each compartment. The air velocity was determined with Hill's catathermometer several times each month.

From the obtained thermohygrograms with the use of the plani metric method, mean weekly air temperatures and humidities were calculated and then, taking into consideration the mean air velocity the mean weekly effective temperature was calculated. In evaluation of microclimatic conditions the scale of effective temperatures after Yaglou with a comfort zone 17 to 22°C was used.

For comparison purposes of the data obtained from the analogical work departments, the number of days with the effective temperature over 22°C and the number of days with the effective temperature below 17°C, both taken as percentage of year, were calculated.

The results have been presented in Table VI.

It can be seen from Table VI that the factory located in rural area "B" possessed a somewhat worse microclimate deriving from higher fluctuations of its components. These differences may have been caused by location of the factories in differently congested areas and by differences of building construction.

Fluctuations of microclimate components during the whole year are presented in Table VII.

b. Microfloral Pollution of Raw Material and of the Air of Factory-rooms.

For the purpose of evaluation of the bacteriological pollution of the raw-material, 50 mg samples of cotton were washed out several times in physiological solution of NaCl. Then, 0.1 cc of solution was cultivated on agar and differential media and microscopically investigated after 24 hours and 48 hours of incubation. The number of colonies was counted and calculated for one gramme of cotton. The identification of colonies was made on the basis of Bergey's key. For each of the slides one hundred fields were investigated in order to calculate various kinds of micro-organisms, expressed in percentage.

The results are presented in Table VIII.

From the data presented in Table VIII it can be seen that the raw material processed in "B" factory was much more polluted than in factory "A". In the spectrum of microflora of "A" factory cocci were more prevalent, whereas in factory "B" a higher percentate of gram-positive bacilli and moulds were observed.

The number of micro-organisms in the air of work departments was calculated from the samples taken on the level of 1.5 m over the floor, using Chirana's aeroscope. Broth agar, broth agar enriched 3% bovine blood, Czapek-Dox and Endo-agar were used as culture media. Calculation of the number of microorganisms per 1 m^3 and the diagnosis of the colonies were made in the above described way.

The results of the bacterial air pollution according to the period of year and work-department-group has been presented in Table IX.

It can be noted that the air in factory "B" was much more polluted with microorganisms than the air in factory "A" (statistically significant differences for $p < 0.001$). No differences for the air pollution were noted in both mills with regard to the year period. At the same time it can be noted that the number of microorganisms in the air diminished with the technological advance of the processing.

The distribution of microorganisms according to the year's period and work-department-group is presented in Table X. From the comparison of Table VIII and Table X it comes out that the source of the microorganism in the air was the microflora carried on the cotton fibers. Therefore, it may also explain the much higher pollution of the air in factory "B" than in factory "A".

As shown in Table IX, participation of the floor origin microorganisms in the air pollution did not play an important role. It was not also possible to detect any infectious microorganisms in the air of the investigated factories.

Our findings of morphological structure of the air microflora of the investigated mills were similar to those described in cotton industry by other authors.

c. In-Factory Dust

Measurements of the dust concentration in the air of in-work halls of both investigated factories were conducted for three years (1966 to 1968). In total 1377 gravimetric samples and 1352 konometric samples were taken.

The obtained results were accepted as cumulative distribution functions and marked on the logarithmic probability paper in order to obtain parameters of distribution: geometric mean concentration ($\tilde{m}g$) and geometric standard deviation (g).

Determinations were also made of SiO_2 content in the gravimetric dust samples and of the geometric mean of particle size distribution. The results obtained for both investigated mills are shown in Table XI.

It can be seen from Table XI that the air pollution with dust was somewhat higher in factory "B" than in factory "A" and showed diminishing tendency in both factories together with advance of the technological process.

The investigated dust in both factories contained on the average 1.1% of free silica (maximum 5%) and did not show any significant differences in the geometric means of particle size distribution which were lower than 3 μ.

3. Medical Studies of the Factories Population

The study comprised 3,395 workers (690 males and 2705 females). Distribution of the investigated population of both mills according to sex, age, and length of service is presented in Tables XII and XIII.

Consideration of both tables shows that males comprised about 24% of total population and that the workers of mill "B" were somewhat younger than the workers of mill "A".

The medical studies encompassed: medical histories including; anamnesis and personal data recorded on the standard questionnaires, approved by Medical Council Committee on Aetiology of Bronchitis (enclosure 1), routine physical examination including, if necessary, microbiological studies of the sputum, fluorography of the chest and spirographic examinations (FVC and FEV_1).

For establishment of diagnosis of chronic non-specific respiratory diseases (CNRD), criteria according to: "Definition and Classification of Chronic Bronchitis for Clinical and Epidemiological Purposes" presented by C.H. Stuart and al. in the Lancet, pp. 775-79, 1965 were accepted.

a. Morbidity of CNRD Survey

The number of examined subjects and diagnosed cases with CNRD according to sex and with relation to the clinical form of the disease is presented in Table XIV. Owing to a too small number of cases which could have been classified as "chronic or recurrent mucopurulent bronchitis", the subjects with such diseases have been included in the group of "chronic obstructive bronchitis".

As it can be read from the data presented in Table XIV, the prevalence of CNRD among the workers in the investigated cotton textile industry amounted to 10.5% (22.6% for men, and 7.5% for women). This prevalence was higher both for men and for women in the mill located in rural areas (13.7%; 25.7% -men, 10.9% - women) than in town district (7.5%; 20.0% - men, 4.1% - women). Among diagnosed cases of CNRD in men as well as in women of both mills the prevalence of chronic bronchitis of recurrent and obstructive form was twice as large as the simple type.

The prevalence of CNRD according to age, sex and length of service in both investigated mills is shown in Tables XV, XVI, XVII, XVIII.

This prevalence rises proportionally with age and length of service in both sexes, but it shows much stronger tendency in men than in women of both mills. The above noted progression for both sexes is faster in the factory located in the rural areas.

Table XIX presents the specific incidence from CNRD and diseases of the circulatory system (percentage for a year) in relation to sex in both investigated mills. It can be noted that the incidence from CNRD was lower in men than in women of both mills, and higher for both sexes in the mill located in rural district.

From the data in Table XIX it can also be concluded that in the mill located in rural district the incidence from CNRD was four times higher than in the mill in town district. The specific incidence from circulatory diseases for both sexes showed similar trend as the incidence from CNRD in both investigated mills.

b. Lung-Function Survey

The lung-function was examined using a modified metabograph in which the speed of recording paper was about 3000 mm/min.

The examinations encompassed FVC, FEV_1 and IMBC, the latter calculated indirectly according to the formula $IMBC = FEV_{0.75} x 30$. The obtained data were recalculated with regard to BTPS. Evaluation of lungs ventilation for FVC was made using predicted values, elaborated by Bold et al., for men and by Sartorelly for women.

The results of spirographic values for men and women in both mills with relation to age are presented in Table XX. Dependency was noted of spirographic findings in accordance with age in both sexes, which were significantly diminishing after 40 years of age. Lower values for $FEV_1\%$ and IMBC were found in men working in the factory located in the rural area than in those working in town district.

Contrary findings were recorded for women.

Spirographic values for men and women with relation to length of service are given in Table XXI. It was noted that FEV_1% and IMBC for both sexes in factory "A" located in town district were significantly lower after 15 years length of service. This was not noted for FVC%. It may be explained that in the examined population the main factor deteriorating lung ventilation might have been that one which in effect led to obstruction. Lack of such an observation in the mill situated in the rural area may have been due to a younger age of the working population.

IMBC for men and women of both cotton mills with relation to work-department-group are presented in Table XXII. From the table it can be noted that in women working in both mills a significantly higher IMBC occurred among those working in departments in which working environments were the best. Such an occurrence did not exist for men population.

The results of spirographic findings in subjects with and free from CNRD for both investigated cotton mills with relation to sex are shown in Table XXIII. It can be seen that in subjects suffering from CNRD there was a stronger tendency to obstructive changes (FEV_1% decreases much more than FVC%).

Impairment of lung ventilation was lower in men working in factory "B" located in rural area. Such a difference was not found among the women of both factories.

c. Sputum Survey

Sputum survey was conducted among 240 subjects (106 men and 134 women) employed in both investigated mills.

The subjects were divided into three groups, namely "healthy", "with simple chronic bronchitis" and "with chronic recurrent and obstructive bronchitis".

The sputum was collected in Petri's plates during the work shift after washing of the mouth with a weak solution of kalium permanganate. Simple and diagnostic cultures were made not later than two hours after collection of the sputum.

A general characteristic of sputum of the examined subjects according to sex and health conditions is presented in Table XXIV.

Table XXV presents the number of subjects in whose sputum were found microorganisms which have also been found in the air of work premises and on the cotton. The microorganisms have been arranged into five diagnostic groups, in accordance with previously accepted classification.

The table shows that the sputum of cotton workers carried live microorganisms similar to those found in the air of the work premises and on the manufactured cotton. These microorganisms appeared in significantly higher proportion in the sputum of workers suffering from CNRD.

It seems that in cotton workers, participation of such microorganisms cannot be excluded from the etio-pathogenesis of CNRD.

4. Sickness-Absenteeism Survey

Studies of sickness-absenteeism in both investigated mills were conducted throughout the whole three years (1966 to 1968).

The indices of total sickness-absenteeism for the particular work-department-groups of both mills are presented in Table XXVI. The Table XXVI shows also the indices of specific sickness-absenteeism in both mills due to chronic bronchitis.

As is shown in Table XXVI, the indices of total sickness-absenteeism for both sexes were higher for the mill situated in town district. The indices of specific incidence for chronic bronchitis from the point of view of location of the mill were similar, and they showed a perceptible dropping tendency with the betterment of working conditions (proportionally to the technological progress).

The indices of total sickness-absenteeism for chronic diseases of respiratory and circulatory systems as well as for remaining diseases in both mills for subjects with diagnosed CNRD for both sexes are presented in Table XXVII.

The table shows that sickness-absenteeism of subjects with diagnosed CNRD was higher for both sexes in both mills from that in the rest of the subjects and was much higher in the mill located in town. This occurrence may have been due to a more severe course of the disease (three times longer average absenteeism) due to higher air pollution or some other precisely unknown social and economic living conditions. A detailed analysis of the absenteeism data showed that:

a. The sickness-absenteeism indices rise with age of working population and the length of service;

b. The sickness-absenteeism among women workers is higher than among men workers and shows a rising tendency parallelly to the number of children;

c. Diminishing tendency of sickness-absenteeism is noted with the increase of the pay-packet;

d. The sickness-absenteeism among smokers is higher than among non-smokers. This occurrence is especially noticeable in specific sickness-absenteeism due to diseases of the respiratory system.

5. Tobacco Smoking and CNRD

The importance of tobacco smoking in the etiology of CNRD has been reported by many authors. This noxious habit has also played a considerable role in impairment of the state of health among the workers of both investigated cotton mills. The influence of smoking on the prevalence of CNRD among workers of both mills with relation to sex is presented in Table XXVIII.

It can be noted that smoking habit had a considerable effect on the incidence of CNRD only in men and may be accepted as a main factor for higher prevalence of CNRD in men than in women. The data recorded for non-smokers showed that sex did not play any role on the level of CNRD prevalence among cotton-worker.

Correctness of the above conclusion may be corroborated by comparison of sickness-absenteeism among smokers and non-smokers which is given in Table XXIX. Sickness-absenteeism due to chronic respiratory tract diseases was higher among smokers than in non-smokers irrespective of sex and mill location and rose parallelly with the number of cigarettes. In our studies smoking habit did not show any effect on the specific absenteeism due to diseases of the circulatory system. Among men workers population smoking habit had a considerable bearing on the level of total sickness-absenteeism.

III. Discussion

The fundamental data characterizing work environment in accordance to prevalence and incidence of CNRD, spirographic values and sickness-absenteeism indices for both sexes of the working population of both investigated mills are summarized in Table XXX.

The analysis of these data permits the hypothesis that the conditions of work environment may be accepted as a main factor in the etio-pathogenesis and prevalence of CNRD. Higher indices for prevalence and incidence of CNRD in work places with the worse work conditions are present, and also higher specific indices for sickness-absenteeism from CNRD as well as spirographic values in the work departments where work environment is more polluted, explicitly speak for such a thesis.

This correlation is true not only for a mill located in rural area but also in town district.

When one compares the work environment in the mill "A" and the mill "B", the work environment in mill "B", which is located in rural area, is worse than in mill "A" located in town district. The poorer health indices for the population of the mill "B" strongly support the thesis that poorer work envionment conditions play a paramount role in development of CNRD among the cotton workers.

The atmospheric air pollution (dust, SO_2 cyclic hydrocarbons) which was of three times higher in urban than in rural district did not have any additional effect on the development (morbidity) of CNRD in cotton workers living in that district.

It could be assumed that the concentration of pollution had been too low to have an important effect in comparison with the degree of pollution of the air in the work environment. On the other hand it may be assumed, based on the longer mean time of sickness-absenteeism due to CNRD of cotton workers domiciled in urban district, that the atmospheric air pollution may have had an additional effect on the course of the disease (more frequent occurrences and more prolonged time of exacerbation of the disease).

From these studies it may be concluded that the main pathological factors in development of CNRD in the cotton industry are high concentration of dust and microorganisms in the air of work environment. Prevalence of CNRD correlated with concentration of these elements in the air of the work rooms, as well as, the results of bacteriological examinations of the sputum corroborates this conclusion. The factor of sex in the development of CNRD does not presumably play an important role as based on the comparison of prevalence data and spirographic values for men and women of non-smoking population.

It must be accepted that tobacco smoking is an essential etiological factor augmenting the prevalence of CNRD among cotton workers. It can be proved by the high difference of prevalence of CNRD and specific indices of sickness-absenteeism between smoking and non-smoking men and increase of these components with the increase of the number of the smoked cigarettes.

The results of the study show that no typical cases of byssinosis were found among the examined population. In the majority of complaints given by the examined workers such as chest pain, tightness and breathlessness which could be regarded as symptoms of byssinotic origin were in fact symptoms of neurotic and cardiac derivation and had no relation with the specific day of the week.

Lack of incidence of byssinosis in the Polish cotton textile industry may be commented, as follows:

a. Six day's work-week, as opposed to the five day week in Anglo-Saxon countries does not give cyclic 2 day's break of work in the form of longer week-ends;

b. Great absorptive power of the labor-market provides an ample opportunity for an easy change of job. That may be why the cotton workers often leave the job, and especially after the first few years of work. The cause may also lie in the fact that those workers who begin to feel the very first symptoms typical to byssinosis (pains in the chest, tightness and breathlessness) leave their jobs in the cotton industry and move to other employment. The assumptions are even more strongly grounded considering that textile workers wages lie at the bottom limit of the average earnings in other branches of the industry.

c. Industrial medical service should be given its role for initial and periodic medical examinations of the candidates and workers. The results are that individuals with poor health are not accepted as new workers and workers with suspected symptoms are shifted to less exposed work departments.

IV. Conclusion

1. Morbidity due to CNRD among the cotton workers depends on the level of concentration of dust and micro-organisms polluting the air in the work environment, irrespective of the location of the factory (town or rural area).

2. Degree of the atmospheric air pollution of the area in which the investigated cotton workers live does not have a significant influence of the increase of prevalence due to CNRD in the cotton mill located in that area.

3. It may be assumed that the atmospheric air pollution may have some effect on the course and duration of CNRD (more frequent occurrences and more prolonged time of exacerbation of the disease).

4. Tobacco smoking plays a paramount role in the development of CNRD in workers of the cotton industry, irrespective of working and living conditions and factory location (town or rural area).

The following participants cooperated in the studies:

Medical Examinations

H. Kuński, M.D., K. Stepien, B.M., A. Maciejewska, B.M., L. Kozłowska, B.M., T. Hankowski, B.M.

Atmospheric Air Pollution and Working Environmental Survey

I. Kesy-Dabrowska, Ph.D., J. Adamiak, M.Sc., J. Swiatczak, M.Sc., E. Wiecek, M.Sc.

Bacteriological Survey

J. Przyłecka, Ph.D.

Sickness-Absenteeism Survey

R. Machnikowski, B.M., G. Sitkiewicz, B.M.

Statistical Elaboration

Cz. Andryszek, M.A.

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Table I

Comparable Work-Department Groups for Two Investigated Cotton Mills.

Work Department Group	COD
Opening Blending Picking Mixing	1
Picking Mixing Carding	2
Drawing	3
Spinning	4

Table II

Mean Monthly Values of Dust Fall in Relation to the Season of the Year From Two Investigated Areas. (Data for 3 Years: 1966-1969)

	Factory A		Factory B	
Month	No. of Samples	Mean Conc. $g/m^2/day$	No. of Samples	Mean Conc. $g/m^2/day$
January	186	0.59	21	0.54
February	178	0.56	18	0.34
March	192	0.70	21	0.53
April	136	0.66	14	0.64
May	136	0.68	13	0.50
June	129	0.82	13	0.75
July	133	0.63	14	0.70
August	132	0.77	20	0.64
September	132	0.57	21	0.69
October	131	0.98	18	0.27
November	125	0.71	18	0.27
December	154	0.49	18	0.36

Table III

Mean Monthly Concentration of SO_2 in the Air in Relation to the Season of the Year for Two Investigated Areas. (Contact Method: Data for 3 Years: 1966-1969)

	Factory A		Factory B	
Month	No. of Samples	Mean Conc. $mg/100\ cm^2 PbO_2$	No. of Samples	Mean Conc. $mg/100cm^2 PbO_2$
January	80	1.37	20	0.71
February	79	1.14	20	0.69
March	76	0.90	21	0.57
April	50	0.69	13	0.40
May	55	0.42	13	0.20
June	55	0.34	14	0.16
July	55	0.35	14	0.14
August	76	0.31	21	0.11
September	80	0.38	21	0.16
October	76	0.62	20	0.28
November	78	0.91	18	0.53
December	82	1.08	20	0.64

Table IV

Results of the Benzo-a-pyren Concentration in the Air, in Two Investigated areas. Data for 2 Years (1967-1968).

Month	Capacity of the Filtrated air m^3	Total Dust Contents mg.	Total Pitch Components contents (percent)	Benza-a-pyren Contents ug/1000 m^3	Factory
January	18902	2527.8	27.2	47.5	
February	15262	2035.4	31.0	54.0	
March	16481	1867.6	18.3	29.7	
April	23557	2497.0	12.8	20.0	
May	26512	2215.2	12.4	9.7	
June	19585	1293.6	11.2	8.7	A
July	20496	1350.6	11.3	6.8	
August	19858	1189.2	10.8	8.6	
September	23265	1791.6	11.4	9,8	
October	18628	1705.8	18.8	25.9	
November	17714	1765.0	25.7	45.1	
December	15737	1889.6	24.9	35.1	
Jan., Feb., Mar.	3493	-	-	11.3	B
May, June, July	8046	534.4	7.5	2.6	

Table V

Mean 24 Hrs. Concentration of SO_2 for Two Measuring Points in the Town District, Colorimetric Method. Data for 3 Years: 1966-1969

Month	1st Measuring Point No. of Samples	1st Measuring Point Mean Concen. $\mu g/m^3$	2nd Measuring Point No. of Samples	2nd Measuring Point Mean Concen. $\mu g/m^3$
January	73	111.8	90	116.40
February	73	93.7	82	72.73
March	88	79.8	90	49.11
April	53	31.1	49	23.51
May	53	14.1	46	9.84
June	58	10.1	44	7.02
July	75	15.9	77	7.56
August	74	11.5	66	9.49
September	84	17.7	85	12.88
October	92	31.9	91	34.46
November	87	48.4	90	62.96
December	79	104.2	81	114.54

Table VI

Microclimate Condition in Investigated Cotton Mills.

Factory	Number of Days w/Effective Temp. Over 22°C (as % of Year)	Mean Effective Temperature (in°C)	No. of Days w/Effective Temp. below 17°C (as % of Year)	No. of Days Outside of the Comfort Zone (as % of Year)
A	18.6	23.2	0	18.6
B	26.2	23.8	6.5	32.7

Table VII

Fluctuation of Microclimate Components During a Year in the Investigated Cotton Mills.

Microclimate Components	FACTORY A	FACTORY B
Air Temperature (°C)	14.0 - 33.0	7.5 - 36.0
Relative Humidity %	40 - 70	38 - 100
Air Velocity m/sec	0.3 - 0.6	0.2 - 0.9

Table VIII

Bacterial Pollution of Raw Material and Kind of Microorganisms in Two Investigated Cotton Mills.

FACTORY	Mean No. of Microorganisms in 1 gr of Raw Material ± st. Dev. in thousands	KIND OF MICROORGANISMS IN %					No. of Samples
		Gram-Postive Bacilli	Gram-Negative Bacilli	Cocci	Moulds	Actinomycetales	
A	3,674 ±8,590	14.2	31.2	47.1	5.2	2.3	248
B	39,540 ±257,775	33.3	30.4	21.5	12.0	2.8	116

Table IX

Bacterial-Air Pollution in the Investigated Cotton Mills According to Year-Period and Work-Department Group.

Period of the Year	Factory	No. of Samples	Mean No. of Microorganisms in/m^3 of air ± St. Dev. (in thousands)	Distribution of Bacterial-air-pollutions According to Work-department-group Mean No. of Microorganisms in/m^3 of air ± St. Dev. (In thousands)				Participation of Floor Origin Microorganisms (in %)
				COD				
				1	2	3	4	
COLD	A	442	18.55 ±19.72	21.08 ±26.46	81.42 ±19.08	14.04 ±25.04	4.19 ±11.89	0.4 - 1.4
	B	420	77.30 ±54.55	102.01 ±60.25	105.88 ±43.25	65.65 ±40.87	31.25 ±36.41	0.1 - 0.3
WARM	A	372	14.36 ±15.32	27.06 ±30.46	32.13 ±10.58	18.00 ±9.60	7.48 ±34.40	0.6 - 1.1
	B	149	59.89 ±92.51	77.66 ±24.70	83.17 ±17.48	85.03 ±27.99	35.14 ±10.95	0.1 - 0.5

Table X

Distribution of Microorganism in Percentage According to Year-Period and Work-Department-Group, in Both Cotton Mills.

Period of Year	COLD								WARM							
Factory	A				B				A				B			
Work-Department Group (COD)	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Gram-Positive Bacilli	28.7	56.4	47.0	79.9	47.0	45.0	59.7	50.7	58.5	62.4	74.4	58.5	75.0	69.4	61.0	64.3
Gram-Negative Bacilli	44.1	20.2	33.6	6.1	32.0	17.0	23.0	24.6	15.2	19.1	11.2	27.0	11.8	18.2	19.8	25.7
Cocci	1.3	5.6	2.5	8.3	0.8	26.0	0.9	4.4	2.8	1.3	3.4	5.0	1.7	2.1	2.5	1.7
Moulds	25.7	16.5	16.1	5.0	19.2	11.0	15.1	19.5	21.8	14.6	10.0	8.9	9.7	9.1	14.0	7.0
Actinomycetales	0.2	1.3	0.8	0.7	1.0	1.0	1.3	0.8	1.7	2.6	1.0	0.6	1.8	1.2	2.7	1.3

Table XI

Geometric Means of Dust Concentration (Gravimetric and Konimetric) ± Geometric St. Dev. No. of Samples in Brackets and Mean Values of SO_2 in % and Particle Size in µ for Two Investigated Cotton Mills.

	FACTORY A			FACTORY B		
Work Department Group (COD)	Dust Concentration: Gravimetric mg/m^3	Dust Concentration: Konimetric No. of Particles/cm^3	Mean Values of SO_2 in % and Particle Size in µ	Dust Concentration: Gravimetric mg/m^3	Dust Concentration: Konimetric No. of Particles/cm^3	Mean Values of SiO_2 in % and Particle Size in µ
1	4.3 ±0.36 (54)	1,700 ±0.24 (54)	1.35 2.9	5.2 ±0.34 (146)	3,000 ±0.22 (145)	1.01 2.9
2	5.6 ±0.25 (257)	2,050 ±0.30 (244)	1.16 2.3	5.2 ±0.27 (186)	2,600 ±0.26 (183)	1.14 2.8
3	3.2 ±0.30 (63)	2,100 ±0.30 (63)	1.15 2.2	4.2 ±0.21 (54)	2,350 ±0.20 (54)	1.00 2.5
4	2.5 ±0.27 (330)	1,700 ±0.28 (328)	1.16 2.3	3.1 ±0.23 (278)	1,800 ±0.23 (281)	1.20 2.5

Table XII

Distribution of the Investigated Subjects According to Sex and Age.

Age Group	A MEN No. of Cases	%	A WOMEN No. of Cases	%	A TOTAL No. of Cases	%	B MEN No. of Cases	%	B WOMEN No. of Cases	%	B TOTAL No. of Cases	%
-19	9	2.4	69	5.0	78	4.5	1	0.3	6	0.4	7	0.4
20-29	36	9.7	262	19.2	298	17.1	30	9.4	476	35.6	506	30.5
30-39	98	26.4	414	30.3	512	29.5	157	49.2	602	45.0	759	45.8
40-49	115	31.1	322	23.6	437	25.1	80	25.1	193	14.4	273	16.5
50-59	78	21.0	235	17.2	313	18.0	32	10.0	49	3.7	81	4.9
60-	35	9.4	65	4.7	100	5.8	19	6.0	12	0.9	31	1.9
Total	371	100.0	1,367	100.0	1,738	100.0	319	100.0	1,338	100.0	1,657	100.0

A = Cotton Mill in Town District

B = Cotton Mill in Rural District

Table XIII

Distribution of the Investigated Subjects According to Sex and Length of Occupation.

Length of Occupation (Yrs.)	A MEN No. of Cases	%	A WOMEN No. of Cases	%	A TOTAL No. of Cases	%	B MEN No. of Cases	%	B WOMEN No. of Cases	%	B TOTAL No. of Cases	%
-4	51	13.8	214	15.7	265	15.2	59	18.5	371	27.7	430	26.0
5-9	58	15.6	241	17.6	299	17.2	69	21.6	553	41.3	622	37.6
10-14	50	13.5	240	17.6	290	16.7	183	57.4	404	30.2	587	35.4
15-19	105	28.3	394	28.8	499	28.7	6	1.9	10	0.8	16	1.0
20-	107	28.8	278	20.3	385	22.2	2	0.6	-	-	2	0.0
TOTAL	371	100.0	1,367	100.0	1,738	100.0	319	100.0	1,338	100.0	1,657	100.0

Table XIV

Number of Investigated and Number of Diagnosed Cases of CNRD with Relation to the Clinical Form.

Investigated Mills	A						B						A + B					
Sex and Number of Cases	Men 371		Women 1,367		Total 1,738		Men 319		Women 1,338		Total 1,657		Men 690		Women 2,705		Total 3.395	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Chronic Bronchitis (Simple)	28	7.5	12	0.9	40	2.3	16	5.0	49	3.7	65	3.9	44	6.4	61	2.2	105	3.1
Chronic Bronchitis (Recurrent and Obstructive)	46	12.5	44	3.2	90	5.2	66	20.7	97	7.2	163	9.8	112	16.2	141	5.3	253	7.4
TOTAL	74	20.0	56	4.1	130	7.5	82	25.7	146	10.9	228	13.7	156	22.6	202	7.5	358	10.5

Table XV

Prevalence of CNRD for Both Sexes with Relation to Age in Cotton Mill. (A) Located in Town District.

Age	No. of Subjects	Chronic Bronchitis Total No.	Total %	Simple No.	Simple %	R&O* No.	R&O* %
		TOTAL					
-19	78	-	-	-	-	-	-
20-29	298	5	1.68	1	0.33	4	1.34
30-39	512	30	5.86	10	1.95	20	3.91
40-49	437	37	8.60	18	4.18	19	4.41
50-59	313	42	13.42	9	2.87	33	10.54
60-	100	16	16.00	2	2.00	14	14.00
TOTAL	1,738	130	7.51	40	2.31	90	5.20
		MEN Chronic Bronchitis					
-19	9	-	-	-	-	-	-
20-29	36	2	5.56	-	-	2	5.56
30-39	98	12	12.24	7	7.14	5	5.10
40-49	115	20	17.70	13	11.50	7	6.19
50-59	78	26	33.33	6	7.69	20	25.64
60-	35	14	40.00	2	5.71	12	34.28
TOTAL	371	74	20.00	28	7.59	46	12.47
		WOMEN Chronic Bronchitis					
-19	69	-	-	-	-	-	-
20-29	262	3	1.14	1	0.38	2	0.76
30-39	414	18	4.35	3	0.72	15	3.62
40-49	322	17	5.34	5	1.57	12	3.77
50-59	235	16	6.81	3	1.28	13	5.53
60-	65	2	3.08	-	-	2	3.08
TOTAL	1,367	56	4.11	12	0.88	44	3.23

* R & O = Recurrent and Obstructive

Table XVI

Prevalence of CNRD for Both Sexes with Relation to Age in Cotton Mill. (B) Located in Rural Area.

Age	No. of Subjects	Chronic Bronchitis Total No.	Total %	Simple No.	Simple %	R&O* No.	R&O* %
		TOTAL					
-19	7	-	-	-	-	-	-
20-29	506	30	5.93	13	2.57	17	3.36
30-39	759	99	13.04	33	4.35	66	8.70
40-49	273	57	20.88	12	4.40	45	16.48
50-59	81	27	33.33	3	3.70	24	29.63
60-	31	15	48.38	4	12.90	11	35.48
TOTAL	1,657	228	13.76	65	3.92	163	9.84
		MEN Chronic Bronchitis					
-19	1	-	-	-	-	-	-
20-29	30	2	6.67	1	3.33	1	3.33
30-39	157	33	21.02	8	5.10	25	15.92
40-49	80	26	32.50	4	5.00	22	27.50
50-59	32	12	37.50	2	6.25	10	31.25
60-	19	9	47.37	1	5.26	8	42.10
TOTAL	319	82	25.71	16	5.01	66	20.69
		WOMEN Chronic Bronchitis					
-19	6	-	-	-	-	-	-
20-29	476	28	5.88	12	2.52	16	3.36
30-39	602	66	10.96	25	4.15	41	6.81
40-49	193	31	16.06	8	4.14	23	11.92
50-59	49	15	30.61	1	2.04	14	28.57
60-	12	6	50.00	3	25.00	3	25.00
TOTAL	1,338	146	10.91	49	3.66	97	7.25

* R & O = Recurrent and Obstructive

Table XVII

Prevalence of CNRD for Both Sexes with Relation to Length of Service in Cotton Mill.
(A) Located in town district.

Length of Service (years)	No. of Subjects	Chronic Bronchitis Total No.	%	Simple No.	%	R&O* No.	%
		TOTAL					
-4	265	7	2.73	3	1.13	4	1.51
5-9	299	16	5.35	4	1.34	12	4.01
10-14	290	19	6.55	6	2.07	13	4.48
15-19	499	34	6.81	16	3.21	18	3.61
20-	385	54	14.03	11	2.86	43	11.17
TOTAL	1,738	130	7.51	40	2.30	90	5.18
		MEN Chronic Bronchitis					
-4	51	6	11.76	3	5.88	3	5.88
5-9	58	9	15.52	2	3.45	7	12.07
10-14	50	7	14.00	4	8.00	3	6.00
15-19	105	18	17.14	12	11.43	6	5.71
20-	107	34	31.77	7	6.54	27	25.23
TOTAL	371	74	20.00	28	7.55	46	12.40
		WOMEN Chronic Bronchitis					
-4	214	1	0.47	-	-	1	0.47
5-9	241	7	2.90	2	0.83	5	2.07
10-14	240	12	5.00	2	0.83	10	4.17
15-19	394	16	4.06	4	1.01	12	3.04
20-	278	20	7.19	4	1.44	16	5.75
TOTAL	1,367	56	4.10	12	0.88	44	3.22

* R & O = Recurrent and Obstructive

Table XVIII

Prevalence of CNRD for Both Sexes with Relation to Length of Service in Cotton Mill (B) Located in Rural Area.

Length of Service (Years)	No. of Subjects	TOTAL Chronic Bronchitis Total No.	%	Simple No.	%	R&O* No.	%
-4	430	29	6.74	10	2.32	19	4.42
5-9	622	82	13.18	23	3.70	59	9.48
10-14	587	112	19.08	30	5.11	82	13.97
15-19	16	4	25.00	2	12.50	2	12.50
20-	2	1	50.00	-	0	1	0
TOTAL	1657	228	13.76	65	3.92	163	9.84
		MEN Chronic Bronchitis					
-4	59	5	8.47	1	1.69	4	6.78
5-9	69	18	26.08	2	2.90	16	23.18
10-14	183	56	30.60	12	6.56	44	24.04
15-19	6	2	33.33	1	16.67	1	16.67
20-	2	1	50.00	-	-	1	50.00
TOTAL	319	82	25.70	16	5.01	66	20.69
		WOMEN Chronic Bronchitis					
-4	371	24	6.47	9	2.42	15	4.04
5-9	553	64	11.57	21	3.80	43	7.78
10-14	404	56	13.86	18	4.46	38	9.40
15-19	10	2	20.00	1	10.00	1	10.00
20-	-	-	-	-	-	-	-
TOTAL	1338	146	10.91	49	3.66	97	7.25

* R & O = Recurrent and Obstructive

Table XIX

Specific Incidence Indices in Percentage for the Year in Relation to Sex in Both Investigated Cotton Mills

Factory	Sex	Specific Incidence Indices (in %) For CNRD	Specific Incidence Indices (in %) For Circulatory System Disease
A	Men	0.2	0.7
	Women	0.3	1.0
	Total	0.3	0.9
B	Men	1.3	0.8
	Women	1.4	2.3
	Total	1.4	1.7

Table XX

Spirographic Values for Men and Women in Both Investigated Cotton Mills with Relation to Age

Factory	Sex	Spirographic Values	Age Groups (in years) 20 - 29	30 - 39	40 - 49	50 -
A	Men	VC %	95.3 ± 11.2	91.4 ± 12.3	87.9 ± 12.9	83.8 ± 12.1
		FEV_1 %	78.3 ± 12.1	74.9 ± 11.5	67.1 ± 12.5	59.3 ± 13.2
		IMBC	123.5 ± 21.7	108.6 ± 20.8	95.0 ± 15.4	78.2 ± 19.5
	Women	VC %	86.2 ± 11.2	88.1 ± 12.4	84.7 ± 13.5	80.9 ± 11.3
		FEV_1 %	71.7 ± 11.9	70.1 ± 12.3	66.7 ± 13.0	62.4 ± 13.9
		IMBC	81.0 ± 14.0	75.1 ± 14.3	68.8 ± 13.9	58.4 ± 13.6
B	Men	VC %	91.3 ± 14.9	90.1 ± 14.7	82.9 ± 18.6	81.2 ± 16.8
		FEV_1 %	74.3 ± 13.0	70.7 ± 15.5	62.1 ± 13.3	59.4 ± 16.3
		IMBC	109.1 ± 24.4	104.2 ± 23.8	89.4 ± 25.5	75.1 ± 21.3
	Women	VC %	87.8 ± 12.2	89.0 ± 12.8	88.1 ± 15.5	84.8 ± 15.8
		FEV_1 %	73.6 ± 13.6	73.1 ± 13.7	64.4 ± 15.0	65.9 ± 15.6
		IMBC	83.8 ± 16.2	80.2 ± 15.3	72.1 ± 15.7	65.0 ± 10.3

1) VC % In men as percentage of predicted values according to Bolt et al.
In women as percentage of predicted values according to Sartorelli et al.
2) FEV_1 % -As percentage of actual VC
3) IMBC -$FEV_{0.75 \times 30}$

Table XXI

Spirographic Values for Men and Women in Both Investigated Cotton Mills with Relation to Length of Service

Factory	Sex	Spirographic Values	Length of Service (in years) 0 - 4	5 - 9	10 - 14	15 -
A	Men	VC %	91.7 ± 11.9	89.4 ± 11.7	91.7 ± 14.3	87.0 ± 12.6
		FEV_1 %	74.3 ± 12.0	69.8 ± 14.4	74.4 ± 13.9	64.7 ± 13.4
		IMBC	108.4 ± 25.0	102.3 ± 27.6	105.9 ± 25.0	89.8 ± 21.0
	Women	VC %	85.1 ± 11.0	85.1 ± 12.8	87.5 ± 12.6	84.5 ± 14.2
		FEV_1 %	71.1 ± 12.4	69.9 ± 12.7	69.6 ± 12.4	65.9 ± 13.4
		IMBC	81.5 ± 14.9	76.5 ± 15.1	73.6 ± 15.0	66.2 ± 15.1
B	Men	VC %	89.6 ± 18.1	87.0 ± 15.4	86.1 ± 16.8	86.7 ± 9.4
		FEV_1 %	70.0 ± 17.2	65.0 ± 15.9	66.9 ± 16.8	66.7 ± 13.3
			99.6 ± 24.5	90.7 ± 21.8	97.7 ± 28.3	82.5 ± 24.4
	Women	VC %	87.8 ± 13.0	88.2 ± 12.9	88.7 ± 13.9	87.2 ± 7.8
		FEV_1 %	73.8 ± 13.8	71.6 ± 14.1	72.3 ± 14.4	73.9 ± 8.8
		IMBC	82.9 ± 23.0	78.3 ± 15.7	78.6 ± 16.8	75.5 ± 10.1

1) VC % In men as percentage of predicted values according to Bolt et al.
In women as percentage of predicted values according to Sartorelli et al.

2) FEV_1 % -As percentage of actual VC

3) IMBC -$FEV_{0.75} \times 30$

Table XXII
IMBC Values for Men and Women in Both Investigated Cotton Mills with Relation to Work-Department Group

Factory	Sex	Work-Department Group (COD) 1	2	3	4
A	Men	100.0 ± 24.3	91.7 ± 25.2	81.0 ± 23.9	97.8 ± 22.9
	Women	67.1 ± 17.9	65.1 ± 16.7	66.9 ± 13.7	74.1 ± 15.4
B	Men	93.9 ± 24.1	96.0 ± 27.9	97.5 ± 36.2	97.6 ± 26.6
	Women	78.5 ± 20.9	73.3 ± 16.7	77.9 ± 15.4	80.3 ± 16.3

Table XXIII

Spirographic Values in Subjects With and Free From CNRD in Both Investigated Cotton Mills with Relation to Sex

Factory	Sex	VC %		FEV_1 %		IMBC	
		Free From CNRD	With CNRD	Free From CNRD	With CNRD	Free From CNRD	With CNRD
A	Men	89.7 ± 12.4	83.4 ± 13.1	77.0 ± 17.2	66.8 ± 16.8	99.7 ± 23.7	82.5 ± 22.1
	Women	85.4 ± 13.2	81.0 ± 14.8	74.0 ± 17.7	67.1 ± 19.5	71.6 ± 15.9	64.2 ± 19.7
B	Men	89.2 ± 16.2	80.7 ± 16.0	69.2 ± 16.5	61.1 ± 15.5	98.9 ± 26.5	88.2 ± 24.2
	Women	88.5 ± 13.1	85.9 ± 13.8	73.0 ± 14.1	67.7 ± 12.9	80.6 ± 16.4	72.3 ± 15.2

Table XXIV

Character of Sputum of Investigated Cotton Workers with Relation to Sex and Health Conditions

	Men										Women									
	Investi-gated Subjects		Sputum								Investi-gated Subjects		Sputum							
			Muco-purulent		Muciform		Saliva		Other				Muco-purulent		Muciform		Saliva		Other	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Healthy	30	100	2	7	19	63	3	10	6	20	76	100	2	3	45	59	21	28	8	10
With Chronic Bronchitis (Simple)	52	100	7	13	39	75	3	6	3	6	35	100	2	6	27	77	5	14	1	3
With Chronic Bronchitis (Recurrent & Obstructive)	24	100	5	21	15	63	2	8	2	8	23	100	6	26	13	57	4	17	-	-

Table XXV

Distribution of Subjects with Relation to the Kind of Microorganisms Found in Sputum and Health Conditions

		Total Number of Inves-tigated Subjects		Without Micro-organisms		Subjects with Relation to Kind of Microorganisms Found in Sputum: Cocci		Gram-negative Bacilli		Gram-positive Bacilli		Acino-mycetales		Moulds		(x)
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Men	Healthy	30	100	20	67	2	7	4	13	6	20	1	3	1	3	0.7
Men	With Chronic Bronchitis (Simple)	52	100	5	10	26	50	21	40	10	19	3	6	-	-	1.2
Men	With Chronic Bronchitis (Recurrent & Obstructive)	24	100	5	21	12	50	12	50	9	37	1	4	4	17	2.0
Women	Healthy	76	100	55	72	5	7	8	10	6	8	1	1	5	7	1.2
Women	With Chronic Bronchitis (Simple)	35	100	4	11	21	60	16	46	5	14	1	3	3	9	1.5
Women	With Chronic Bronchitis (Recurrent & Obstructive)	23	100	2	9	14	61	8	35	8	35	1	4	4	17	1.7

(x) -Number of microorganism - groups (above-mentioned for one person)

Table XXVI

Sickness-Absenteeism for Men and Women in Both Investigated Cotton Mills with Relation to Work-Department Groups.

Work Department Group (COD)	Factory A Absenteeism Indices Men	Women	Total	(x)	Factory B Absenteeism Indices Men	Women	Total	(x)
1	4.27	6.46	4.97	0.29	4.13	3.03	3.85	0.15
2	5.32	6.18	5.92	0.14	3.68	3.69	3.69	0.18
3	5.85	6.18	6.16	0.08	2.02	4.53	4.49	0.08
4	4.55	5.06	4.99	0.01	2.72	3.03	2.94	0.06
Total	4.70	5.40	5.25	0.12	2.95	3.15	3.09	0.07

(x) - percentage rate for chronic bronchitis (for both sexes)

Table XXVII

Sickness-Absenteeism Indices in Cotton Workers Employed in Two Investigated Mills Relation to Sex and Diagnosed CNRD.

FACTORY	Cause of sickness-absenteism	Grand Total					Men					Women				
		Chronic Bronchitis			Total		Chronic Bronchitis			Total		Chronic Bronchitis			Total	
		Total	Simple	Recurrent and Obstructive	With chr. bronchitis	Free from bronchitis	Total	Simple	Recurrent and Obstructive	With chr. bronchitis	Free from bronchitis	Total	Simple	Recurrent and Obstructive	With chr. bronchitis	Free from bronchitis
A	Chronic respiratory tract diseases *	0.89	0.77	3.04	2.32	0.77	1.05	0.81	3.22	2.34	0.73	0.84	0.70	2.84	2.28	0.78
	Remaining other diseases	4.33	5.24	5.56	5.46.	4.24	3.75	5.80	3.76	4.50	3.57	4.49	4.19	7.67	6.76	4.40
	TOTAL	5.22	6.01	8.60	7.78	5.01	4.80	6.61	6.98	6.84	4.30	5.33	4.89	10.51	9.04	5.18
B	Chronic respiratory tract diseases *	0.41	0.67	0.70	0.69	0.36	0.44	0.68	0.61	0.63	0.38	0.40	0.66	0.75	0.72	0.36
	Remaining other diseases	2.83	3.60	3.71	3.68	2.80	2.75	2.91	2.91	2.91	2.71	2.96	3.83	4.18	4.06	2.82
	TOTAL	3.34	4.27	4.41	4.37	3.16	3.19	3.59	3.52	3.54	3.09	3.36	4.49	4.93	4.78	3.18

* According to International Classification of Diseases.

Table XXVIII

Influence of Tobacco-smoking on Prevalence of CNRD in Cotton Workers with Relation to Sex

Sex	Men			Women		
	Number of Investigated Subjects	Number of Cases With CNRD	Prevalence of CNRD (in percent)	Number of Investigated Subjects	Number of Cases With CNRD	Prevalence of CNRD (in percent)
Smokers	513 (80.5%)	164 (91.6%)	(1) 32.0	225 (8.9%)	34 (10.9%)	(2) 15.1
Non Smokers	124 (19.5%)	15 (8.4%)	(1) 12.1	2305 (91.1%)	278 (89.1%)	(2) 12.1
Total	637 (100%)	179 (100%)	(x) 16.7	2530 (100%)	312 (100%)	(x) 12.8

(x) Standarized according to smoking distribution in total population (difference statistically significant; ($p<0.001$)

(1) Difference statistically significant ($p<0.001$)

(2) Difference statistically not significant ($p>0.005$)

Table XXIX

Sickness-Absenteeism Indices in Cotton Workers Employed in Two Investigated Mills with Relation to Sex and Smoking Habit.

FACTORY	Cause of sickness absenteism	Grand Total					Men					Women				
		Total	Non Smokers	Smokers (total)	Smokers smoking cigarettes — Nr		Total	Non Smokers	Smokers (total)	Smokers smoking cigarettes — Nr		Total	Non Smokers	Smokers (total)	Smokers smoking cigaretts — Nr	
					1 - 14	15 -				1 - 14	15 -				1 - 14	15-
A	Chronic respiratory tract diseases *	0.89	0.70	1.48	1.30	1.65	1.05	0.50	1.22	0.94	1.40	0.84	0.72	2.07	1.76	2.92
A	Circulatory system diseases*	0.48	0.54	0.29	0.12	0.47	0.44	0.61	0.39	0.16	0.54	0.49	0.54	0.08	0.08	0.08
A	Remaining other diseases	3.85	3.76	4.12	4.80	3.44	3.31	3.37	3.29	3.25	3.32	4.00	3.79	6.03	6.77	4.05
A	TOTAL	5.22	5.00	5.89	6.22	5.56	4.80	4.48	4.90	4.35	5.26	5.33	5.05	8.18	8.61	7.05
B	Chronic respiratory tract diseases *	0.41	0.39	0.46	0.50	0.43	0.44	0.34	0.47	0.60	0.42	0.40	0.40	0.42	0.40	0.49
B	Circulatory system diseases*	0.12	0.13	0.13	0.12	0.14	0.11	0.07	0.13	0.06	0.15	0.13	0.13	0.15	0.18	0.03
B	Remaining other diseases	2.80	2.72	3.07	3.39	2.84	2.64	2.32	2.74	3.00	2.64	2.83	2.75	3.92	3.76	4.48
B	TOTAL	3.33	3.24	3.66	4.01	3.41	3.19	2.13	3.34	3.66	3.21	3.36	3.28	4.49	4.34	5.00

* According to International Classification of Diseases.

Table XXX

Summarized Data Characterizing Work Environment with Relation to Prevalence and Incidence of CNRD. Spirographic Values and Sickness-Absenteeism Indices for Both Investigated Cotton Mills.

Factory	Work Dept. Group (COD)	No. of Particles in cm^3	Dust Conc. mg/m^3	Mean Nr. of Microorganism in m^3	Prevalence of CNRD (x)	MEN VC% $\bar{x}\pm ox$	MEN FEV_1% $\bar{x}\pm ox$	MEN IMBC* $\bar{x}=ox$	WOMEN VC% $\bar{x}=ox$	WOMEN FEV_1% $\bar{x}=ox$	WOMEN IMBC* $\bar{x}\pm ox$	Incidence of CNRD	Specific Absenteeism Index for CNRD
A	1	1,700	4.3	27,060	15.3	90±12	70±14	100±24	80±14	66±13	67±18	0.3	0.29
	2	2,050	5.6	32,130	8.2	85±14	64±14	92±25	82±13	64±14	65±17	0.5	0.14
	3	2,100	3.2	18,000	5.4	81±16	62±16	81±24	83±14	65±13	67±14	0.3	0.08
	4	1,700	2.5	7,480	7.0	91±12	70±13	98±23	87±13	69±13	74±15	0.2	0.10
B	1	3,000	5.2	77,660	14.0	85±18	66±18	94±24	84±16	67±15	78±21	2.2	0.15
	2	2,600	5.2	83,170	21.1	85±15	66±15	96±28	88±16	69±14	73±17	3.1	0.18
	3	2,350	4.2	85,030	17.5	88±25	72±15	97±36	89±13	73±13	78±15	2.2	0.08
	4	1,800	3.1	35,140	12.6	89±17	68±17	98±26	88±13	73±14	80±16	1.1	0.06

(x) = Standarized according to age-distribution in both mills.

(*) = In liters per minute.

Figure 1.

Mean monthly values of dust fall in relation to the season of the year for two investigated areas. (Data for 3 years: 1966 – 1969)

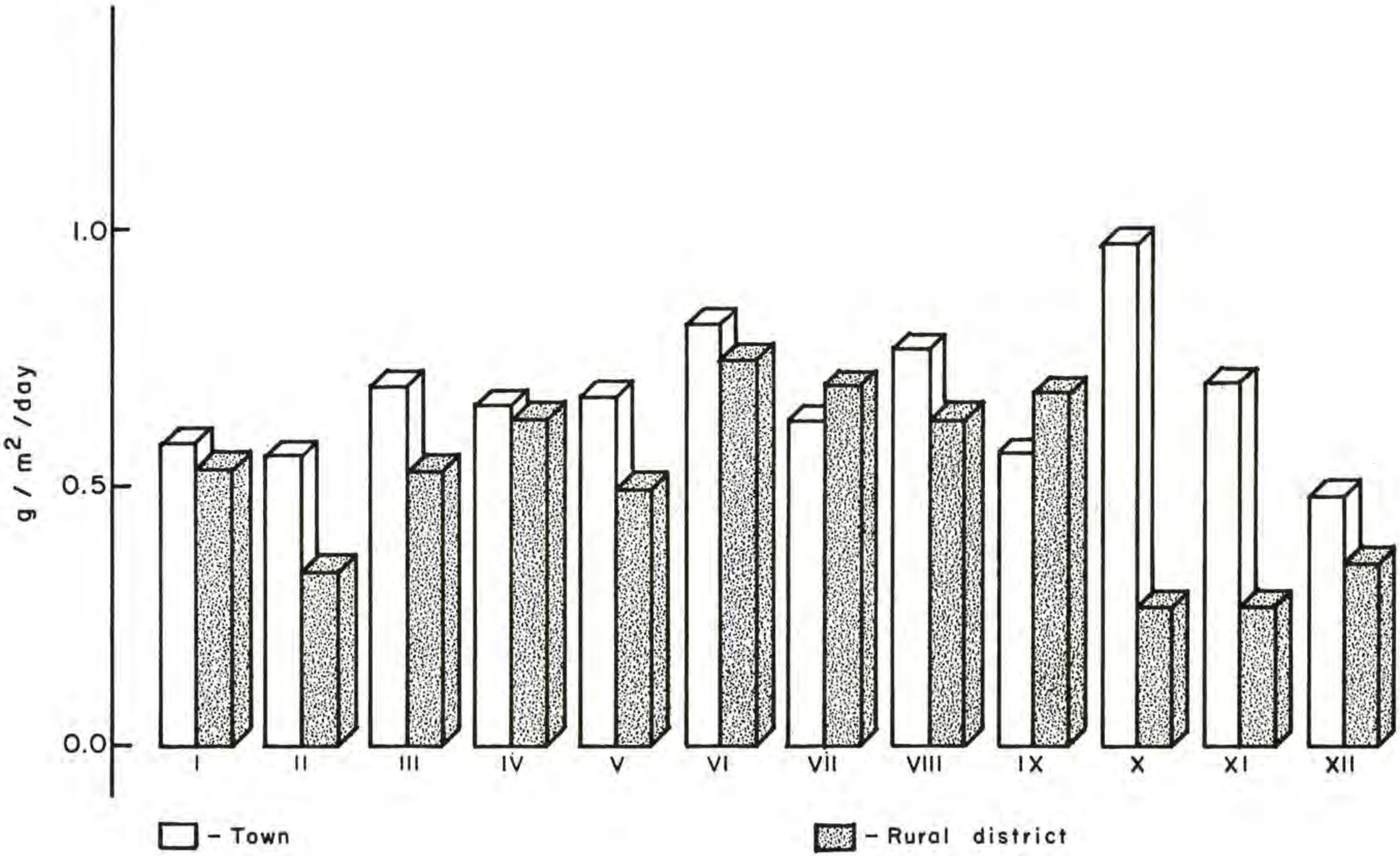


Figure 2.

Mean monthly concentration of SO_2 in the air in relation to the season of the year for two investigated areas. (Contact method, Data for 3 years: 1966 – 1969)

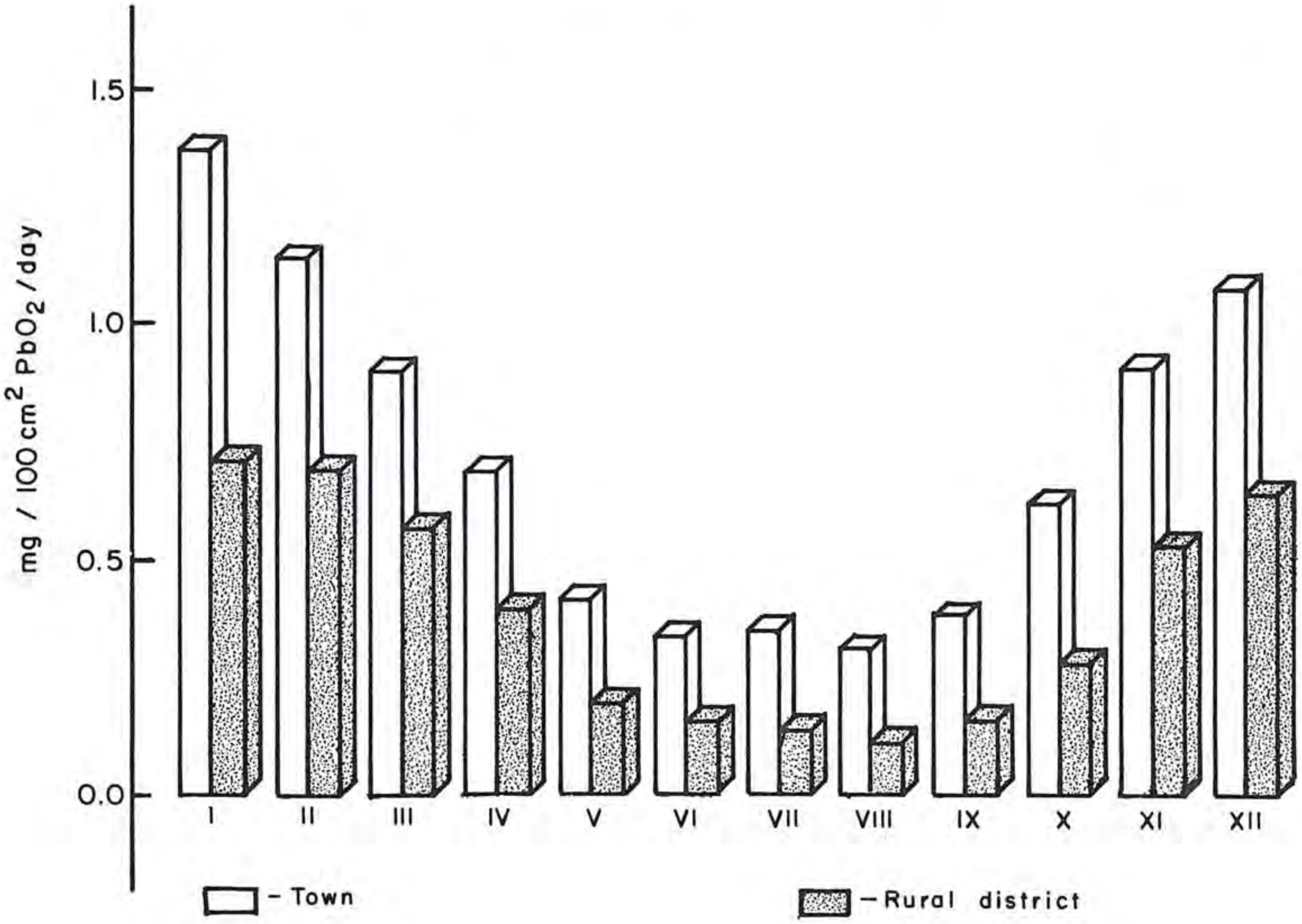


Figure 3.

Results of the benzo-a-pyren concentration in the air, in two investigated areas. (Data for 2 years: 1967 - 1968)

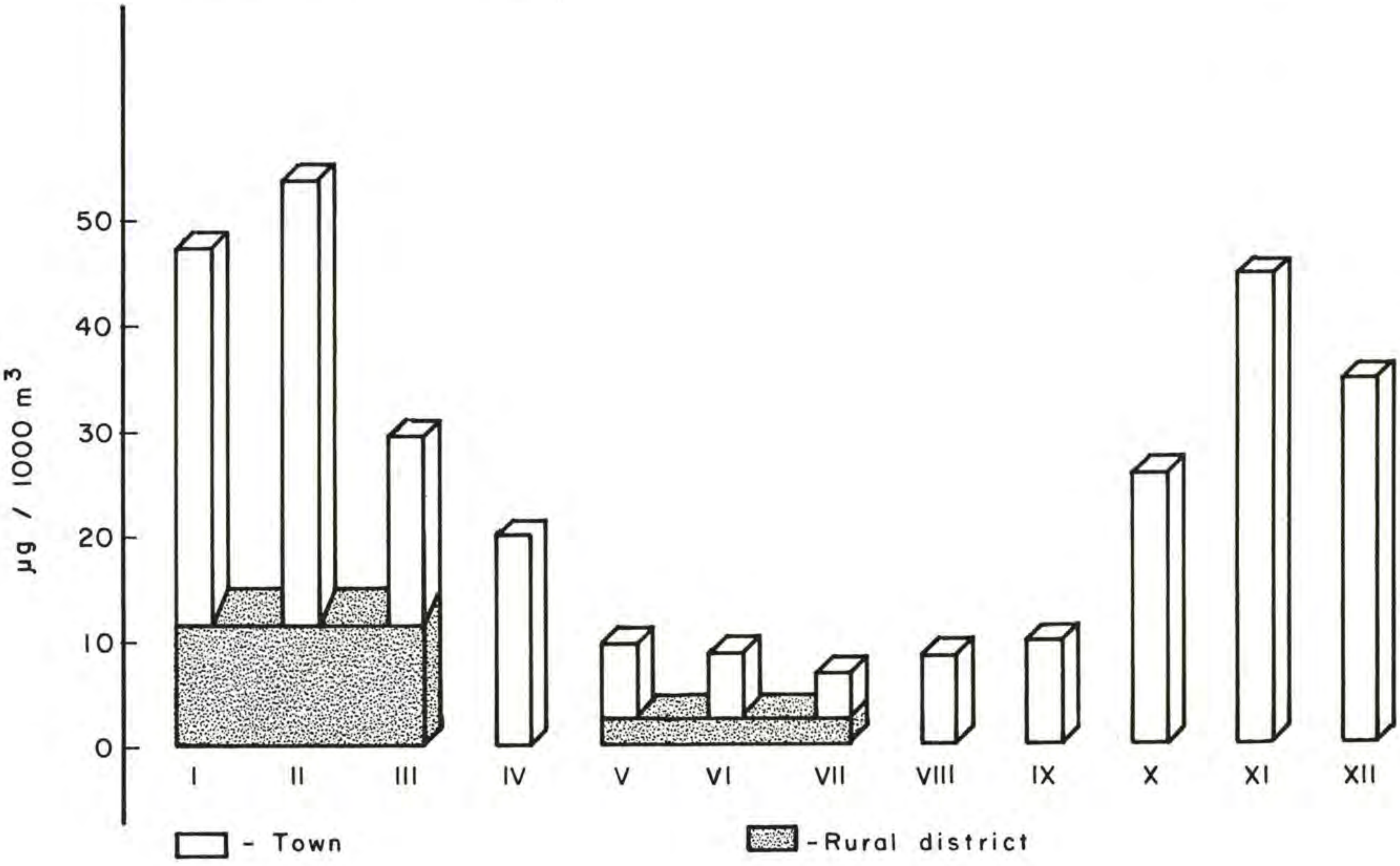
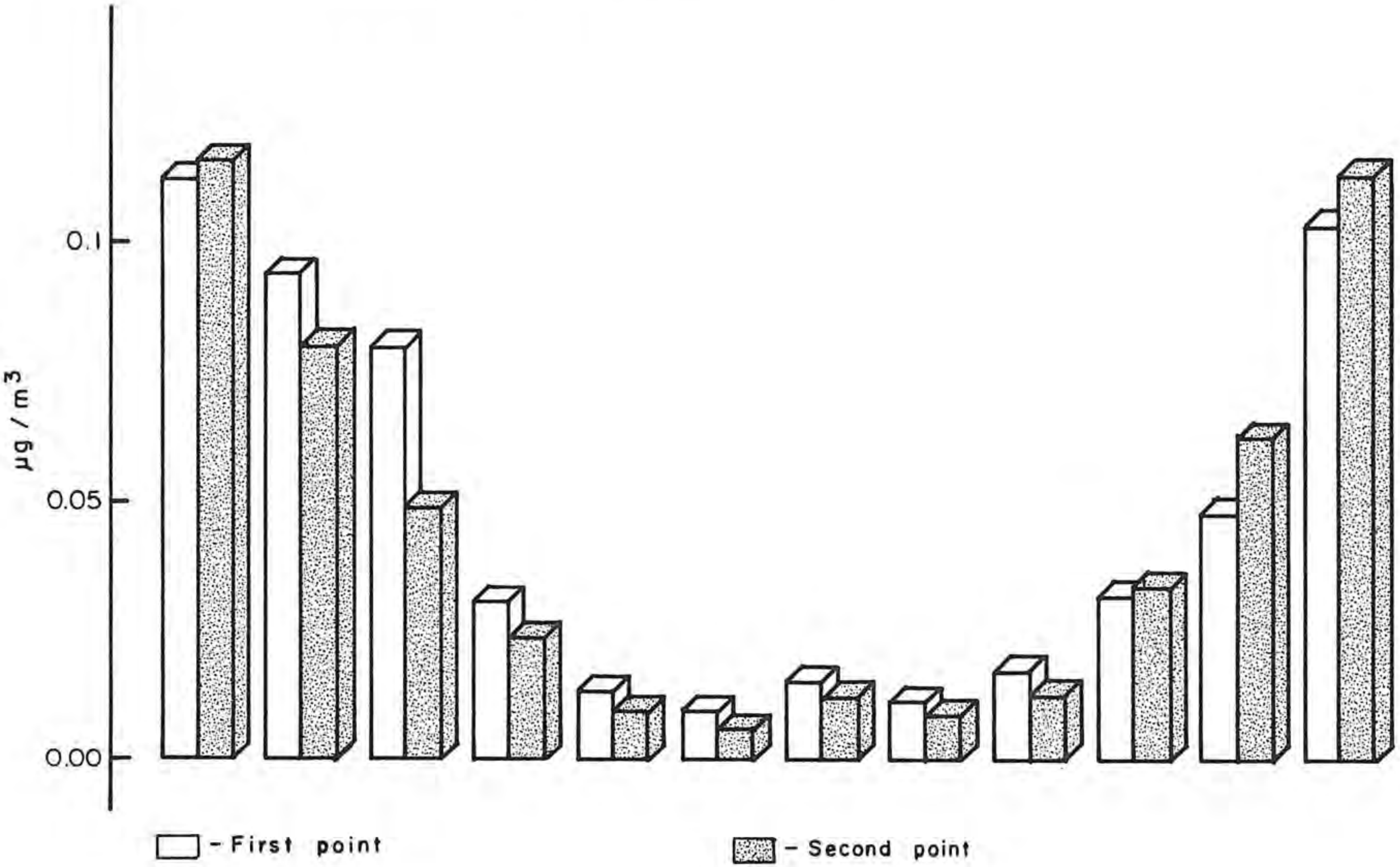


Figure 4.

Mean 24 hrs. concentration of SO_2 for two measuring points in the town district; colorimetric method. (Data for 3 years: 1966–1969)



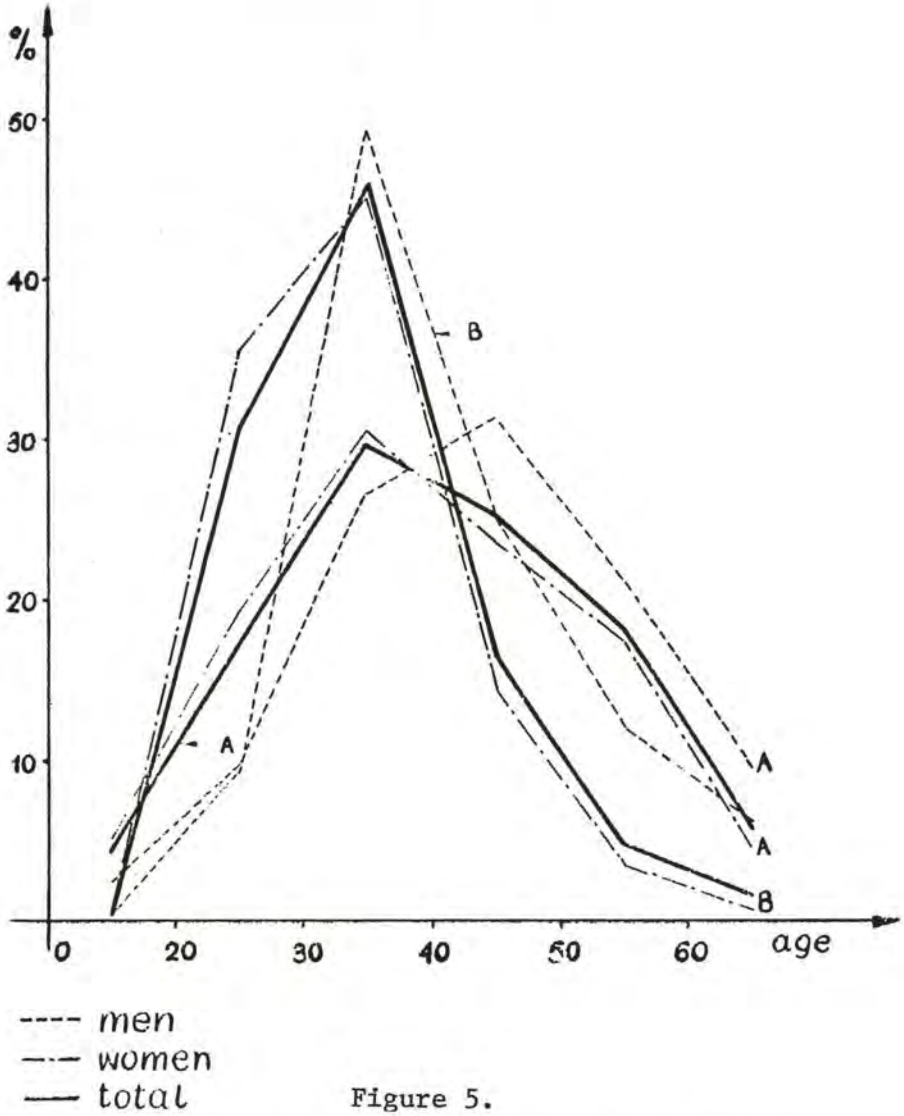


Figure 5.

Distribution of the investigated subjects according to sex and age.

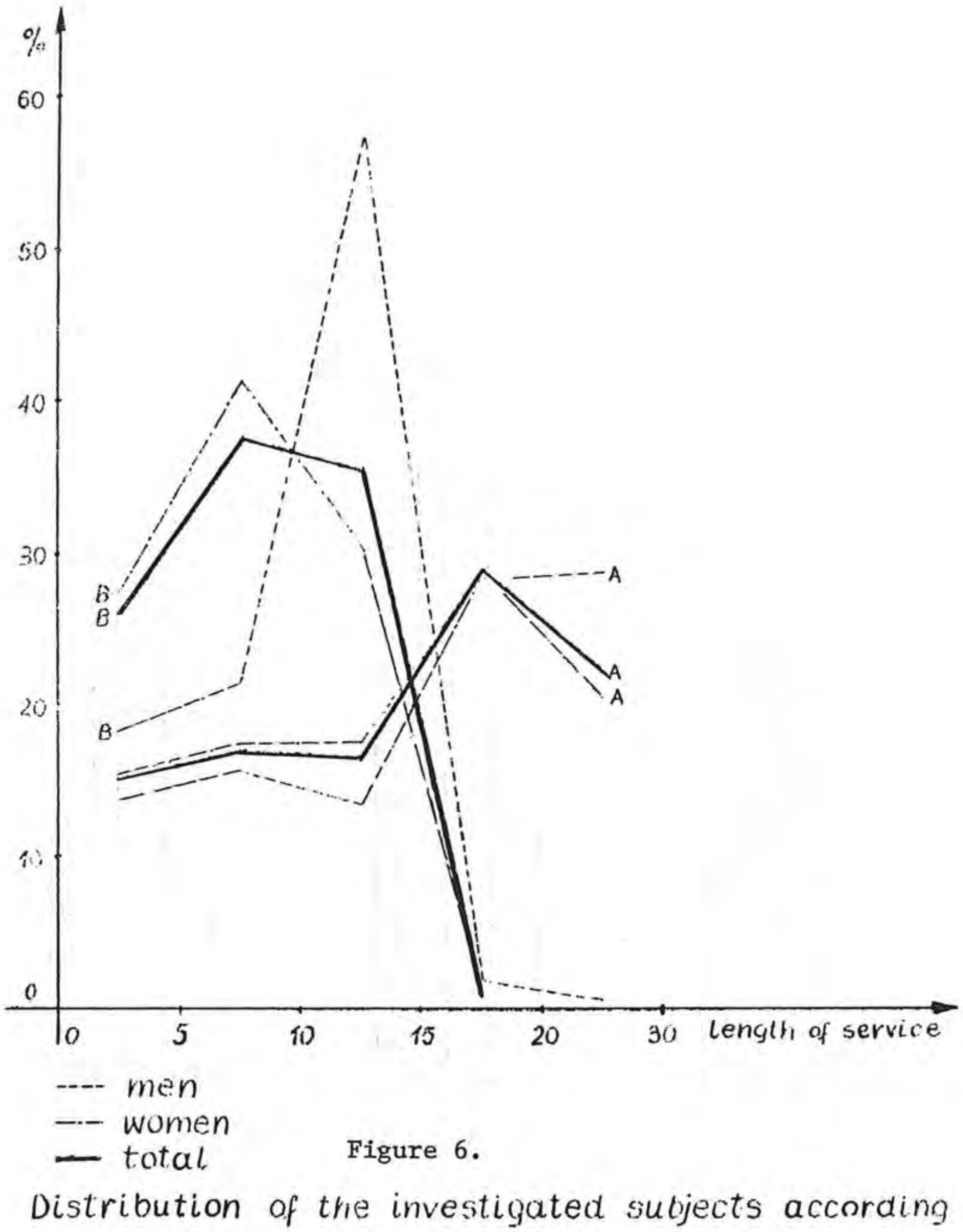
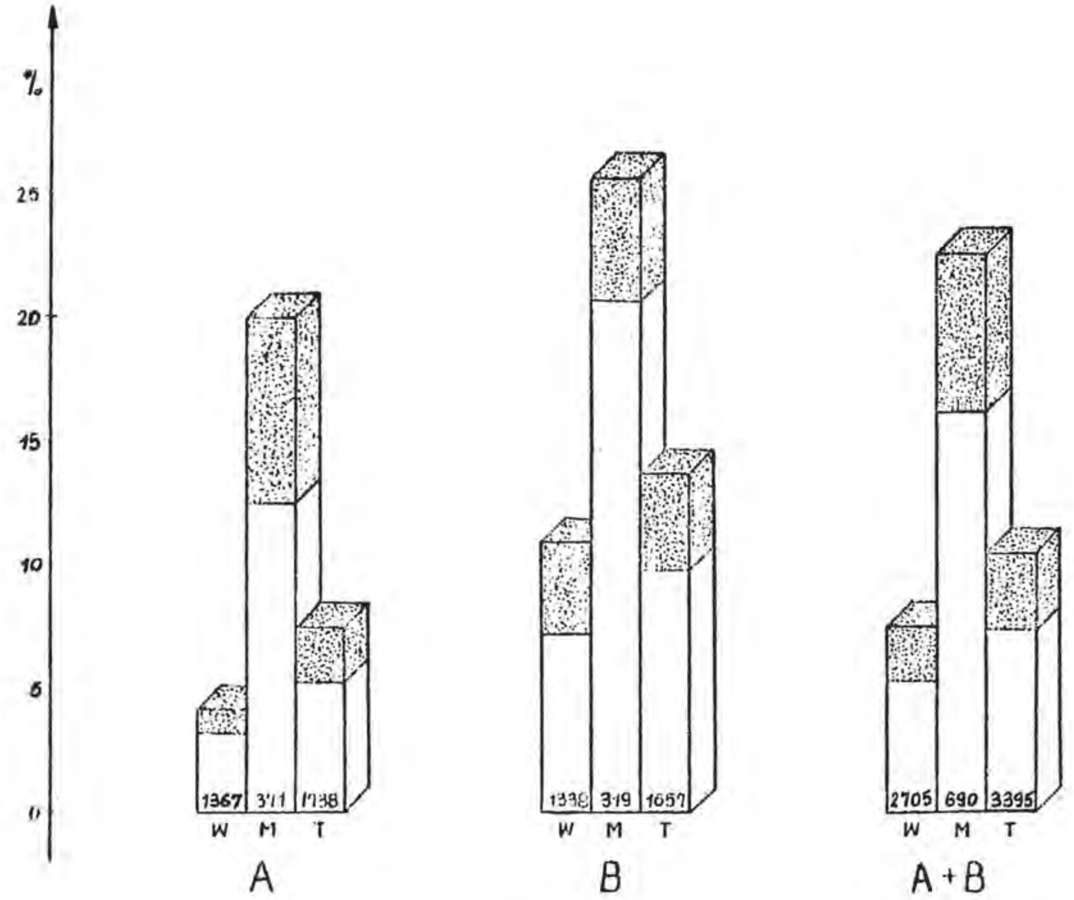


Figure 6.

Distribution of the investigated subjects according to sex and length of service.



▒ – Chronic bronchitis (simple)

☐ – Chronic bronchitis (recurrent obstructive)

Prevalence of CNRD with relation to the clinical form of the disease for men and women in two cotton mills (in percent)

Figure 7.

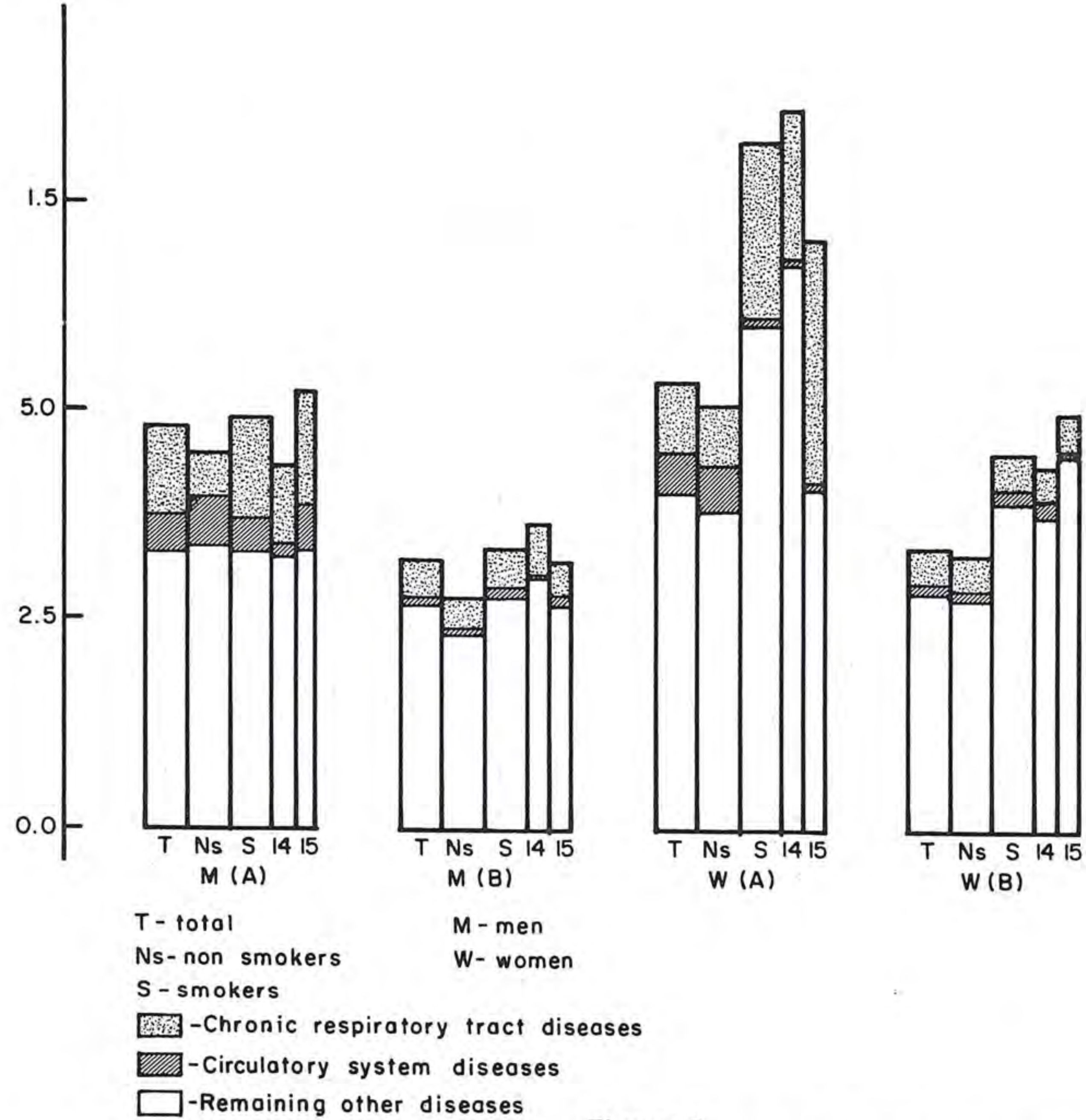


Figure 8.

Sickness-absenteism indices in cotton workers employed in two investigated mills with relation to sex and smoking habit.

Discussion after Dr. Szymczykiewicz's Paper

Dr. Zuskin: In regard to the prevalence of respiratory symptoms, I wonder if you asked the question about chest tightness on Monday or on the first working day which would be consistent with byssinosis. In addition, do you have data about the dyspnea prevalence in examined cotton workers?

Dr. Szymczykiewicz: In the answers given by our examined workers, we did not receive tightness on Monday or on other first working days more than usually, and therefore, our subjects could not be diagnosed as byssinotics. The prevalence of dyspnea was found in about 10% of the investigated subjects, and the repeatability of this symptom was 78%.

Dr. Valic: Was there a positive correlation between the indoor dust concentration and bacterial indoor air pollution in the working environments studied? If so, then you can not conclude that there was an association between bacterial air pollution and development of nonspecific respiratory disease. In some of our studies we found no significant correlation between the prevalence of nonspecific respiratory disease and the concentration of microorganisms in air. We did, however, find significant positive correlations between the prevalence of non-specific respiratory disease and mean dust concentration in many instances.

Dr. Szymczykiewicz: We found positive correlation between pollution of the in-factory air (microflora and dust) and prevalence of CNRD. We also found that the sputum of cotton workers carried live microorganisms which were similar to those found in the air of the work premises and as well as in the manufactured cotton. These microorganisms appeared in significantly higher numbers in the sputum of workers suffering from CNRD. In accordance with the above, it seems that in cotton workers, such microorganisms can not be excluded from etio-pathogenesis of CNRD.

Dr. Potkonjak: As you have shown, you had divided your subjects into two groups: healthy and bronchitis, simple and purulent. Certainly, it was done on the ground of data obtained by interview -- you said that the standardized questionnaire had been used. I wonder whether you noticed disagreement in the answers obtained in the two successive years.

Dr. Szymczykiewicz: The repeatability of main pathological symptoms from the anamnesis (cough, expectoration and dyspnea) obtained at reexamination of the same patient after two years ranges from 80 to 100% for men and 77 to 100% for women.

STUDY OF THE EFFECTS OF NON-SILICEOUS MINERAL DUSTS ON CHRONIC RESPIRATORY DISEASE

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This study was performed on the miners from lignite and brown coal mines and on the workers employed in the production of cement. The basis for a study in these two industries we have had in literature. The authors such as Carstens (2), Hyatt (11) and some others suggested that occupational exposure in miners, particularly the exposure to dusts, besides producing pneumoconiosis, may also induce nonspecific respiratory impairment. Later on, Higgins, et al. (9) came to a conclusion that smoking habit represents a more significant factor in the development of respiratory impairment than occupation. Enterline and Lainhart (5), like Higgins, et al. (8) found a higher rate of respiratory symptoms in miners than in control workers, but found the same for miners' wives. The problem of chronic bronchitis and occupation was dealt with in a Medical Research Council's Report in Great Britain in 1966.

The results of the so far performed clinico-epidemiological studies into the frequency of chronic nonspecific lung disease in cement workers also differ. Guilliani and Belli (7) report that 84% of workers in a cement plant in Italy suffered from lung emphysema, chronic bronchitis or other chronic respiratory disorders. Vyskocil (19) points out that by examining 104 workers he found nasopharyngitis in almost each worker, chronic bronchitis in 75 and lung emphysema in 21 workers. The Soviet authors Tarnopoljskaja and Ostenskij (18) also report a high rate of lung emphysema in male and female cement workers. The high rate of emphysema, chronic bronchitis, chronic pharyngitis and chronic rhinitis in these workers was equally pointed out by some other authors including the Yugoslav authors Karajovic, et al. (14), and Popovic (16). Gardner and collaborators (6) on the contrary, did not find chronic respiratory infections in cement workers any more frequently than in the rest of the population. The same experience was reported by Jenny, et al. (13) as well as by Sander (17) and Hudson (10).

More or less controversial opinions in the literature concerning the role of exposure in coal mines and cement production in the occurrence of chronic nonspecific lung disease led us to examine the problem in a group of miners and cement workers in Yugoslavia. In either case i.e., among both the cement workers and miners a selection was made among those with five or more years of work experience in underground work and cement production.

The investigation was planned and carried out as a cross sectional study. As controls served the workers from the same area, of corresponding age and socio economic structure who had never been occupationally exposed to dusts or chemical irritants. The examinations were performed in the same season (spring). The method of examination consisted of the questionnaire of the Committee on the Aetiology of Chronic Bronchitis of the British Medical Research Council, FVC and $FEV_{1.0}$ determinations, clinical examination, X-ray of the lungs and analysis of the volume and consistency of the early morning sputum. Beside, basic anthropometric measures were taken, electrocardiography was performed and blood pressure measured.

Examinations also included miners' and cement workers' wives as well as the wives of the corresponding control workers. These examinations were planned as an attempt to evaluate the influence of nonoccupational factors on the rate of respiratory impairment.

Data were analyzed according to criteria worked out by the authors of the questionnaire (Lancet, 1965). Chronic bronchitis was defined as phlegm production in the morning in winter or during day and/or night for at least three months per year, in the course of the last two years or longer. Exacerbations were defined as periods of increased phlegm or cough lasting three weeks or more during the last three years. Categories of smokers were determined according to the number of years of smoking and the number of cigarettes smoked per day (Brinkman and Coates) (1).

The obtained values of forced expiratory volumes are expressed as percentage of the values expected with regard to age and height of subjects (12) For women they are presented in relation to normal values established by Kory et al. (15).

As far as $FEV_{1.0}/FVC(\%)$ values are concerned as reduced were regarded the values of 79% and lower for the 20-29 age group, 76% and lower for the 30-39 age group, 74% and lower for the 40-49 age group and 71% and lower for the 50-59 age group.

The study has been in its final stage.* In this paper the most important results are presented separately for groups of miners and cement workers.

Miners

A group of 904 miners from 3 lignite and brown coal mines was examined. All examined miners are presented together and not separately by mines because there was no essential difference in the method of work or working conditions. The excavating of coal was relatively mechanized. The problem of exposure to free SiO_2 did not exist in any of the mines. As control served a group of 342 workers.

Besides, 418 miners' wives and 122 control workers' wives were also examined.

In Table 1 are presented general data about examined miners and control workers.

In Table 9 are presented data about the prevalence of different respiratory symptoms in nonsmokers-miners and nonsmokers-control workers.

Tables 10 and 11 show the prevalence of the same respiratory symptoms in miners - nonsmokers and smokers by length of mining experience.

Table 12 summarizes $FEV_{1.0}$ values expressed as percentage of those expected for respective age and height in control workers and miners according to years of mine work and smoking habit.

Table 13 shows $FEV_{1.0}$ data expressed as percentage of expected values in control workers and miners grouped by smoking categories and years of mine work.

Table 14 shows $FEV_{1.0}$/FVC (%) ratio in control workers and miners by length of mining experience and smoking habit.

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* The results obtained earlier have been presented in two theses and several papers: Ivo Kalacic: Chronic nonspecific lung disease in cement workers, Ph.D. Thesis, Zagreb, 1970.
Slavica Palaic: Nonspecific respiratory impairment in coal miners, M.Sc. Thesis, Zagreb, 1970.
Kalacic, I.: Ventilatory lung function in cement workers, International Occupational Safety and Health Congress, Geneva, 1969.
Saric, M., and Slavica Palaic: The prevalence of respiratory symptoms in a group of miners and the relationship between symptoms and some functional parameters, III. International Symposium on Inhaled Particles, London, 1970, Preprints, 11.1 1.

Discussion

From the data presented in the above tables it is evident that respiratory symptoms and their combinations which have been dealt with occur as a rule more frequently in miners than in control workers. Data from Table 1 and 2 show a good agreement with regard to main characteristics between the group of miners and control workers.

Chronic bronchitis has been found to occur more often in older age groups (miners) and particularly in smokers. However, it is important to note that among miners - smokers the rate of chronic bronchitis is higher than among control workers - smokers. The same is valid for the nonsmoking miners as compared to nonsmoking control workers (Tables 6-8).

The rate of chronic bronchitis is also associated with the length of work experience in the mine (Table 5). Here, however, age is a factor that should be taken into consideration - older miners usually have longer work experience in the mine.

Individual respiratory symptoms presented separately for nonsmokers (Table 9) also show a higher prevalence in miners than in control workers. Some of them were associated at the same time with the length of mining experience. Cough, phlegm production, shortness of breath and wheezing were found at the highest rate in miners with the longest duration of work in mine, more often in those who smoked than in nonsmokers (Tables 10 and 11).

The presented functional parameters do not show such a good correlation with the length of work experience in the mine, smoking habit and the number of cigarettes smoked.

As shown in Table 12 $FEV_{1.0}$ values are not greatly reduced if compared with those expected. However, in the miners with longest exposure (more than 20 years) who are also smokers considerably reduced $FEV_{1.0}$ values were found with the exception of light smokers (Table 13).

$FEV_{1.0}/FVC$ (%) ratio was also lowest in miners with the longest mining experience (Table 14). It is interesting to mention that the mean $FEV_{1.0}/FVC$ (%) values were very similar in smokers and nonsmokers with the exception of the group of miners with mining experience from 10 to 19 years in which this functional parameter showed lower mean value in smokers than in nonsmokers. However in none of the groups or subgroups compared was the $FEV_{1.0}/FVC$ (%) ratio greatly reduced.

A certain disproportion between the rate of respiratory symptoms indicative of chronic bronchitis and the spirometric values indicative of obstructive impairment might be due to a certain selection which occurred in the examined groups. Namely ill workers quit the job and retired with a disability pension without having been included in the examined groups. Records of disability pension insurance indicate that particularly in the coal mines which have recently been through economic difficulties a relatively great number of workers retire with a disability pension. On the other hand it should be taken into consideration that a number of workers with recorded bronchitic symptoms need not instantly develop obstructive disorders. Besides, the applied tests represent relatively rough functional parameters so that a number of slighter changes were probably not recorded at all.

The wives of miners and control workers had considerably less respiratory symptoms than their husbands and there was no significant difference in the prevalence of chronic bronchitis between miners' and control workers' wives. Among functional parameters the mean $FEV_{1.0}$ value expressed as percentage of expected value was somewhat reduced in miners' wives. In spite of the fact that - due to technical difficulties - we could not examine all the miners' and control workers' wives we think that those examined represent the actual relationship between the two groups.

It is important to note that among miners' as well as the control workers' wives there is only a small percentage of smokers - 5.3% miners' and 10.6% control workers' wives are present smokers. Among miners' wives four smoked for more than 20 years and only one more than 20 cigarettes per day; among control workers' wives all smoked for less than 20 years and only one smoked more than 20 cigarettes per day.

The results of the examinations performed agree with a number of observations cited in the introduction about the association between the prevalence of various symptoms indicative of chronic nonspecific lung disease and occupation. In spite of the observed association between the prevalence of respiratory symptoms and mine work (in addition to smoking habit and age to some extent) it is not possible to make a definite conclusion about the causal relationship between mine work and respiratory impairment. The fact that miners' and control workers' wives do not significantly differ in this respect supports the assumption about the potential role of the occupational factor, though the possibility that some other factors besides those observed and controlled might also contribute to the differences found cannot be excluded.

Cement Workers

This part of the study was conducted in three cement-manufacturing plants. In total 847 cement workers were examined. The control group consisted of 460 workers. The cement plants from which the workers were selected manufacture portland cement while one of them also manufactures white cement.

Since among the examined groups of workers there were no great mutual differences regarding the prevalence of respiratory disorders, the conditions of production showing no essential difference either, the groups are presented together.

Besides cement and control workers their wives were also examined.

In Table 17 are presented general data about examined exposed and control workers.

Tables 27 and 28 show the prevalence of the same respiratory symptoms in nonsmokers and smokers respectively with various length of exposure in the manufacture of cement.

In Table 29 are shown FVC values (expressed as percentage of expected values) in the control and exposed group according to the length of work experience and smoking habit.

Table 30 shows the $FEV_{1.0}$/FVC (%) in control and exposed workers by length of work experience and smoking habit.

In Table 31 are shown the values of the mean air velocity in the third fourth of FVC (E_{50-75}) expressed in mil/sec in nonsmoking control and exposed workers.

Discussion

If it is assumed that selective factors did not differ in the exposed and control group, the higher rate of symptoms and some objective findings characteristic of chronic nonspecific lung disease in exposed workers should be regarded as expression of an association between the exposure in cement production and the occurrence of the disease. However, it is not possible to give a definite answer to the question whether the connection is causal as it is not known whether the two groups of workers were comparable with regard to all factors with the exception of the factor of incriminated exposure.

Smoking as one of these factors appeared at a somewhat higher rate in the exposed group, what might have contributed to the higher rate of the disease in this group (Table 18). However, in nonsmokers too chronic bronchitis as well as some of the separately presented respiratory symptoms were more often found in the exposed group clearly indicating that smoking, even if it was a reason, could not have been the only one accounting for the differences found between the total sample of the control and experimental group (Table 22).

The mean age of the exposed workers was approximately the same as that of the controls (Table 17) so that it seems that the effect of age on the occurrence and frequency of respiratory symptoms may be disregarded.

From a rough estimate that the economic condition and medical welfare of control and exposed workers did not essentially differ and considering the fact that there were no basic differences in the rate of respiratory symptoms and findings between the wives of control workers and those of the exposed (Tables 32, 33), it is possible to assume that socio-economic, nonoccupational factors did not affect significantly the results obtained. Naturally, the effect of some other factors which it was not possible to have under control in the course of examination cannot be eliminated with certainty.

In spite of the observed differences in the rate of respiratory impairment between the cement workers and their controls the connection between the rate of prevalence and the degree of exposure does not seem to be particularly close. The nonsmokers with longer exposure showed, it is true, a somewhat higher rate of symptoms and findings than the nonsmokers with shorter exposure (Table 27), but the differences were not statistically significant in spite of the older age of workers with longer exposure. These results led us to assume that the occurrence of the disease might depend more on hereditary predisposition than on the occupational factor. The workers predisposed to respiratory impairment may get ill already in the first years of exposure.

Still, with regard to the relatively small number of workers in the compared groups and the possibility of a stronger influence of the selective factor in workers with longer exposure, from the results obtained it cannot be claimed that the duration of exposure is not associated with the onset of the disease. In smokers the differences in the rate of symptoms with regard to the length of exposure were more noticeable and in most cases statistically significant (Table 28). In the evaluation of these differences the fact that smokers with longer exposure were advanced in age being also heavier smokers should by all means be taken into account. It might be therefore assumed that the observed differences are more a result of the additive effect of age, smoking habit and exposure than a sole result of the length of exposure. It is equally possible that they be a result of the interaction of all the three factors.

Considering which is the most probable causal factor of exposure in the cement production, cement dust should by all means be put in the first place followed by other factors such as kiln gases and possibly abrupt temperature changes to which the workers are exposed.

The functional tests showed that the ventilatory impairment of the obstructive type is more often found than that of the restrictive type (Tables 29 and 30). It appears already in the first years of exposure and seems to be directly associated with the length of exposure. This connection with the duration of exposure is more evident if the impairment is determined by measuring air velocity in the third fourth of the forced vital capacity (Table 31) than by measuring the volume in the first second of the forced expiration. It seems that the mechanism of ventilation is more impaired in workers with smaller than in those with larger lung volumes.

Analyzing the results of the functional tests it is interesting to note that by none of the three spirometric parameters used was it possible to prove the differences in the ventilatory impairment between nonsmoking and smoking cement workers. It is difficult to provide a convincing explanation of this phenomenon. It might be assumed however, that dust during work provokes cough and that expectoration helps clear the lungs of cement dust thus eliminating or decreasing its noxious effect on ventilatory function.

Conclusion

It is our opinion that it would be useful to continue i.e., to extend a part of investigations. After the completion of the studies of the cross sectional type the planning of follow-up investigations with detailed clinical analysis of particular categories of already examined workers seems desirable (workers without symptoms, those with respiratory symptoms, workers who quit the job, etc.).

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Table 1

General Data about Examined Miners and Control Workers

	Summoned for examination	Examined	Mean age		Mean height in cm		Mean weight in kg	
			$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Miners	967	904 (93.5%)	38.2	6.0	169.9	5.7	68.1	7.9
Controls	396	342 (86.4%)	35.2	6.9	171.1	5.6	70.1	9.6

Table 2

Smoking Habit in Miners and Control Workers

	Miners N=904	Controls N=342
Nonsmokers	213 (23.6)	94 (27.5)
Past smokers	106 (11.7)	41 (12.0)
Present smokers		
Light	180 (19.9)	76 (22.2)
Moderate	362 (40.0)	108 (31.6)
Heavy	43 (4.8)	23 (6.7)

Note: Numbers in parenthesis in this and the following tables are percentages.

Table 3

Miners by Length of Work Experience

	Miners N=904
< 10 yrs	185 (20.5)
10 - 19 yrs	488 (54.0)
> 20 yrs	231 (25.5)

Table 4

General Data about Examined Miners' and Control Workers' Wives

	Summoned for examination	Examined	Mean age		Mean height in cm		Mean weight in kg	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Miners' wives	537	418 (77.8%)	40.1	7.3	158.4	5.6	65.2	12.2
Controls' wives	164	122 (74.4%)	36.9	8.1	159.0	4.8	66.6	10.3

Table 5

Chronic Bronchitis in Control Workers and Miners by Length of Mining Experience

		N	Chronic bronchitis I	Chronic bronchitis with exacerbations II	Chronic bronchitis with reduced $FEV_{1.0}/FVC$ (%) III	Chronic bronchitis with mucopurulent or purulent sputum IV
Controls	(1)	342	21 (6.1)	3	8 (2.3)	10 (2.9)
Miners	< 10 yrs (2)	185	34 (18.4)	5 (2.7)	17 (9.2)	18 (9.7)
	10-19 yrs (3)	488	116 (23.8)	16 (3.3)	40 (8.2)	69 (14.1)
	> 20 yrs (4)	231	84 (36.4)	13 (5.6)	40 (17.3)	41 (17.7)

I 1-2,3,4 $P<0.01$; III 1-2,3,4 $P<0.01$; IV 1-2,3,4 $P<0.01$

Note: In this and in the following tables percentages were not calculated for less than five cases

Table 6

Chronic Bronchitis in Miners and Control Workers
by Age

	N	Chronic bronchitis I	Chronic bronchitis with exacerbations II
Miners			
39 yrs or less (1)	525	107 (20.4)	14 (2.7)
40 yrs or more (2)	379	127 (33.5)	20 (5.3)
Controls			
39 yrs or less (3)	245	13 (5.3)	1
40 yrs or more (4)	97	8 (8.2)	2

I 1-2 $P < 0.01$; 1-3 $P < 0.01$; 2-4 $P < 0.01$

Table 7

Chronic Bronchitis in Miners by Smoking Habit

		N	Chronic bronchitis I	Chronic bronchitis with exacerbations II
Nonsmokers (1)		213	22 (10.3)	2
Past smokers (2)		106	23 (21.7)	9 (8.5)
Present smokers	Light (3)	180	47 (26.1)	6 (3.3)
	Moderate (4)	362	119 (32.9)	13 (3.6)
	Heavy (5)	43	23 (53.5)	4

I 1-2 $P<0.05$; 1-3 $P<0.01$; 1-4 $P<0.01$;
1-5 $P<0.01$

Table 8

Chronic Bronchitis in Control Workers by Smoking Habit

		N	Chronic bronchitis I	Chronic bronchitis with exacerbations II
Nonsmokers (1)		94	2	-
Past smokers (2)		41	2	-
Present smokers	Light (3)	76	5 (6.6)	1
	Moderate (4)	108	7 (6.5)	2
	Heavy (5)	23	5 (21.7)	-

Table 9

Respiratory symptoms in Miners and Control Workers: Nonsmokers

	Miners N=213	Controls N=94	
Cough - part day[x]	24 (11.3)	6 (6.4)	-
Cough - whole day[xx]	30 (14.1)	1	-
Phlegm - part day[x]	22 (10.3)	5 (5.3)	-
Phlegm - whole day[xx]	27 (12.7)	3	-
Shortness of breath			
Grade 2-5	119 (55.9)	24 (25.5)	P<0.01
Grade 3-5	12 (5.6)	2	-
Wheezing			
Only occasionally	73 (34.3)	17 (18.1)	P<0.01
Most days (or nights)	9 (4.2)	-	-
With attacks of shortness of breath	8 (3.8)	-	-
Nasal cathar 3 months per year	38 (17.8)	8 (8.5)	P<0.05
Chest illness during the last 3 years	19 (8.9)	7 (7.4)	-
Shortness of breath due to the effect of weather	48 (22.5)	4	-

[x]Part day - in the morning or during the day (or night) on most days for 3 months (in winter).

[xx]Whole day - in the morning and during the day (or night) on most days for 3 months or longer (in winter).

Table 10

Respiratory Symptoms in Miners - Nonsmokers by Length of Mining Experience

	Length of mining experience <10 yrs N=51 (1)	10-19 yrs N=110 (2)	> 20 yrs N=52 (3)	1-2	1-3	2-3
Cough - part day[x]	5 (9.8)	13 (11.8)	6 (11.5)	-	-	-
Cough - whole day[xx]	9 (17.6)	9 (8.2)	12 (23.1)	-	-	$P<0.05$
Phlegm - part day[x]	2	8 (7.3)	12 (23.1)	-	-	$P<0.05$
Phlegm - whole day[xx]	10 (19.6)	12 (10.9)	5 (9.6)	-	-	-
Shortness of breath						
Grade 2-5	21 (41.2)	61 (55.5)	37 (71.2)	-	$P<0.01$	$P<0.05$
Grade 3-5	2	6 (5.5)	4	-	-	-
Wheezing						
Only occasionally	16 (31.4)	32 (29.1)	25 (48.1)	-	-	$P<0.05$
Most days (or nights)	-	5 (4.5)	4	-	-	-
With attacks of shortness of breath	-	4	4	-	-	-
Nasal cathar 3 months per year	11 (21.6)	14 (12.7)	13 (25.0)	-	-	-
Chest illness during the last 3 yrs	2	12 (10.9)	5 (9.6)	-	-	-
Shortness of breath due to the effect of weather	3	27 (24.5)	18 (34.6)	-	-	-

[x]Part day - in the morning or during the day (or night) on most days for 3 months (in winter).

[xx]Whole day - in the morning and during the day (or night) on most days for 3 months or longer (in winter).

Table 11

Respiratory Symptoms in Miners-Smokers by Length of Mining Experience

	Length of mining experience					
	< 10 yrs N=134 (1)	10-19 yrs N=378 (2)	> 20 yrs N=179 (3)	1-2	1-3	2-3
Cough - part day[x]	28 (20.9)	65 (17.2)	19 (10.6)	-	P<0.05	P<0.05
Cough - whole day[xx]	36 (26.9)	119 (31.5)	92 (51.4)	-	P<0.01	P<0.01
Phlegm - part day[x]	27 (20.1)	67 (17.7)	25 (14.0)	-	-	-
Phlegm - whole day[xx]	33 (24.6)	116 (30.7)	81 (45.2)	-	P<0.01	P<0.01
Shortness of breath						
Grade 2-5	72 (53.7)	240 (63.5)	149 (83.2)	P=0.05	P<0.01	P<0.01
Grade 3-5	11 (8.2)	35 (9.2)	38 (21.2)	-	P<0.01	P<0.01
Wheezing						
Only occasionally	63 (47.0)	206 (54.5)	130 (72.6)	-	P<0.01	P<0.01
Most days (or nights)	17 (12.7)	58 (15.3)	58 (32.4)	-	P<0.01	P<0.01
With attacks of shortness of breath	14 (10.4)	35 (9.2)	28 (15.6)	-	--	P<0.05
Nasal cathar 3 months per year	29 (21.6)	91 (24.1)	43 (24.0)	-	-	-
Chest illness during the last 3 years	25 (18.6)	55 (14.6)	30 (16.8)	-	-	-
Shortness of breath due to the effect of weather	29 (21.6)	124 (32.8)	88 (49.2)	P<0.01	P<0.01	P<0.01

[x]Part day - in the morning or during the day (or night) on most days for 3 months (in winter).

[xx]Whole day - in the morning and during the day (or night) on most days for 3 months or longer (in winter).

Table 12

$FEV_{1.0}$ (% of expected values) in Control Workers and Miners by Length of Mining Experience and Smoking Habit

		Smokers I			Nonsmokers II			Total		
		N	$\bar{X}$	SD	N	$\bar{X}$	SD	N	$\bar{X}$	SD
Controls (1)		248	98.9	14.6	94	99.6	14.3	342	99.1	14.4
Miners	$<$ 10 yrs (2)	134	101.6	15.7	51	101.7	13.9	185	101.6	14.8
Miners	10-19 yrs (3)	378	98.9	14.6	110	100.6	15.7	488	99.3	15.2
Miners	$>$ 20 yrs (4)	179	91.2	15.3	52	96.0	12.6	231	92.3	14.0

I 1-4 $P<0.01$

III 1-4 $P<0.01$

I-II : 4 $P<0.05$

Table 13

$FEV_{1.0}$ (% of expected values) in Control Workers and Miners by Smoking Categories and Length of Mining Experience

		Past smokers I			Light smokers II			Moderate smokers III			Heavy smokers IV		
		N	$\bar{X}$	SD	N	$\bar{X}$	SD	N	$\bar{X}$	SD	N	$\bar{X}$	SD
Controls	(1)	41	103.3	13.2	76	101.5	15.5	108	96.6	16.7	23	93.1	13.2
Miners	$<$ 10 yrs (2)	18	103.9	15.0	48	101.7	14.3	68	101.0	17.8	-	-	-
Miners	10-19 yrs (3)	69	100.7	16.7	105	97.7	17.3	189	99.7	15.4	15	89.8	9.1
Miners	$>$ 20 yrs (4)	19	87.5	16.2	27	96.1	13.9	105	91.4	18.4	28	88.5	12.7

I 1-4 $P < 0.01$
III 1-4 $P < 0.05$

Table 14

$FEV_{1.0}/FVC$ (%) in Control Workers and Miners by Length of Mining Experience and Smoking Habit

		Smokers I			Nonsmokers II			Total III		
		N	$\bar{X}$	SD	N	$\bar{X}$	SD	N	$\bar{X}$	SD
Controls	(1)	248	79.1	7.6	94	81.5	7.3	342	79.8	7.4
Miners	< 10 yrs (2)	134	79.4	8.0	51	81.7	7.2	185	80.0	7.6
Miners	10-19 yrs (3)	378	76.8	8.2	110	79.8	7.0	488	77.5	7.6
Miners	> 20 yrs (4)	179	74.0	8.6	52	75.1	9.3	231	74.2	9.0

I 1-3 $P < 0.01$; 1-4 $P < 0.01$

II 1-4 $P < 0.01$

III 1-3 $P < 0.01$; 1-4 $P < 0.01$

I-II 3 $P < 0.01$

Table 15

Chronic Bronchitis in Miners' and Control Workers' Wives

	N	Chronic bronchitis	Chronic bronchitis with exacerbations
Miners' wives	418	14 (3.3%)	3
Control workers' wives	122	7 (5.7%)	1

Table 16

Forced Expiratory Volumes in Miners' and Control Workers' Wives

	N	FVC *I		$FEV_{1.0}$ II		$FEV_{1.0}$/FVC (%) III	
		$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Miners' wives	418	109.7	15.4	89.6	15.5	79.2	6.8
Control workers' wives	122	111.3	13.5	101.0	16.6	80.4	8.4

II $P < 0.01$

Table 17

General Data about Examined Cement and Control Workers

	Summoned for examination	Examined	Mean age		Mean height in cm		Mean weight in kg	
			$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Cement workers	914	847 (92.7%)	39.9	6.8	172.4	5.8	76.0	11.0
Controls	526	460 (87.5%)	37.6	8.1	172.8	6.2	75.0	10.8

Table 18

Smoking Habit in Cement Workers and Control Workers

	Cement workers N=847	Controls N=460
Nonsmokers	197 (23.3)	137 (29.8)
Past smokers	155 (18.3)	75 (16.3)
Present smokers		
Light	89 (10.5)	63 (13.7)
Moderate	290 (34.2)	141 (30.6)
Heavy	116 (13.7)	44 (9.6)

Table 19

Cement Workers by Length of Work Experience

	Cement workers N=847
<10 yrs	329 (38.8)
10 - 19 yrs	371 (43.8)
>20 yrs	147 (17.4)

Table 20

General Data about Examined Cement Workers' and Control Workers' Wives

	Summoned for examination	Examined	Mean age		Mean height in cm		Mean weight in kg	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Cement workers' wives	274	214 (78.2%)	40.6	8.4	160.9	5.8	69.4	11.6
Controls' wives	164	133 (81.1%)	37.7	9.2	160.3	4.6	67.4	11.2

Table 21

Smoking Habit in Cement Workers' and Control Workers' Wives

	Cement workers' wives N=214	Controls' wives N=133
Nonsmokers	203 (94.8)	126 (94.7)
Past smokers	1	1
Present smokers		
Light	8 (3.7)	5 (3.8)
Moderate	1	1
Heavy	1	-

Table 22

Chronic Bronchitis in Control Workers and Cement Workers by Length of Work Experience

		N	Chronic bronchitis I	Chronic bronchitis with exacerbations II	Chronic bronchitis with reduced $FEV_{1.0}/FVC\%$ III	Chronic bronchitis with mucopurulent or purulent sputum IV
Controls	(1)	460	44 (9.6)	8 (1.7)	20 (4.3)	22 (4.8)
Cement workers	< 10 yrs (2)	329	44 (13.4)	4 (1.2)	29 (8.8)	33 (10.0)
	10-19 yrs (3)	371	80 (21.6)	8 (2.2)	47 (12.7)	56 (15.1)
	> 20 yrs (4)	147	37 (25.2)	2	19 (12.9)	27 (18.4)

I 1-3 $P<0.01$; 1--4 $P<0.01$

III 1-2 $P<0.01$; 1-3 $P<0.01$; 1-4 $P<0.01$

IV 1-2 $P<0.01$; 1-3 $P<0.01$ 1-4 $P<0.01$

Table 23

Chronic Bronchitis in Cement Workers and Control Workers by Age

	N	Chronic bronchitis I	Chronic bronchitis with exacerbations II
Cement workers			
39 yrs or less (1)	463	60 (13.0)	7 (1.5)
40 yrs or more (2)	384	101 (26.3)	7 (1.8)
Controls			
39 yrs or less (3)	288	18 (6.2)	5 (1.7)
40 yrs or more (4)	172	26 (15.1)	3

I 1-2 $P<0.01$; 3-4 $P<0.01$; 1-3 $P<0.01$; 2-4 $P<0.01$

Table 24

Chronic Bronchitis in Cement Workers by Smoking Habit

			N	Chronic bronchitis I	Chronic bronchitis wirh exacerbations II
Nonsmokers		(1)	197	23 (11.7)	2
Past smokers		(2)	155	18 (11.6)	1
Present smokers	Light	(3)	89	15 (16.8)	1
	Moderate	(4)	290	64 (22.1)	5 (1.7)
	Heavy	(5)	116	41 (35.3)	5 (4.3)

I 1-3 $P>0.10$; 1-4 $P<0.01$; 1-5 $P<0.01$

Table 25

Chronic Bronchitis in Control Workers by Smoking Habit

		N	Chronic bronchitis I	Chronic bronchitis with exacerbations II
Nonsmokers		137	3	-
Past smokers		75	6 (8.0)	2
Present smokers	Light	63	6 (9.5)	3
	Moderate	141	18 (12.8)	1
	Heavy	44	11 (25.0)	2

Table 26

Respiratory symptoms in Cement Workers and Control Workers: Nonsmokers

	Cement workers N=197	Controls N=137	
Cough - part day[x]	22 (11.2)	7 (5.1)	P< 0.05
Cough - whole day[xx]	27 (13.7)	5 (3.6)	P< 0.01
Phlegm - part day[x]	18 (9.1)	8 (5.8)	
Phlegm - whole day[xx]	29 (14.7)	5 (3.6)	P< 0.01
Shortness of breath			
Grade 2-5	78 (39.6)	34 (24.8)	P< 0.01
Grade 3-5	11 (5.6)	-	-
Wheezing			
Only occasionally	50 (25.4)	35 (25.5)	-
Most days (or nights)	9 (4.6)	3	-
With attacks of shortness of breath	6 (3.0)	1	-
Nasal cathar 3 months per year	20 (10.2)	17 (12.4)	-
Chest illness during the last 3 years	35 (17.8)	26 (19.0)	-
Shortness of breath due to the effect of weather	16 (8.1)	4 (2.9)	P< 0.05

Table 27

Respiratory Symptoms in Cement Workers - Nonsmokers by Length of Work Experience

	Length of work experience < 10 yrs N=74 (1)	10-19 yrs N=85 (2)	> 20 yrs N=38 (3)	1-2	1-3	2-3
Cough - part day[x]	7 (9.4)	9 (10.6)	6 (15.8)	-	-	-
Cough - whole day[xx]	7 (9.4)	13 (15.3)	7 (18.4)	-	-	-
Phlegm - part day[x]	7 (9.4)	7 (8.2)	4 (10.5)	-	-	-
Phlegm - whole day[xx]	10 (13.5)	12 (14.1)	7 (18.4)	-	-	-
Shortness of breath						
Grade 2-5	22 (29.7)	34 (40.0)	22 (57.9)	-	P<0.05	P<0.05
Grade 3-5	3	5 (5.9)	3	-	-	-
Wheezing						
Only occasionally	20 (27.0)	19 (22.4)	11 (28.9)	-	-	-
Most days (or nights)	2	4	3	-	-	-
With attacks of shortness of breath	1	3	2	-	-	-
Nasal cathar 3 months per year	8 (10.8)	5 (5.9)	7 (18.4)	-	-	-
Chest illness during the last 3 years	11 (14.9)	15 (17.6)	9 (23.7)	-	-	-
Shortness of breath due to the effect of weather	3	8 (9.4)	5 (13.2)	-	-	-

Table 28

Respiratory Symptoms in Cement Workers - Smokers by Length of Work Experience

	Length of work experience					
	< 10 yrs N=255 (1)	10-19 yrs N=286 (2)	> 20 yrs N=109 (3)	1-2	1-3	2-3
Cough - part day[x]	44 (17.2)	55 (19.2)	17 (15.6)	-	-	-
Cough - whole day[xx]	52 (20.4)	90 (31.5)	45 (41.3)	P<0.01	P<0.01	-
Phlegm - part day[x]	46 (18.0)	52 (18.2)	18 (16.5)	-	-	-
Phlegm - whole day[xx]	49 (19.2)	77 (26.9)	31 (28.4)	P<0.05	-	-
Shortness of breath						
Grade 2-5	99 (38.8)	147 (51.4)	70 (64.2)	P<0.01	-	P<0.05
Grade 3-5	21 (8.2)	28 (9.8)	12 (11.0)	-	-	-
Wheezing						
Only occasionally	105 (41.2)	127 (44.4)	59 (54.1)	-	P≈0.05	-
Most days (or nights)	16 (6.3)	26 (9.1)	12 (11.0)	-	-	-
With attacks of shortness of breath	7 (2.7)	9 (3.1)	3	-	-	-
Nasal cathar 3 months per year	45 (17.6)	45 (15.7)	25 (22.9)	-	-	-
Shest illness during the last 3 years	41 (16.1)	50 (17.5)	23 (21.1)	-	P<0.01	-
Shortness of breath due to the effect of weather	24 (9.4)	38 (13.3)	23 (21.1)	-	P<0.01	-

Table 29

FVC (% of expected values) in Control Workers and Cement Workers by Length of Work Experience and Smoking Habit

		Nonsmokers I			Smokers II			Total III		
		N	$\overline{X}$	SD	N	$\overline{X}$	SD	N	$\overline{X}$	SD
Controls	(1)	132	92.4	13.6	308	93.0	12.5	440	92.8	13.0
Cement workers	< 10 yrs (2)	71	94.5	12.2	246	95.9	13.7	317	95.6	13.0
	10-19 yrs (3)	80	91.9	15.1	263	92.5	14.2	343	92.4	14.6
	> 20 yrs (4)	36	86.2	9.6	104	87.7	14.8	140	87.3	12.2
	Total (5)	187	91.8	12.3	613	93.0	14.2	800	92.8	13.3

I 1-4 $P < 0.05$; 3-4 $P < 0.05$

II 1-4 $P < 0.01$; 3-4 $P < 0.01$

III 1-2 $P < 0.01$; 1-4 $P < 0.01$; 2-3 $P < 0.01$; 2-4 $P < 0.01$;
2-5 $P < 0.01$; 3-4 $P < 0.01$; 4-5 $P < 0.01$

Table 30

$FEV_{1.0}/FVC(\%)$ in Control Workers and Cement Workers by Length of Work Experience and Smoking Habit

		Nonsmokers I			Smokers II			Total III		
		N	$\overline{X}$	SD	N	$\overline{X}$	SD	N	$\overline{X}$	SD
Controls	(1)	132	77,7	9.3	308	76.8	8.3	440	77.1	8.8
Cement workers	< 10 yrs (2)	71	74.4	8.8	246	74.3	9.3	317	74.3	9.0
	10-19 yrs (3)	80	75.0	9.7	263	72.6	10.0	343	73.2	9.8
	> 20 yrs (4)	36	72.4	7.0	104	72.1	10.2	140	72.2	8.6
	Total (5)	187	74.3	8.5	613	73.2	9.8	800	73.5	9.1

I 1-2 $P < 0.05$; 1-4 $P < 0.01$; 1-5 $P < 0.01$

II 1-3 $P < 0.01$; 1-4 $P < 0.01$; 1-5 $P < 0.01$

III 1-3 $P < 0.01$; 1-4 $P < 0.01$; 1-5 $P < 0.01$

Table 31

E_{50-75} in Control Workers and Cement Workers by Length of Work Experience and Smoking Habit

		Nonsmokers I				Smokers II			
		N	Age	Height	E_{50-75}	N	Age	Height	E_{50-75}
Controls	(1)	50	36.2	174.2	3.070	104	37.6	173.2	2.650
Cement workers	$<$ 10 yrs (2)	28	36.0	173.8	2.580	83	36.4	171.9	2.380
	10-19 yrs (3)	39	39.0	172.0	2.510	91	40.9	173.2	2.420
	$>$ 20 yrs (4)	14	50.8	171.0	1.620	40	46.7	171.5	1.960
	Total (5)	81	40.0	172.4	2.380	214	40.2	172.4	2.320

I 1-2 $P < 0.05$; 1-5 $P < 0.05$; 2-4 $P < 0.05$; 3-4 $P < 0.05$

II 1-3 $P < 0.01$; 2-4 $P < 0.01$; 3-4 $P < 0.01$

I-II 1 $P < 0.05$

Table 32

Chronic Bronchitis in Cement Workers' and Control Workers' Wives

	Chronic bronchitis	Chronic bronchitis with exacerbations
Cement workers' wives	3	–
Control workers' wives	6 (4.5)	2

Table 33

Forced Expiratory Volumes in Cement Workers' and Control Workers' Wives

	FVC I		$FEV_{1.0}$ II		$FEV_{1.0}$/FVC (%) III	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Cement workers' wives	106.2	15.0	94.5	14.3	77.1	6.6
Control workers' wives	104.7	16.2	94.1	15.2	78.9	7.7

DISCUSSION AFTER DR. SARIC'S PAPER:

Dr. Szymczykiewicz:

This is a very interesting question that the cement and coal dust are much more irritating agents than the cotton dust and the prevalence in these industries is very similar to the prevalence of chronic bronchitis in the cotton industry; comparison with my results to results of Dr. Saric.

Dr. Saric:

It's very difficult to say whether this is only the question of the exposure to dust, for example, in mines. As I already mentioned the study is still in progress. So we haven't completed the analysis of data about the working environment in mines. But from some of the preliminary results I can say that the concentration of dust was a little bit higher than the maximum allowable concentration here in this country. But in mines there is also the problem of exposure, at least in some cases, to irritating gases also. And perhaps the problem of climatic condition in mines has to be taken into consideration, as well. As you probably realized from this paper and from the tables I showed, I always avoided speaking about the exposure to dust but used instead the term underground work experience in mines because I don't really know whether the dust exposure is the main factor. In the case of cement production if there is any relationship between the prevalence rate of chronic bronchitis and the occupation, I think that dust is probably the most important factor. But even in this case, for example, climatic conditions have to be taken into consideration and there may be to some extent exposure to gases also.

Dr. Lainhart:

Do either you or possibly Dr. Szymczykiewicz have an idea on how the knowledge that dust may produce disease, may affect the way a miner answers questions regarding his health? May the fact that a man may be a farmer or a small businessman or some such other control who may already know that he doesn't have to worry about dust causing him disease, effect the way he may answer a question.

Dr. Saric:

It is not easy to answer this question. The coal mines from which we took coal miners for examination had economic difficulties in the last years. There was a tendency to reduce a number of workers. So, the miners answered the questionnaire probably under rating their conditions, because they didn't want to leave mines. This is probably one of the selective factors in our case. But on the other hand, of course, there is possibility that some of them prefer to leave the mine and to get a pension because some of them are still connected with land. They work in the mine and to some extent they are at the same time farmers. So this is a complicated question and this, I agree, has to be taken into account. But it's difficult to give a very definite answer.

Dr. Potkonjak:

I should like to ask you whether you have examined lung function, particularly FEV test or some other test, after having applied bronchodynamic drugs? It is considered that subjects exposed to cement dust particularly, show bronchial instability.

Dr. Saric:

We didn't use this but we plan to do this especially if we continue with this study.

Dr. Potkonjak:

I just wanted to say the reason for asking. Perhaps, you will have the same difficulties that I have had. Also, for example, how are the results to be interpreted. There are great disagreements. Some authors consider that positive bronchospasm can be diagnosed if more than 10% difference of FEV is obtained. Some others consider the test as positive if the difference is more than 20% and there are some in Germany who consider the difference of 35% as positive.

Dr. Lainhart:

You may have to make you own standards. Since this literature doesn't agree, you may just have to develop your own, then let them disagree with you.

Dr. Rafalski:

On one of your slides I saw differences between the control group and examined group according to the age, weight, and height. May I know whether those differences also apply to the genetics or economical status of populations?

Dr. Saric:

It is very difficult to find corresponding controls, especially in the case of miners. We tried to get for controls those people living in the same areas with similar social-economical conditions, with similar housing conditions and so on. And this was a difficult problem, because we utilized people who weren't working in the mines. But later we realized that some of them had a certain period of mining experience, so we had to exclude them from the control groups. Because these are areas with long mining tradition, it's very difficult to find people who never have worked or do not work in the mines. We couldn't take farmers as controls for one simple reason; we do not have the same health insurance system for private farmers and employees and their families and this, of course, may influence the result.

THE EFFECT OF VEGETABLE DUST ON RESPIRATORY FUNCTION OF INDUSTRIAL WORKERS

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I. Introduction

Although a great many papers have been published on byssinosis and related subjects, the whole syndrome of byssinosis is still far from being definitely elucidated. Byssinosis is an industrial pulmonary disease which affects certain groups of workers exposed to certain types of airborne vegetable dust. In spite of the fact that it was Ramazzini himself, years ago, who described a typical respiratory disease of textile workers, the very existence of byssinosis is still disputed in many circles. Today it is only the cotton-caused byssinosis that is generally accepted as an occupational disease and even included in the list of compensable occupational diseases in a few countries, while byssinosis caused by other vegetable dusts, such as hemp and flax, has not yet received general recognition.

The diagnostic basis of byssinosis is not yet definitely defined. Byssinosis is characterized by symptoms of chest tightness, cough, wheezing and shortness of breath, usually accompanied by the acute reduction of ventilatory lung capacity during the work shift (1). The symptoms are by far most evident on the first day after absence from work, particularly so at the initial stages of the disease, while on the following days they may not be felt at all. Some authors claim that there is no acute reduction of ventilatory capacity during the work shift except on first exposure to dust after absence from work (Monday effects). As there are no radiological changes specific to byssinosis at the early stages of the disease, the early diagnosis is most often based upon the characteristic Monday symptoms and, far less often, on the basis of acute reduction of ventilatory capacity, particularly the timed forced vital capacity. In later stages the clinical picture of byssinosis is in fact identical to that of chronic bronchitis or emphysema so that it is impossible to differentiate between these diseases except on the basis of the past occupational history.

The etiopathogenesis of byssinosis is not yet clarified either. There have been indications that there may be some biologically active agents (bronchoconstrictors) contained in certain vegetable dusts that affect smooth muscles of bronchi, and of bronchioles in particular, producing, in this way, a spastic reduction of ventilatory capacity (2). As the content of these contractor substances in airborne vegetable dust has generally been found too low to account for the very significant acute reductions of ventilatory capacity, it has been proposed that it is some agents contained in vegetable dust that release histamine from lung tissue, thus causing, in this indirect way, a histamine-produced contraction of smooth muscles in the respiratory system (3).

There is still some dispute on whether the term byssinosis should be used to describe the reversible condition caused by the acute effects of vegetable dusts or the irreversible pathological condition developing after chronic exposure to active vegetable dusts through a longer period of time (as a rule, after at least 8-10 years of exposure). It may not be unreasonable to accept the former definition which results in an earlier diagnosis of the disease and allows the prevention of the development of its chronic stages.

There are three main objectives of the present study:

1. To verify whether cotton, flax, hemp, and jute dust should be considered as byssinogenic and to establish the relative potency of these dusts to the reduction of ventilatory capacity and development of non-specific respiratory disease.

2. To study the preventive and curative effects of bronchodilators and possibly antihistaminics in workers exposed to vegetable dusts.

3. To attempt the isolation and identification of biologically active agents contained in airborne dusts and to develop methods for the quantitative or at least semi-quantitative assessment of the contraction potency of these agents.

II. Methods

Dust Assessment

Airborne dust samples are collected by means of a modified Hexhlet apparatus and the dust concentrations are determined in two particle size groups; the total dust fraction and the respirable dust fraction.

Respiratory Symptoms

Respiratory symptoms are recorded by using the British Medical Research Council questionnaire (4) completed with some questions relating to such characteristic symptoms of byssinosis as the chest tightness or breathlessness on Monday and effort intolerance (5).

Lung Function Tests

The one-second forced expiratory volume ($FEV_{1,0}$), the forced vital capacity (FVC) and in some cases the peak expiratory flow (PEF) are determined before and after the work shift on Mondays and Thursdays. The lung function tests are carried out by the Bernstein type spirometers. Five measurements are performed in each subject during each spirometric examination, and the highest value is taken as the result of the test.

The measurements also include basic anthropometric parameters measured in each subject (standing height, sitting height, and body weight).

Byssinosis is diagnosed on the basis of the following:

1. Established exposure to vegetable dust.

2. Chest tightness and breathlessness on Mondays and/or other days in the week.

Byssinosis is graded as described by Shilling, Vigliani, Lammers, Valić and Gilson (6):

Grade 1/2 - Occasional chest tightness on the first working day of the working week.

Grade 1 - Chest tightness on every first day of the working week.

Grade 2 - Chest tightness on the first and other days of the working week.

Grade 3 - Grade 2 symptoms accompanied by evidence of permanent incapacity from effort intolerance.

Chronic bronchitis is diagnosed on the basis of the definition proposed by Fletcher et al (7), i.e., as "a condition characterized by a productive cough on most days for at least three months in the year during at least two consecutive years, provided other pulmonary diseases have been excluded."

Dyspnea is recorded on the basis of the recommendations of the Medical Research Council's Committee on the Etiology of Chronic Bronchitis (7).

III. Population

The results are presented of the studies on 102 female workers exposed to hemp dust, three samples totalling 184 female workers exposed to cotton dust (Mill A - 70, Mill B - 53, and Mill C - 60), 92 female workers exposed to jute dust, and 30 female workers exposed to flax dust.

All the workers were chosen among non-smokers.

IV. Results

Hemp Exposure

Prevalence of byssinosis, persistent cough, persistent phlegm and dyspnea (Grade 2 and 3) with corresponding mean dust concentrations are summarized in Table 1. Of 102 female hemp workers 39 percent had symptoms of byssinosis similar to those found in the previous studies (8), carders: 49 percent, spinners: 32 percent, difference not significant). No significant difference was found between carders and spinners in prevalence of any respiratory symptoms except for persistent cough which was higher in carders ($p < 0.05$). Younger and older workers, with short and long durations of exposure, had symptoms of byssinosis. Subjects with byssinosis had significantly higher prevalence ($p < 0.01$) of persistent cough (78 percent), persistent phlegm (65 percent), dyspnea (38 percent) and chronic bronchitis (56 percent) than those without byssinosis (37, 31, 26, and 30 percent respectively) (Table 2). Of eight workers exposed to hemp dust for about a year, four had characteristic symptoms of byssinosis. Of 39 byssinotics, 16 had byssinosis Grade 1/2, 15 Grade 1, and 8 Grade 2. As is seen in Table 2, prevalences of all respiratory symptoms (persistent cough, persistent phlegm, dyspnea, chronic bronchitis) were significantly higher in hemp workers than in controls irrespective of simultaneous development of byssinosis.

To assess the acute effects of hemp bust on ventilatory function, changes in $FEV_{1.0}$ and FVC during the work shift were measured (Table 3). While no changes were recorded in control workers either in $FEV_{1.0}$ or FVC, both parameters decreased significantly ($p < 0.01$) in both byssinotic and non-byssinotic hemp workers on Mondays and Thursdays (with the exception of Thursday FVC in byssinotics). The reductions of ventilatory parameters were significantly higher on Mondays than on Thursdays ($p \leq 0.01$). The mean Monday $FEV_{1.0}$ reduction was found significantly greater in byssinotics than in hemp workers without byssinosis ($p < 0.01$). This, however, was not the case on Thursdays.

In Tables 4 and 5 the effects of Alupent* inhalation before and after the work shift are presented. In the first week Alupent was administered to 44 female hemp workers on Monday at the end of the shift after lung function testing. As is shown in Table 4, the significant $FEV_{1.0}$ reduction during the shift proved to be reversible to a great extent by the administration of the bronchodilator. In fact, the mean $FEV_{1.0}$ was increased to its initial pre-shift values after the inhalation of Alupent with the exception of $FEV_{1.0}$ in byssinotics in whom the mean $FEV_{1.0}$ value remained significantly lowered in spite of the bronchodilator application. The acute effect of hemp dust inhalation in byssinotics appears to have been so strong that the administration of the bronchodilator, in spite of a significant increase of $FEV_{1.0}$, could not reverse it to its pre-shift value.

The effects of the Alupent administration before the shift are shown in Table 5. Alupent produced no change in the normal $FEV_{1.0}$ measured before exposure to hemp dust. Repeated measurements of ventilatory function the same day after the work shift showed that the inhalation of the bronchodilator before the shift decreased the reduction of $FEV_{1.0}$ during the shift. The difference between the $FEV_{1.0}$ values measured before and after the shift, in the case of Alupent administration before the shift, was, however, still significant both in byssinotic and non-byssinotic hemp workers. The application of the bronchodilator before the shift did significantly diminish the reduction of ventilatory function though not completely preventing it.

In order to assess the possible chronic effects of hemp dust on ventilatory function, a comparison was made of the values of $FEV_{1.0}$, FVC, and $FEV_{1.0}$/FVC percent obtained on Mondays before the shift in workers exposed to hemp dust and in the controls. The subjects in the control groups were selected in such a way as to almost fully match the respective exposed groups (byssinotics and non-byssinotics) in the age and height distribution. It has been assumed that the changes of ventilatory capacities in the exposed groups on Mondays before the shift, prior to the superposition of acute effects of exposure to hemp dust during the work shift, represent chronic changes in ventilatory function due to the long-term exposure to hemp dust. As can be seen from the results presented in Table 2 all three ventilatory function parameters were found significantly lower ($p < 0.01$) in subjects exposed to hemp dust than in the controls, except for FVC in byssinotics.

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* Orciprenaline: 1-/3.5-dihydroxyphenyl/-2-isopropyl aminoethanol sulphate.

Cotton Exposure

The results of the determination of the airborne cotton dust in cotton mill A are presented in Table 6. The concentrations are expressed as total dust and as respirable dust, the latter being defined as the particle size fraction that passes the Hexhlet horizontal laminar plate elutriator which conforms to the requirements of the British Medical Research Council for the particle retention in the upper respiratory tract (9). As can be seen from Table 6, the mean total dust concentration was very high.

The prevalence of chronic bronchitis and dyspnea (Grade 3 and 4) is shown in Table 7. Not a single case of byssinosis was registered among the examined workers in spite of the comparatively very high mean total dust concentration. Fourteen percent of the examined female workers had symptoms of chronic bronchitis and eighteen percent suffered from dyspnea Grade 3 or 4.

Mean $FEV_{1.0}$ and FVC changes over work shift measured in the group of 70 female cotton workers are shown in Table 8. Comparatively small but significantly ($p < 0.01$) reductions of $FEV_{1.0}$ and FVC were measured on the first day after Sunday break. The reductions were significantly smaller on Thursdays than on Mondays.

As seen from the Table 9, the mean total dust concentration in Mill B was considerably lower than in Mill A, while the mean respirable dust concentrations were similar in both mills.

The prevalence of byssinosis in workers of Mill B was found to be 20.8 percent. The prevalence of chronic bronchitis was 54.5 percent in byssinotic and 26.1 in non-byssinotic cotton workers, while the prevalences of dyspnea Grade 3 and 4 were 18.1 and 2.4 percent respectively (Table 10).

In spite of the lower mean total dust concentration in Mill B, Monday and Thursday $FEV_{1.0}$ and FVC changes over shift in workers from that mill were much more pronounced than in workers from Mill A (Table 11).

The falls of both ventilatory parameters were statistically significant on Mondays and Thursdays in byssinotic and non-byssinotic cotton workers of Mill B (Table 12). The reductions were greater in byssinotics than in non-byssinotic workers but these differences were not significant ($p > 0.05$), which can probably be explained by the comparatively small number of the workers involved.

Dust concentrations measured in cotton mill C are presented in Table 13. The mean dust concentrations, both total and respirable, were similar to those measured in cotton mill B and the respirable dust concentration did not differ much from that measured in cotton mill A. In spite of the almost three times lower mean total dust concentration as compared with that in cotton mill A, the prevalence of byssinosis was found to be 28.3 percent (Table 14). The prevalence of both chronic bronchitis and dyspnea was higher in byssinotics than in non-byssinotics.

Changes in ventilatory function values over shift in female workers employed in cotton mill C are presented in Table 15. The changes in $FEV_{1.0}$ and PEF were significantly higher on Mondays than on Thursdays ($p < 0.05$). Mean reductions of all ventilatory parameters over shift were higher in byssinotics than in non-byssinotics but the differences were not found to be statistically significant, probably because of the small number of the workers examined (Table 16).

Jute Exposure

Total and respirable dust concentrations determined in a jute mill are presented in Table 17. Although neither the mean airborne total dust concentration nor the respirable dust concentration differed much from those in cotton mills B and C, no case of byssinosis was registered in workers exposed to jute dust. The prevalence of chronic bronchitis and dyspnea was very low (Table 18).

Slight but significant changes of mean ventilatory functions were observed in jute workers both on Mondays and Thursdays (Table 19). Mean changes of all the parameters were greater on Mondays. The mean length of exposure to jute dust in the examined group of workers was only 3 years.

Flax Exposure

Thirty flax workers have so far been surveyed. Their average age was 32 years and the average length of exposure 10 years. The byssinosis prevalence was found to be 40 percent and the prevalence of chronic bronchitis 47 percent (Table 20).

There was a very significant difference in the prevalence of chronic bronchitis between byssinotics and non-byssinotics (75 percent and 28 percent respectively, $p < 0.01$) (Table 21).

The results of the measurements of Monday lung function changes over shift in 30 flax workers and 60 controls are shown in Table 22. Very significant reductions of ventilatory capacities were found in flax workers while there were no changes in the control group. The control group fully matched the exposed group in height and age distribution. In fact, the subjects of the control group were selected in such a way that each subject in the exposed group had two pairs of the same body height and age in the control group.

Although byssinotics suffered from much higher reductions of ventilatory capacities over shift than non-byssinotics, the differences were not significant, this probably being due to the small number of workers in both groups (Table 23). The acute $FEV_{1.0}$ reductions were greater both in byssinotics ($p < 0.05$) and non-byssinotics ($p < 0.01$) on Mondays than on Thursdays.

V. Discussion

The results obtained in non-smoking female workers exposed to airborne dust of hemp, flax, cotton, and jute give the basis for assuming that soft hemp, flax, and cotton dust may cause byssinosis. The significantly higher prevalence of symptoms of non-specific respiratory disease (chronic bronchitis, persistent cough, persistent phlegm, dyspnea) in byssinotic, than in non-byssinotic workers exposed to hemp, flax, and cotton dust suggests an association between the development of byssinosis and non-specific respiratory disease other than byssinosis. The mean length of exposure of our jute workers (3 years) was too short to allow any meaningful conclusion to the development of byssinosis.

Significant acute reductions of ventilatory parameters in workers exposed to vegetable dust on Mondays and Thursdays indicate that there is an acute effect of vegetable dusts on bronchial muscles which, although more pronounced at the beginning of the working week after the Sunday break, persists practically throughout the week. This can probably be explained by the lower depot of histamine in the lung tissue in the latter part of the week. Significant acute reductions of ventilatory capacities were found in workers exposed to vegetable dusts irrespective of whether or not they had developed symptoms of byssinosis but these reductions were, as a rule, more pronounced in byssinotics than in non-byssinotics.

In order to establish the rank of biological activity of vegetable dusts, five groups of female workers exposed to similar mean concentrations of hemp, flax, cotton, sisal*, and jute airborne dust respectively were compared as to the prevalence of byssinosis, chronic respiratory symptoms and acute $FEV_{1.0}$ and FVC changes over Monday shift. The five groups were selected in such a way as to approximately match in the distribution of age and length of exposure to the respective dust.

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* The results obtained on sisal workers have not yet been completely analyzed, detailed data are therefore not reported.

As seen from Figure 1 hemp and flax dust caused approximately equal prevalence of byssinosis (42 and 46 percent respectively), cotton caused considerably lower prevalence (24 percent) while no byssinosis was caused by either jute or sisal dust. Highest prevalence of chronic respiratory symptoms was recorded in hemp workers (36 percent), followed by flax (31 percent) and cotton workers (26 percent) while prevalence in sisal (14 percent) and jute workers (11 percent) was lower. The highest relative mean acute Monday reduction of $FEV_{1.0}$ was measured in hemp workers (19 percent), followed by flax (11 percent), cotton (8 percent), sisal (7 percent) and jute workers (5 percent). As the number of workers in each group was small it would be premature to attempt drawing definite conclusions as to the relative activity of the five types of vegetable dust examined. Investigations in the forthcoming stages of the project by increasing the number of subjects studied, will probably more clarify this question.

The experiments made with the inhalation of Alupent in hemp workers after shift have shown that the acute effects of vegetable dust exposure are at least partly reversible. By applying Alupent before the shift we have succeeded in significantly diminishing the reduction of ventilatory capacities during the shift, although not preventing it completely. The subjects interviewed as to their subjective feelings during the shift after Alupent inhalation claimed they felt better. We are planning to repeat the experiments with the greater doses of Alupent to be applied before the shift in order to find out if this could enhance the preventive effect of the bronchodilator.

It is difficult to interprete the byssinosis prevalence found in the three cotton mills. The three mills did not differ in the mean concentration of the respirable airborne dust, yet they differed very markedly in the prevalence of byssinosis. In Mill A the mean total dust concentration was found almost four times as high as the concentration in Mill B, yet the prevalence of byssinosis in the former was found to be nil and 20.8 percent in the latter. The mean duration of exposure to cotton dust was longer in Mill B which, to some extent, might have accounted for the higher byssinosis prevalence. In Mill C, however, with practically the same respirable dust concentration as in Mill A and with the almost four times lower total dust concentration, the prevalence of byssinosis was found to be 28.3 percent, although the mean duration of exposure of the workers examined was only 5 years. This unexpected relationship between airborne dust concentrations and the byssinosis prevalence (and to some extent the magnitude of acute reductions of ventilatory capacity over shift) raises doubts as to whether either the total or the respirable (as currently defined) dust concentration could be used as an index of the dust hazard level. The definition of "respirable particle fraction" is based on the assumption of alveolar particle deposition which is justified for pneumoconiosis-producing particles but not for particles causing changes in bronchi or broncioles. The size-selective characteristics of the first stage of two-stage dust samplers for sampling the latter kind of dust should differ from those used for pneumoconiosis-producing dust sampling.

In addition, it is obvious that the biologic activity of dust should also be taken into account when dealing with the pharmacodynamic dusts. The biologic activities of cotton dust in the three cotton mills studied are likely to have differed significantly in their respective biologic activity. The results of our preliminary testing appear to support this assumption.

The assessment of vegetable dust hazard will most likely have to be done on the basis of specific respirable airborne dust determination considering, at the same time, the biologic activity of dust.

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Table 1

Byssinosis and Respiratory Symptoms in Hemp Workers

		Carders	Spinners	Total
N		42	60	102
Ave. Age (yrs.)		33	35	34
Ave. Exposure (yrs.)		10	10	10
Mean Dust Concentrations (mg/m^3)	Total	26.93	6.24	
	Respirable*	0.87	2.66	
Byssinosis (percent)		49	32	39
Persistent Cough (percent)**		67	43	53
Persistent Phlegm (percent)		55	37	44
Dyspnea 2 and 3 (percent)		37	27	31

* Respirable - fine dust collected on filter after elutriation.

** The only significant ($p < 0.05$) difference in prevalence of respiratory symptoms between carders and spinners.

Table 2

Prevalence of Respiratory Symptoms and Mean Values of Ventilatory Function Tests in Hemp Workers and Control Subjects on Monday Before Work

	With Byssinosis	Control	P	Without Byssinosis	Control	P
N	40	33		62	45	
Age (yrs)*	32 18-51	30 18-50		35 18-49	34 18-51	
Height (cm)*	158 147-173	159 149-172		158 148-174	158 150-170	
Persistent Cough (%)	78	15	< 0.01	37	9	< 0.01
Persistent Phlegm (%)	65	18	< 0.01	31	13	< 0.05
Chronic Bronchitis (%)	56	12	< 0.01	30	11	< 0.05
Dyspnea Grade 2 and 3 (%)	38	0	< 0.01	26	4	< 0.01
$FEV_{1.0}$ (ml)	2753	3295	< 0.01	2755	3215	< 0.01
FVC (ml)	3504	3742	NS	3395	3584	< 0.01
$FEV_{1.0}$/FVC (%)	78.6	88.1	< 0.01	81.2	87.3	< 0.01

* Mean and range.
NS - not significant ($p > 0.05$).

Table 3

Ventilatory Function Tests in Exposed and Control Workers

Group	N	Test	Day	Mean Lung Function Data (ml) Before Shift	After Shift	Change	p
Hemp Workers with Byssinosis	40	$FEV_{1.0}$	Monday	2753	2177	-576	<0.01
						<0.01	
			Thursday	2737	2621	-116	<0.01
		FVC	Monday	2504	3355	-149	<0.01
						<0.01	
			Thursday	3450	3452	+2	NS
Hemp Workers Without Byssinosis	62	$FEV_{1.0}$	Monday	2755	2451	-304	<0.01
						<0.01	
			Thursday	2690	2578	-112	<0.01
		FVC	Monday	3395	3244	-151	<0.01
						<0.01	
			Thursday	3360	3309	-51	<0.01
Controls	78	$FEV_{1.0}$	Monday	3247	3249	+2	NS
			Thursday	3255	3258	+3	NS
		FVC	Monday	3707	3700	-7	NS
			Thursday	3707	3705	-2	NS

NS - not significant ($p > 0.05$).

Table 4

Effect of Bronchodilator Application After Shift

Group	N	Test	Before Shift 1	After Shift 2	t_{1-2}	After Broncho-dilator 3	t_{1-3}
With Byssi-nosis	20	Mean $FEV_{1.0}$	2825	2007	10.7*	2580	3.7*
		Mean FVC	3600	3379	5.0*	3584	1.6⁻
No Byssi-nosis	24	Mean $FEV_{1.0}$	2850	2135	7.8*	2780	1.3⁻
		Mean FVC	3610	3423	4.4*	3590	0.6⁻

* Statistically significant difference ($p < 0.01$).

⁻ Not significant ($p > 0.05$).

Table 5

Effect of Bronchodilator Application Before Shift

Group	N	Test	Before Shift: Before Broncho-dilator 1	Before Shift: After Broncho-dilator 2	After Shift 3	t_{1-3}
With Byssi-nosis	20	Mean $FEV_{1.0}$	2825	2825	2410	4.9*
		Mean FVC	3600	3620	3548	2.7**
No Byssi-nosis	24	Mean FEV	2850	2850	2576	6.3*
		Mean FVC	3610	3622	3560	2.1**

* Statistically significant ($p < 0.01$).

** Statistically significant ($p < 0.05$).

Table 6

Dust Concentrations in the Cotton Mill A

Concentrations (mg/m^3)	Total Dust (mg/m^3)	Respirable Dust (mg/m^3)
Mean	15.23	0.43
Maximum	82.35	1.32
Minimum	0.71	0.03

Table 7

Prevalence of Respiratory Symptoms Among Male and Female Cotton Workers in Mill A

Group	N	Chronic Bronchitis		Dyspnea Grade 3 and 4	
		N	%	N	%
Men	18	4	22.2	1	5.6
Women	70	10	14.3	13	18.6
Total	88	14	15.9	14	15.9

No case of byssinosis was registered.

Table 8

Mean $FEV_{1.0}$ and FVC Changes Over Shift in Female Cotton Workers in Mill A

Group	N	Test	Day	Mean Values (ml)			
				Before Work	After Work	Reduction	P
Women	70	$FEV_{1.0}$	Monday	2772	2635	-137	<0.01
			Thursday	2787	2746	-41	<0.05
		FVC	Monday	3552	3519	-133	<0.01
			Thursday	3529	3492	-37	NS

Table 9

Dust Concentrations in the Cotton Mill B

Concentrations (mg/m^3)	Total Dust (mg/m^3)	Respirable Dust (mg/m^3)
Mean	4.05	0.48
Maximum	9.11	0.60
Minimum	1.06	0.24

Table 10

Prevalence of Chronic Bronchitis and Dyspnea in Mill B

Group	N	Chronic Bronchitis N	Chronic Bronchitis %	Dyspnea Grade 3 and 4 N	Dyspnea Grade 3 and 4 %
With byssinosis	11	6	54.5	2	18.1
Without byssinosis	42	11	26.1	1	2.4
Total	53	17	32.1	3	5.7

Prevalence of byssinosis 20.8%.

Table 11

Ventilatory Function Changes in Cotton Workers in Mill B

Group	N	Test	Day	Mean Values (ml) Before Work 1	After Work 2	Reduction	P 1-2
Exposed Workers	53	$FEV_{1.0}$	Monday	2796	2487	-309	< 0.01
			Thursday	2786	2710	-76	< 0.01
		FVC	Monday	3546	3380	-166	< 0.01
			Thursday	3501	3432	-69	< 0.01

Table 12

Mean Reductions in Lung Function Values on Monday and Thursday in Cotton Mill B

Group	N	Monday 1	Thursday 2	p 1-2	Monday 1	Thursday 2	p 1-2
With byssi-nosis	11	-515	-77	<0.01	-189	-73	<0.01
Without byssi-nosis	42	-255	-75	<0.01	-160	-66	<0.01
With byssi-nosis without byssinosis		NS	NS		NS	NS	

NS - not significant ($p > 0.05$).

Table 13

Dust Concentrations in the Cotton Mill C

Concentrations (mg/m^3)	Total Dust (mg/m^3)	Respirable Dust (mg/m^3)
Mean	4.18	0.64
Maximum	12.73	1.09
Minimum	0.63	0.10

Table 14

Prevalence of Respiratory Symptoms in Mill C

Group	N	Chronic Bronchitis N	Chronic Bronchitis %	Dyspnea Grade 3 and 4 N	Dyspnea Grade 3 and 4 %
With Byssinosis	17	5	29.4	2	17.6
Without Byssinosis	43	9	20.9	4	9.3

Prevalence of Byssinosis 28.3%

Table 15

Ventilatory Function Test in Female Cotton Workers in Mill C on Monday and Thursday (N = 60)

Days	Mean Changes					
	$FEV_{1.0}$ ml	p	FVC ml	p	PEF (1/min)	p
Monday	-138	<0.01	-108	<0.01	-23	<0.01
	0.05		NS		0.05	
Thursday	-92	<0.01	-78	<0.01	-13	<0.01

NS - not significant ($p > 0.05$).

Table 16

Ventilatory Function Tests in Female Cotton Workers
Over the Shift on Monday in Mill C

Group	N	Mean Reductions					
		FVC		$FEV_{1.0}$		PEF	
		ml	p	ml	p	l/min	p
With Byssinosis	17	-134	<0.01	-174	<0.01	-27	<0.01
		NS		NS		NS	
Without Byssinosis	43	-98	<0.01	-123	<0.01	-22	<0.01
Total	60	-108	<0.01	-138	<0.01	-23	<0.01

Table 17

Dust Concentrations in Jute Mill

Concentrations	Total Dust (mg/m^3)	Respirable Dust (mg/m^3)
Mean	3.22	0.73
Maximum	7.62	1.09
Minimum	0.68	0.11

Table 18

Prevalence of Chronic Bronchitis in Jute Mill

N	Chronic Bronchitis N	Chronic Bronchitis %	Dyspnea Grade 3 and 4 N	Dyspnea Grade 3 and 4 %
92	13	14.2	1	1.1

No case of byssinosis registered.

Table 19

Ventilatory Function Test in Jute Workers
on Monday and Thursday (N = 92)

Days	Mean Changes					
	$FEV_{1.0}$ (l)	P	FVC (l)	P	PEF (l/min)	P
Monday	-0.12 < 0.01	< 0.01	-0.06 < 0.05	< 0.01	-15 < 0.01	< 0.01
Thursday	-0.07	< 0.01	-0.03	< 0.01	-11	< 0.01

Table 20

Mean Total Dust Concentration and Prevalence of Respiratory Symptoms in Flax Workers

N	Ave. Age (yrs)	Ave. Exposure (yrs)	Mean Dust Concentration (mg/m^3)	Byssinosis %	Chronic Bronchitis %
30	32	10	6.368	40	47

Table 21

The Prevalence of Chronic Bronchitis in Byssinotic and Non-Byssinotic Flax Workers

Group	N	Ave. Age (yrs)	Ave. Exposure (yrs)	Chronic Bronchitis %
With Byssinosis	12	34	12	75
				< 0.01
Without Byssinosis	18	31	9	28

Table 22

Mean Changes of Ventilatory Capacity During Shift in Flax Workers

Group	No	Day	FVC (ml)				$FEV_{1.0}$ (ml)			
			Before Shift	After Shift	Change	t	Before Shift	After Shift	Change	t
Exposed	30	Mon.	3623	3417	-206	5.7*	2875	2454	-421	7.1*
		Thurs.	3555	3423	-132	4.6*	2771	2589	-182	6.6*
Control	60	Mon.	3705	3704	-1	0.2**	3254	3258	+4	0.9**
		Thurs.	3704	3705	+1	0.3**	3262	3260	-2	0.1**

* Statistically significant change ($p < 0.01$).
** Not significant ($p > 0.05$).

Table 23

Mean Changes in $FEV_{1.0}$ and FVC Over Shift on Monday and Thursday in Flax Workers

Group	N	$FEV_{1.0}$ (ml) Monday	Thursday	P	FVC (ml) Monday	Thursday	P
With Byssinosis	12	-468	-245	< 0.05	-245	-141	NS
		NS	NS		NS	NS	
Without Byssinosis	18	-391	-140	< 0.01	-181	-126	NS

NS - not significant ($p > 0.05$).

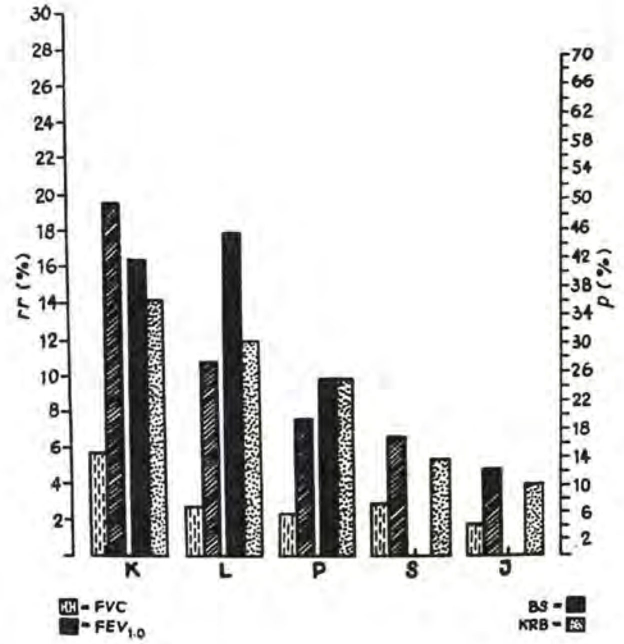

Figure 1. Comparison of the results obtained in workers exposed to five different types of vegetable dust.

FVC	:	Forced Vital Capacity
$FEV_{1.0}$	:	Forced Expiratory Volume in the first second
BS	:	Byssinosis
KRB	:	Chronic Respiratory Symptoms
p (%)	:	Prevalence in percentage
rr (%)	:	Relative reduction in percentage

DISCUSSION AFTER DR. VALIC'S PAPER:

Dr. Saric:

Will you, for comparative reasons, say a few words about the definition of chronic bronchitis you used in these tables you showed to us, and also the definition of byssinosis.

Dr. Valić:

The definition of chronic bronchitis that we used is the well-known definition of Fletcher of the British Medical Research Council, that we take it as a condition characterized by a productive cough at least three months in the year during at least two consecutive years provided other pulmonary diseases have been excluded. This is a well-recognized definition of chronic bronchitis. The definition of byssinosis is a little bit more difficult because, in fact, there is no definitely accepted definition of byssinosis. But what we take as byssinosis is probably what the majority of the workers in byssinosis in the world take now and that is a pulmonary disease characterized by a chest tightness on first working day and subsequent working days after a short break in exposure of a day or more and accompanied by a sharp reduction of ventilatory capacity. But the second part of the definition is put in brackets because many people do not take that into consideration. As a matter of fact I must say that even we did not definitely always consider the second part. So the significant acute change ventilatory capacity.

Dr. Potkonjak:

I apologize, perhaps I missed hearing you, but I really didn't hear whether your subjects were smokers or non-smokers or both? Because I think some correlation between byssinosis and chronic bronchitis, it seems to me, is possibly only in non-smokers.

Dr. Valić:

Thank you Dr. Potkonjak to have put it so nicely that you may be mishearing me because I really didn't say it. All our subjects were non-smokers, and I fully agree with you that the correlation between byssinosis prevalence and chronic bronchitis probably in the pure form of correlation is to be expected only for non-smokers.

Dr. Cralley:

I agree with Dr. Valić on the definition of the term "respirability." It may be different depending on the type of dust and the area of the respiratory system affected. The type of sampling instrument will have to take these facts into consideration. As I recalled the concentrations of a dust from the flax, hemp, and cotton were all at the same general levels. I don't recall any similar data you have on the sisal or jute. Are different industrial operations concerned with these than those of the flax, hemp, and cotton as far as production is concerned?

Dr. Valić:

The technology that we found in the plants where we studied jute and sisal workers is different than the technology of flax, hemp, and cotton workers and especially in the initial stages of the process. As a matter of fact, there are not initial stages of the process in the jute and sisal workers.

Dr. Szymczykiewicz:

I should like to speak again about micro-organism problems. Are you sure Dr. Valić that these constrictor compounds, which you obtained from the cotton dust, are really of cotton origin, because we extracted the bacteria living in the cotton dust and we know that these bacteria produce endotoxins which have had the same constrictor effect on muscles like these obtained in your investigation. For my opinion, it is impossible to separate bacteria from the dust. I suppose that effect in your investigations may be also produced by the endotoxin extracted from bacteria living on the surface of organic dust.

Dr. Valić:

I cannot answer you from the basis of our results because we do not have our results. When I made the remark in respect to the bacterial pollution I did not speak about cotton then, because our experience in this respect is not connected to cotton. It is connected to completely different sort of organic dusts. That is number one. Secondly, speaking of endotoxin, now we have not seen the experiments in Italy and had the chance of discussing the endotoxin theory proposed for pathogenesis of byssinosis. I think that the main weak point of the endotoxin in vegetable dust is that it cannot be so high as to produce such very strong acute effects as we found in here.

Dr. Szymczykiewicz:

The bacteria, living in the cotton dust are simply saprophytes living mainly on the surface of the earth and in the water. I am not sure that these bacteria don't exist in other dust, for instance, jute, flax, and so on. I didn't investigate it in the flax and jute, but it seems to me that these same bacteria are living in all organic dusts.

Dr. Valić:

I really cannot say anything about cotton in this respect. I just don't know. You may be right. I think that the results published so far do indicate that the bacterial action would not cause such strong acute effects as that found in this cotton dust. So it is probably a pharmacological action, rather than the bacteriological action. Our other experiments speak against these questions of bacteria.

Dr. Potkonjak:

As I remember, you showed that decrease of FEV values is parallel with the increase of the years of work. Don't you consider that some other factors could come into the play. For example: the role of the age, the role of the emphysema developed. Therefore, I would like to ask you: it is now emphasized that FEV is a non-physiological test which can give us false results. Especially in a subject of higher age and it is suggested that only measurement of the airway resistance can be really useful.

Dr. Valić:

First of all as to your question whether age could be responsible for the effect, I think we excluded this effect. We carried out multiple regression analyses and made allowance for the age effect. We did it in another way besides this mathematical correlation by having control groups. Matching the exposed group and control group in age we did not find the same effect of the drop of FEV. That is number one. Now your second remark of course whether we should consider FEV as a satisfactory basis for testing some lung function. That is another point. As you know the majority of researchers use FEV tests as the simpliest test for epidemiological studies.

CARDIOPULMONARY FUNCTION IN COAL MINERS EXPOSED TO COAL DUSTS OF DIFFERING COMPOSITION

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As only the second year of the Research Program was finished, the information given in this survey will only illustrate data collected in the past two years, and the prospective plans of the future works. It was not considered as opportune to give the statistical analysis of the data collected, so no conclusion was attempted to be drawn. The only intention is to show what has been done in the past two years and it is hoped that your suggestions and comments will strengthen the next three years work.

I. THE OBJECT OF THE STUDY

The general objective of the present study is to examine the cardiopulmonary function and work capacity of coal miners exposed to different amounts and compositions of coal dust, and relate these exposures to impairments such as pulmonary emphysema, chronic bronchitis, pneumoconiosis and allergic conditions.

In fact the essential purpose of the foregoing study would be "to evaluate the impairment of the health and working capacity of coal miners as related to length and type of exposure histories and to establish the importance of the coal miner's occupational environment in affecting his health and working capacity."

From this study we will try to answer the following questions:

1. Is there any signficant impairment in the pulmonary function in miners which could be observed as due to their occupation?

2. If any, what kind of impairment is present?

3. Is the comparison of findings between miner groups and control groups statistically significant?

4. What is the relationship between the pulmonary function impairment and,
   - the exposure history,
   - cigarette smoking, and
   - chronic non-specific lung diseases?

5. What are the effects of the air-borne dust on the pulmonary structures?

6. Are there any statistically significant effects of working conditions of miners on their morbidity and mortality rate?

Material and Plan of the Study

According to the Research Agreement the study is conducted in two different mines:

a. A hard coal mine with a low percentage of free silica dust, and

b. A soft coal mine with no free silica dust.

"A sample of 150 miners has to be selected for each of the two mines and a like-sized control group of non-miners..." The selection of subjects "may be based on chest X-ray evidence of presence or absence of pulmonary involvement or other clinical signs and symptoms."

Effective period of the study is five years.

As for the observations to be made they are as follows:

a. Examinations of the subjects according to the defined program.

b. Examinations of working conditions including a description of of the general working conditions and measurements of all exposure factors such as dust, temperature, humidity, air velocity, effective temperature, air gases, analysis of dust samples for free silica and other materials, and measurement of light and noise conditions.

c. Morbidity and mortality experiences of the subject groups during the study have to be compared with those of the general population to determine whether any trends referrable to the objectives of the study occur.

II. IMPLEMENTATION OF THE PROGRAM

In accordance with above defined plan the study started in the soft coal mine "Kostolac" and the hard coal mine "Baljevac na Ibru" (Ibar hard coal mines Baljevac).

Characteristics of the Mines

In "Kostolac" both underground and surface operations are conducted. The thickness of the underground coal layers is 1-3 meters. The mining is both manual and mechanized. The ventilation is artificial, being furnished by a ventilator of the large capacity situated at the entry to the pit. The coal masses are of younger age, very humid - humidity up to 47%; the free silica content is as low as 1.2%.

In the "Ibar hard coal mines Baljevac" there are two pits "Jarando" and "Ušće" having similar characteristics. The content of the free silica both in the coal masses and in the ingredients ranged from 11.2% to 26.6%.

Subjects and Testing Procedures

To form the subject groups nearly all miners present were examined. The actual selection of the subjects to be used for the follow-up study was made on the basis of the following criteria; excluded were those who previously worked in any other mine where they could be exposed to agents affecting the cardiopulmonary system; who showed any clinical, electrocardiographical or radiological abnormality that could affect the cardiopulmonary function such as deformities of the thorax, marked pleural adherences, previous allergic conditions, sequellae of a previous chest disease, heart abnormalities etc; and who were likely to retire in the five years period.

Surface workers in "Kostolac" and surface workers of a magnesite mine in "Baljevac" were used to form corresponding control groups. The selected workers were examined in the same way as the miners and the same selection criteria were used. The only difference between subjects used as controls and the miners, is that the control subjects were not exposed to the inhalation of dust. Therefore, all subjects who previously worked underground were excluded.

The number of subjects selected in each group to be followed-up is greater than planned to be available in the fifth year. This was done because a reduction in number in the five-year period is to be expected.

As the age is definitely related to the pulmonary function, each of the studied groups was divided into sub-groups matched for age. Furthermore, in each group smokers are considered separately from non-smokers.

Procedures used in this study are in accordance with the program defined by the Research Agreement but some modifications and extension of the program were made. Details of individual procedures are as follows:

- examinations of working conditions will be carried out each year and will include all important compartments of the mines;

- the occupational and general history are based on the standardized questionnaire made by the experts of the European Community for Coal and Steel and modified and adapted for actual conditions;

- clinical examinations are oriented towards the cardio-pulmonary system including all other structures relatable to the cardiopulmonary status;

- an X-ray examination is not planned to be repeated in each year over the period of the study, since significant X-ray changes cannot be expected in one year intervals; therefore, the standard X-ray examination done in the first year would be repeated in the third and fifth year over the period of the study; a special X-ray examination would be done whenever indicated, for instance, paranasal spaces were examined in cases where it could be presumed these structures were relatable to the bronchial status;

- electrocardiography - standard, bipolar and unipolar peripheral and precordial leads;

- spirometry tests, FVC, $FEV_{1.0}$, IMBC, air trapping test with bronchodilating and bronchoconstricting drugs, if indicated will be repeated in each year;

- standard blood examination, red cells, haemaglobin, eosinophiles, will be repeated in one year intervals, whereas;

- the sputum examination for eosinophiles; and

- routine diagnostic skin tests using also allergens prepared from materials to which subjects are exposed, would be repeated in the third and fifth year.

In order to follow-up the progression of the impairment of the lung function in more details, special groups of subjects both miners and non-miners were formed. In total, 207 subjects were selected, subjects who were considered to be very likely available for the reexamination over the period of the next several years.

For these subjects an extended program of examination will be repeated once in two years. In addition to the general program of works described, the extended examinations of these subjects will include:

- measurement of the residual volume and the functional residual capacity;
- tests of alveolar gas mixing (single breath test, multiple breaths, N_2 wash-out, lung clearance index);
- blood gases analysis at rest and during efforts (pH, pCO_2, standard and actual bicarbonates, total CO_2, pO_2, SaO_2);
- capnographic tests (pCO_2 in the alveolar gas, arterioalveolar CO_2 gradient, single breath test);
- static compliance test;
- haematocrit and cholinesterase in peripheral blood;
- 17-keto-steroides in urine and parasites in feces; and
- cytology, bacteriology and mycology of sputum.

Experimental animals were used to verify eventually present morphological changes due to the effects of dusts which inhalation miners are exposed. For this purpose 40 albino-rats of Wistar type were used and exposed to the dust in the experimental chanber six hours daily. The dust from the two mines was used and passed through a system of sieves before application. In this way we obtained dust nearly identical to the dust to which miners are exposed during their regular work: 95% of particles having a diameter less than 5 microns and 85% less than 1 micron.

The objective was to verify the eventual changes in airways during a six month exposure.

A routine evidence of the morbidity of the subjects selected, both miners and non-miners, is regularly conducted in local medical offices. As a number of miners retired in 1969, their medical documentation is separately under the scope.

III. RESULTS

Measurements of Exposure Factors

"KOSTOLAC" - UNDERGROUND

Measurements in 11 compartments

Ts	from	18°	to	27°C
Tv̄	from	19°	to	26.2°C
Rv	from	70%	to	97%
W	from	0.28	to	3 m/sec.
ET	from	18°	to	24°C

Outside conditions measured:

Ts - 18°C
Tv - 14°C
Rv - 65%
W - 2.5 m/sec.
ET - 12°C.

Diameter of particles/ Microns	No. of Particles in 1 cm3	%
0.3	180 - 726	33.86 - 65.75
0.8	48 - 276	16.96 - 26.65
1.0	50 - 114	5.90 - 16.40
1.5	20 - 42	2.81 - 6.71
2.0	10 - 32	1.30 - 6.25
2.5	8 - 8	0.93 - 4.23
3.0	5 - 24	0.50 - 3.23
4.2	5 - 24	0.00 - 4.60
5.6	0 - 24	0.00 - 5.74
6.9	0 - 24	0.00 - 8.04

Free silica in dust 1.2%
Gravimetry: 1-5 mg/m^3

Legend: Ts - dry temperature
Tv - wet temperature
Rv - relative humidity
W - air velocity
ET - effective temperature

Nitrous oxides: 0.0 - 2.4 mg/m^3
Carbon monoxide: 0.0 - 78 ppm

"IBAR HARD COAL MINES" - UNDERGROUND

Measurements in 34 different compartments

	1° "Jarando"	2° "Ušće"
Ts	from 12° to 29°C	17.5° to 21°C
Tv	from 10° to 20°C	16° to 19°C
Rv	from 63% to 98%	83% to 91%
W	from 0.01 to 1.70 m/sec	0.20 to 1.20 m/sec
ET	from 11.7° to 28.5°C	13.7° to 19°C

Outside conditions measured:

Ts - 3°C
Tv - 3°C
Rv - 100%
W - 1.10 m/sec
ET

Outside conditions measured:

Ts - 7°C
Tv - 6°C
Rv - 87%
W - 0.30 m/sec
ET - 4.5°C

Measurements of dust (both pits - "Jarando" and Ušće")

Diameter of particles (microns)	No. Of particles in 1 cm^3	%
0.3	168 - 2.816	39.37 - 61.08
0.8	84 - 848	15.94 - 24.32
1.0	48 - 256	6.22 - 13.48
1.5	24 - 174	1.94 - 8.04
2.0	15 - 152	0.96 - 3.84
2.5	10 - 108	0.81 - 3.44
3.0	5 - 84	0.48 - 3.44
4.2	5 - 84	0.97 - 4.45
5.6	5 - 84	0.86 - 4.48
6.9	0 - 108	0.00 - 6.74

Free silica in dust from 4.6% to 26%
Gravimetry: "Jarando": 1-8 mg/m^3
"Ušće": 1-16/410/mg/m^3

	"Jarando"	"Ušće"
Nitrous oxides	: 0.0 - 4.4 mg/m^3	0.0 - 0.8 mg/m^3
Carbon monoxide	: 0.0 - 83 ppm	20 - 104 ppm

Microclimate measurements in "Kostolac" showed:

- a temperature ranging from 20°C to 27°C, the effective temperature being from 18°C to 23°C;
- a relative humidity exceeding 90% except in transportation corridors where it ranged from 70% to 78%;
- air flow velocity being as low as 0.55 to 3 m/sec. and in new corridors 0.38 to 0.50 m/sec. only;
- insufficient lighting, 3-8 lux.

The characteristics are: an increased effective temperature in the zone of comfort; an increased humidity; a low air flow velocity in new corridors; lighting on the lowest permissible level.

The air-borne dust concentrations are very low - below the MAC, probably as the consequence of a high content of water ingredients in the coal masses (up to 47%).

The low content of free silica - 1.2% only - is in accordance with the fact that silicosis was never found.

Microclimate measurements in "Baljevac" showed:

- a slightly increased temperature ranging from 22°C to 29°C, the effective temperature ranging from 20°C to 28.5°C in one of the two pits; a little lower in the other one - temperature from 17°C - 20°C, the effective temperature from 13°C to 18°C;
- an increased relative humidity exceeding 80% (up to 90%);
- a low air flow velocity, particularly in blind corridors: below 1.3 m/sec.;
- insufficient lighting: 3-5-10 lux.

The concentrations of the air borne dust exceeded the MAC as the coal masses are dry, giving rise consequently to the abundance of dust present in the air.

The content of free silica in the coal masses is low - 4.6%, whereas in laporous admixtures it ranges from 11.2% to 26.6 , "Ibar coal mines - Boljevac" are not known as silicosogenic.

Subjects Selected

Obviously, the number of subjects available for reexaminations from year to year is decreasing, but from the numbers given in Table 1 it is reasonable to believe that at the end of the fifth year the number of subjects available will be as planned (Table 1).

The most numerous is the age sub-group 30-40 years. That is quite normal but it can be noticed that in the second year (Table 2) the number of subjects of that sub-group increased on account of the antecedent group. Such a tendency may be expected in the years to come. As for the tobacco smoking habit it can be observed that the incidence of smokers, ex-smokers and non-smokers showed a relative stability (Table 3). Small differences in the second year can be attributed to real variabilities and also to the fact that a given subject may not give the same answers year to year.

Results of the Examinations of Subjects

The X-ray examination was the basis for selection and obviously no significant X-ray abnormality was present in the selected subject. Increased lung field markings, fibrosis (L or Z type), pleural adhesions, a deformities of the thorax or spine which were registered were of an insignificant degree and of no effect on the cardiopulmonary function.

There was not a single case with an abnormal ECG or blood picture (one case of WPW syndrome and one with a left bundle branch block were rejected). As the lowest level of red cells count 3.8 million per cmm was taken and for hemoglobin 80%. All selected subjects were above that level.

Skin tests for allergy were positive in a small number of subjects in both mines. Out of 302 examined miners, in 64 skin tests, one, two or more were positive but no relationship with eosinophiles in blood and sputum could be established. Moreover, no relationship was established with past and family history, with wheezing either.

A standard questionnaire for chronic bronchitis was given, and along with chronic coughers bringing up phlegm, there was a number of dry coughers (some 10% to 15% of all chronic coughers).

The incidence of cough is shown in Table 4. There is no significant difference between the incidence in 1968 and 1969. Small variations are due to the real occurrences in different groups but the different answers may also have some effect. Some difference in the incidence of cough may be observed between miners and controls in both mines. It is not yet possible to evaluate the significance of these differences.

Tables 5, 6, and 7 show a comparative survey of spirometry findings in two succeeding years. The graduation of these findings is given according to the Paris' school (Dr. D. Brille). A slightly abnormal $FEV_{1.0}$ means one standard deviation less than 100%, or Tiffeneau test, i.e., $FEV_{1.0}$, FVC, less than 73%; moderately abnormal $FEV_{1.0}$ two standard deviations less than 100% or Tiffeneau test less than 65%; abnormal $FEV_{1.0}$ more than two standard deviations less than 100%, or Tiffeneau test less than 50%. It should be remarked here that only a decrease of the $FEV_{1.0}$ was found (obstructive type of abnormality), whereas no case of the reduction of the vital capacity alone was found.

When comparing the findings from 1968 with those from 1969 it can be observed that the incidence of abnormalities in 1969 is higher than in 1968 and that those abnormalities were found in the cases with normal finding in 1968. Some subjects with abnormalities found in 1968 showed a worsening in 1969. Of course, differences are noticed only, and no conclusion about their significance is to be drawn.

The comparison of findings in miners with those in controls show the abnormalities are in higher incidence in miners, in all groups (Table 5).

When this parameter is considered in relation with the tobacco smoking habit it may be said as obvious; that the abnormalities are more prevalent in smokers and that the control subjects show normal findings in a higher incidence when considered separately, both in the group of smokers and non-smokers (Table 6).

The $FEV_{1.0}$ considered in relation with cough (Table 8) suggests some relationship. An abnormal $FEV_{1.0}$ is present only in chronic coughers in general. Of course, further follow-up findings will either confirm such a conclusion or not.

Bronchospasm is verified by the bronchodilating and bronchoconstricting drugs applied. If the difference between a before drug $FEV_{1.0}$ value and value after the drug has been applied was higher than 10%, spastic mechanism was considered to be present. In this way bronchospasm was found in 44.2% of all subjects (Table 9). Out of all subjects with verified bronchospasm, 70.1% had a normal $FEV_{1.0}$; 26.8% of them were non-coughers (Table 9).

The measurement of the residual volume was accomplished by the nitrogen wash-out method and the results obtained were compared with normal values calculated by the experts of the European Community for Coal and Steel. The measurements showed a prevalence of subjects with an increased residual volume; such findings were also observed in younger age groups (Table 10).

As it was previously stated the blood gases analyses included measurement of pH, CO_2 content, pCO_2, total CO_2, O_2, pO_2, SaO_2, bicarbonates (actual, standard, total). As an illustration the incidence of increased and normal pCO_2 and the increase of pO_2 after the effort is presented in Table 11. It can be noticed that 46.9% of all examined showed an increased pO_2 more than 10% after the effort; such a finding cannot be related with $FEV_{1.0}$, cough or RV. Succeeding examinations will have to clarify this.

It was observed that a number of subjects gave positive answers to the usual question "Do you cough first thing in the morning...", but they insisted it was a dry cough. To the questions concerning expectoration their answers were generally negative, they denied phlegm production. Some 8% to 15% of all chronic coughers resisted all efforts of the questioner to find out any hidden phlegm production.

A number of chronic coughers without and with phlegm production were selected for bronchoscopic examination. The objective was to apply this method to such subjects to visualize the inside aspect of the bronchial tree and to try to reveal the presence or absence of signs of inflammation. Along with that histological observations of mucosal samples and cytology and bacteriology of bronchial smears were done.

Bronchoscopic examinations revealed the signs of inflammation of bronchial structures both in the productive and dry coughers; hyperemia and edema of the mucosa, flushing of the blood vessels, flattening or swelling of the mucosa and variously shaped mucus in the productive coughers; in the dry coughers mucus was absent as a rule or present in the form of small and viscus patches. Both in the productive and in the dry coughers multiple scars and coal infiltrations were found; in some dry coughers atrophic mucosa was a characteristic finding.

Cytological examination of bronchial smears showed also chronic inflammatory elements both in the productive and in the dry coughers. Figures 1 and 2 show microscopical findings in a case of the chronic productive cough: polynuclear granulocytes predominant and mucus of filament structure; also macrophagocytes and columnar epithelial cells bearing cilia are seen. Figures 3 and 4 show different microscopical elements, columnar or cuboidal cells bearing cilia, flattened epithelial cells, macrophagocytes with dust particles inside, but no polynuclear granulocytes, no filaments of mucus. The subject with these findings is a dry cougher.

Figures 5 and 6 are biopsy specimens of a dry cougher. It can be seen that there is bronchial gland atrophy with small acini with minimal evidence of mucus production and some acini transformed into the fibrous structures; sporadically metaplastic changes of the epithelium with rounded cells of varying size and pleomorphic nuclei; and chronic inflammatory cells.

As the preliminary conclusion it may be said that in dry coughers the inflammatory signs are present but further investigation would be required to prove that conclusion.

Findings are given in Table 12. It can be observed that the presence of signs of inflammation are common in subjects with productive cough (positive finding in 39 subjects, negative in 9, the relation being 4.3:1); that these signs are present in dry coughers (in 12 out of 16 subjects; relation 3:1); that they can be found in subjects who do not cough. Again, the plausibility of answers obtained must be considered with suspicion.

The cytology of the bronchial smear is less convincing, although more suggestive in subjects with productive cough. The cytology of the sputum itself does not mean an absolute diagnostic test. This is well known and the unreliability of the cytology sputum findings may be considered as due to many factors including the technical difficulty in obtaining the proper material. It can be seen that positive findings were obtained even in the sputum of subjects who do not cough as well as those with a dry cough (Table 13).

The mycology sputum examination revealed different fungi mostly of saprophytic nature. Further examination should supply more information.

Experimental animals were exposed to the dust from mines, the time of exposure being six weeks.

Some differences between animals exposed to the dust from "Kostolac" and control animals could be noticed, although a significant prevalence of the involvement of the bronchiolar tree was not established; only a higher incidence of changes in the lung parenchyma in the exposed group was found (signs of inflammation and thickening of the inter-alveolar septa).

Almost the same can be said for animals exposed to the dust from "Baljevac." A six week exposure proved to be much to short for conspicuous changes to be developed. Therefore a new experiment is planned where the exposure should be longer.

IV. COMMENT

The two years work revealed quite a number of difficulties, technical failures, and imperfections in the methodology and execution. Fortunately, omissions and handicaps can be overcome and this is the opportunity to point to those we are aware of.

The fluctuation of the subjects selected was not unexpected, and in fact, it did not present itself as a problem. The migration of miners in both mines is of a minimum degree and that is one of the reasons why "Kostolac" and "Baljevac" mines were selected. Yet, a number of miners included in the group to be followed-up was unexpectedly lost, they retired because of non-medical conditions. The unfavorable consequence was in fact, that a number of those miners were from the younger age groups.

Another problem to be mentioned, is the problem of the adequacy of cooperation of the subjects examined. First of all, it is related to the data obtained by the interview. Not infrequently there was a disagreement between answers taken by questionnaires in 1968 and 1969. For example, it happened that a questioner registered the answer to the question "Do you smoke?" as "yes, 10 years"; while the same subject declared one year before that he had been a non-smoker!. Certainly, the difficulty will be overcome in the subsequent years, as the same question will be asked several times.

The same goes for the spirometry examination, for instance, where a good cooperation is the condition sine qua non. As for that examination, different circumstances can compromise the results obtained, different atmospheric conditions or a different actual health situation in two subsequent years can make the results incomparable.

Many other tests used can be spoiled by unforeseen factors and make the results useless. For example, different respiration rates have different effects on the results in the capnographic examination; or a single breath test in the nitrographic examination can give different results depending on whether this test was accomplished before or after the multiple breath test. Compliance testing can be affected by the fact whether stomach was full or not.

It is not always possible to control all these factors under conditions of a field examination.

The subject of this Research Program involves the collection of data reflecting the chronic condition of subjects examined. By a physical (clinical) examination the signs of abnormality obtained may be the same both in chronic and acute disease.

The comparison of the morbidity of miners with that of the general population has not yet been made. Certainly, the time is too short for such a consideration, but besides this another reason must be mentioned the influenza epidemic compromised the adequacy of the observations.

The examination of the subjects involve quite a number of pulmonary function tests some of which can be performed in a specially equipped laboratory only. The technical and financial reasons compeled us to (1) reduce the number of subjects to be examined, (those selected were those with the highest probability of being available for the re-examination all during the time); (2) to reduce the accomplishment of these tests to the two year period.

V. CONCLUSIONS

As it was emphasized, this is a follow-up study where a phenomenon is observed on the function of time. Consequently, the time passed was used for data collecting only; moreover, the same is valid for the subsequent periods, and the significance of data available up to now may be estimated only in terms of impressions.

On the ground of experiences gained it is possible to establish some fundamentals for the further works:

1. The procedures used in the study will not be essentially changed; possible modifications would serve to eliminate the parasite and spoiling factors only.

2. Everything possible should be done to have the same assistants for the same tasks up to the termination of the program.

3. All possible corrections will be done without delay; the experimental work will be repeated in the proper way.

4. The criteria for the selection of data collected will be established on the proper ground; likewise, the criteria for the interpretation will be adapted to the current concepts.

5. The problem of dry coughers needs a further elaboration due to their functional and structural nature.

6. The firm basis for the expectation that the number of subjects to be followed will be greater than planned should be secured; this because there is an impression that the effective period of five years seems to be much too short and the indications and possibilities for the extension of the research should be reviewed.

Table 1

Nŏ OF SUBJECTS EXAMINED

KOSTOLAC

GROUP	1968		1969	1970	1971	1972
	EXAMI-NED	SELEC-TED				PLANEDCCA
MINERS	432	302	302 (280+22)			150
CONTROLS	295	214	198 (103+95)			150

BALJEVAC

GROUP	1968 EXAMINED	1968 SELECTED	1969	1970	1971	1972 PLANEDCCA
MINERS	464	411 (321+90)	411	351		150
CONTROLS	265	181	146	146		150

Table 2

SUB-GROUPS MATCHED FOR AGE (PERCENTAGE)

KOSTOLAC

MINERS

	30 >	31 - 40	41 - 50	50 <
1968	22.8%	46.4%	26,5%	4,3%
1969	15.7%	55.4%	26.8%	1,1%

CONTROLS

	30 >	31 - 40	41 - 50	50 <
1968	25.3%	50,9%	21,0%	2,0%
1969	20,4%	48,6%	24,3%	6,7%

BALJEVAC

MINERS

	30 >	31 - 40	41 - 50	50 <
1968	23,4%	51,1%	24,3%	1,2%
1969	17,0%	57,2%	22,4%	3,4%

CONTROLS

	30 >	31 - 40	41 - 50	50 <
1968	27,1%	47,5%	20,4%	5,0%
1969	17,1%	41,8%	36,9%	4,2%

Table 3

TOBACO SMOKING HABIT (PERSENTAGE)

KOSTOLAC

miners	1968	1969
non-smoker	34,1%	36,1 %
ex-smoker	8,1%	8,2 %
smoker	57,8%	55,7%

controls	1968	1969
non-smoker	31,8 %	36,8 %
ex-smoker	7,3 %	6,9 %
smoker	60,9 %	56,3 %

BALJEVAC

miners	1968	1969
non-smoker	49,7%	53,0%
ex-smoker	7,9%	8,3 %
smoker	42,4%	38,7 %

controls	1968	1969
non-smoker	50,9 %	48,0 %
ex-smoker	13,1 %	10,2 %
smoker	36,0%	41,8 %

Table 4

INCIDENCE OF COUGH IN 1968 & 1969

KOSTOLAC

MINERS

yr \ cough	No	Dry	Exp.
1968	47,9 % (134)	8,5 % (24)	43,6 % (122)
1969	51,1 % (143)	5,7 % (16)	43,2 % (121)

CONTROLS

No	Dry	Exp.
62,1 % (64)	6,8 % (7)	31,1 % (32)
68,0 % (70)	3,8 % (4)	28,2 % (29)

BALJEVAC

MINERS

yr \ cough	No	Dry	Exp.
1968	59,6% (245)	6,0 % (25)	34,4 % (141)
1969	65,2 % (268)	3,4 % (14)	31,4 % (129)

No	Dry	Exp.
70,5% (103)	1,4 % (2)	28,1 % (41)
74,7 % (109)	2,0 % (3)	23,3% (34)

Table 5

FEV1.0 FOUND IN 1968 & 1969 RESPECTIVELY

KOSTOLAC

MINERS

YR (FEV1.0) SPIROGRAF.	NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	62,5 % (175)	31,1 % (87)	5,4 % (15)	1,0 % (3)
1969	60,4 % (169)	32,1 % (90)	6,8 % (19)	0,7 % (2)

total: 280

CONTROLS

YR	NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	68,9 % (71)	29,1 % (30)	1,0 % (1)	1,0 % (1)
1969	65,0 % (67)	30,1 % (31)	3,9 % (4)	1,0 % (1)

total: 103

BALJEVAC

MINERS

YR (FEV1.0) SPIROGRAF.	NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	79,1 % (325)	18,5 % (76)	1,9 % (8)	0,5 % (2)
1969	70,3 % (289)	24,3 % (100)	4,6 % (19)	0,8 % (3)

total: 411

CONTROLS

YR	NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	77,4 % (113)	19,9 % (29)	2,7 % (4)	0,0 % (-)
1969	69,9 % (102)	24,0 % (35)	5,5 % (8)	0,6 % (1)

total: 146

Table 6

FEV1.0 IN YEARS 1968 & 1969 (Smokers & non-smokers)

KOSTOLAC

MINERS

YR (FEV1.0)		NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	SMOKERS	59,0 % (92)	32,7 % (51)	7,7 % (12)	0,6 % (1)
1968	NON SMOKERS	66,9 % (83)	29,0 % (36)	2,4 % (3)	1,7 % (2)
1969	SMOKERS	54,5 % (85)	35,9 % (56)	8,3 % (13)	1,3 % (2)
1969	NON SMOKERS	67,8 % (84)	27,4 % (34)	4,8 % (6)	0,0 % (0)

SMOKERS - 156
NON-SMOKERS - 124

CONTROLS

YR		NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	SMOKERS	67,3 % (39)	31,0 % (18)	0,0 % (0)	1,7 % (1)
1968	NON SMOKERS	71,1 % (32)	26,7 % (12)	2,2 % (1)	0,0 % (0)
1969	SMOKERS	60,4 % (35)	34,5 % (20)	3,4 % (2)	1,7 % (1)
1969	NON SMOKERS	71,1 % (32)	24,4 % (11)	4,5 % (2)	0,0 % (0)

SMOKERS - 58
NON-SMOKERS - 45

BALJEVAC

MINERS

YR		NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	SMOKERS	74,8 % (119)	21,4 % (34)	3,1 % (5)	0,7 % (1)
1968	NON SMOKERS	65,6 % (40)	31,1 % (19)	3,3 % (2)	0,0 % (0)
1969	SMOKERS	66,0 % (105)	27,0 % (43)	5,7 % (9)	1,6 % (2)
1969	NON SMOKERS	57,4 % (35)	31,1 % (19)	9,8 % (6)	0,7 % (1)

SMOKERS - 159 NON-SMOKERS - 61

CONTROLS

YR		NORMAL	ABNORMAL SLIGHT	ABNORMAL MODERATE	ABNORMAL HAEVY
1968	SMOKERS	81,7 % (206)	16,7 % (42)	1,2 % (3)	0,4 % (1)
1968	NON SMOKERS	85,9 % (73)	11,8 % (10)	2,3 % (2)	0,0 % (0)
1969	SMOKERS	73,0 % (184)	22,2 % (57)	4,0 % (10)	0,8 % (1)
1969	NON SMOKERS	78,8 % (67)	18,8 % (16)	2,4 % (2)	0,0 % (0)

SMOKERS - 252 NON-SMOK. 85

Table 7

MEAN VALUES OF $FEV_{1.0}$ IN YEARS 1968 & 1969

Kostolac

YR.		MINERS		CONTROLS	
1968	SM.	3704 /3736/ 98,5%	3741 /3712/ 101%	3732 /3742/ 99,5%	3889 /3731/ 104%
	N.SM.	3778 /3689/ 102,5%		4046 /3719/ 109%	
1969	SM.	3600 /3702/ 97,5%	3633 /3682/ 98,5%	3625 /3727/ 97%	3787 /3716/ 102%
	N.SM.	3666 /3663/ 100%		3949 /3706/ 106,5%	

Miners: SD 1968 SM. 594,9; N.SM 439,8 — 1969 SM 687,2; N.SM 765,4

Controls: SD 1968 SM 669,5; N.SM 23,1 — 1969 SM 696,9; N.SM 474,3

Baljevac

YR.		MINERS		CONTROLS	
1968	SM.	3922 /3917/ 100%	4053 /3923/ 103,5%	3912 /3831/ 102%	4020 /3897/ 103%
	N.SM.	4184 /3929/ 106,5%		4129 /3964/ 104%	
1969	SM.	3864 /3895/ 99,5%	3940 /3905/ 101%	3779 /3802/ 99,5%	3953 /3871/ 102%
	N.SM.	4016 /3916/ 102,5%		4027 /3947/ 102%	

Miners: SD 1968 SM. 713,0; N.SM. 676,3 — 1969 SM 628,5; N.SM 630,1

Controls: SD 1968 SM 1185,9; N.SM 638,9 — 1969 SM 1202,6; N.SM 679,7

SM = smokers
N.SN. = non - smokers

Table 8

$FEV_{1.0}$ IN RELATION WITH COUGH

KOSTOLAC

	MINERS				CONTROLS			
$FEV_{1.0}$	cough			TOTAL	cough			TOTAL
	Nõ	Dry	Exp.		Nõ	Dry	Exp.	
normal	16,3%	3,4 %	25,0 %	44,7 %	1,9 %	1,0 %	2,9 %	5,8 %
abnormal	1,0%	1,9 %	4,8 %	7,7 %	- %	- %	1,0 %	1,0%

BALJEVAC

normal	15,9 %	2,9 %	12,0 %	30,8 %	0,5 %	0,5 %	2,4 %	3,4 %
abnormal	- %	2,4 %	3,8 %	6,3 %	- %	- %	0,5 %	0,5 %

Table 9

FEV1.0 AND COUGH IN SUBJECTS WITH BRONCHOSPASME

FEV1.0 \ cough	Nō	Dry	Exp	TOTAL
normal	25,8%	6,2 %	38,1 %	70,1 %
abnormal	1,0 %	8,2%	20,7 %	29,9 %
total	26,8 %	14,4 %	58,8 %	100,0%

BRONCHOSPASME+ FOUND IN 44,2% EXAMINED

Table 10

RESIDUAL VOLUME IN DIFFERENT AGE GROUPS

30		31 40		41 50		50		total	
n	+	n	+	n	+	n	+	n	+
4,8%	10,6 %	15,0%	34,8%	7,2%	25,6%	1,5 %	0,5%	28,5%	71,5 %

Table 11

BLOOD GASES AFTER EFFORT IN RELATION WITH FEV$_{1.0}$, COUGH, AND RV

			FEV$_{1.0}$		cough			RV		total
			normal	descreased	no	dry	exp.	normal	increased	measured
effort	pCO_2	normal	51,2%	7,3%	22,2%	7,3 %	29,0%	19,4 %	39,1%	58,5 %
		increased	34,3%	7,2%	13,0%	5,3 %	23,2 %	9,1 %	32,4 %	41,5 %
TOTAL			85,5%	14,5 %	35,3%	12,0%	52,7 %	28,5 %	71,5%	100,0 %
effort	$P^{O}_{a}2$ Sa	not increased	45,4 %	7,7 %	18,9 %	6,7 %	27,5%	13,0%	40,1%	53,1 %
		increased	41,1%	5,8%	16,4 %	5,9 %	24,6%	15,5%	31,4 %	46,9 %

Table 12

BRONCHOSCOPIC FINDINGS IN RELATION WITH COUGH

			№ of subjects	SIGNS OR SEQUELLAE OF INFLAMMATION						CYTOLOGY OF BRONCHIAL SMEAR (SIGNS OF INFLAMM.)	
				no	slight	marked	sequellae	total			
								θ	+	θ	+
cough	NO		6	5	–	1	–	5	1	3	3
	Dry		16	4	5	5	2	4	12	10	6
	phlegm	trace	5	1	2	1	1	1	4	1	4
		moderate	28	7	12	8	1	7	21	8	20
		marked	15	1	5	9	–	1	14	5	10
TOTAL			70	18	24	24	4	18	52	27	43

Table 13

CYTOLOGY OF SPUTUM IN RELATION WITH COUGH

		Nō of subjects	1	2	3	4	5	6	total Nō of subjects with finding: ∅	total Nō of subjects with finding: +
cough	Nō	6	–	–	–	1	1	–	4	2
cough	Dry	16	4	2	2	9	–	6	14	2
cough – phlegm	trace	5	1	–	3	6	–	–	5	–
cough – phlegm	spoon	21	6	–	9	16	5	9	14	7
cough – phlegm	coffee cup	7	3	1	4	7	4	2	4	3
cough – phlegm	more	15	7	1	2	13	2	6	5	10
	TOTAL	70	21	4	20	52	12	23	46	24

1.Low ciliated & cuboid cells.
2.Cells from lower parts of airways.
3.Basal, parabasal & goblet cells.
4.Red & white blood cells (poly, mono, ly).
5.Agitated & destroyed cells.
6.Mycro & macrophagocytes, coniocytes, multinuclear, giantcells

in any group (1-6): metoplasia, necrobiosis, destruction, degeneration, nude nuclei.

DISCUSSION AFTER DR. POTKONJAK'S PAPER:

Dr. Lainhart: Have you any preliminary data to show whether your dry coughers are also smokers or are they all non-smokers?

Dr. Potkonjak: For investigation, I eliminated smokers. Only non-smokers are investigated.

EVALUATION OF ISONIAZID AS A PROPHYLAXIS AGAINST PROGRESSIVE MASSIVE FIBROSIS

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and Radiological Health, Belgrade

I. Objective and Background

Miners pneumoconiosis represents the main problem of occupational pathology in a great part of Yugoslavia, particularly because of the accompanying diseases (1,2,3). Pneumoconioses are complicated with tuberculosis in 15-28% and specially in advanced cases this percentage increases to 65% (4).

Studies conducted in the U.S.A. among persons at risk treated prophylactically with isoniazid at 5 mg. per kg. of body weight per day for a period of one-year reduced the incidence of tuberculosis over a five-year period by about 50% (Amer. Rev. of Resp. Diseases, Vol. 85, No. 6, June 1962; Vol. 88, No. 2, August 1963; Vol. 85, No. 4, April 1962; New England Journal of Medicine, Vol. 265, October 1961, Pp. 713-721). It is believed by many authors that in most cases complicated pneumoconiosis (progressive massive fibrosis (PMF)) is the result of a tuberculous infection.

To date controlled experiments on the effect of isoniazid in preventing complicated pneumoconioses have not been reported in the literature. Only some preliminary communications were published (5,6,7). P. Galy, et al (7) treated 19 patients with INH (300-500 mg. per day), and 23 others with Streptomycine, or PAS or Trecator+Viocine. Thirty-five of them had not progressed to PMF, while seven were complicated with tuberculosis. Some attempts were made with BCG vaccine (8,9). The role of tuberculin sensitivity in coal miners with pneumoconiosis was studied (10).

We decided to do a study with the objective to determine to what extent the prophylactical administration of isoniazid can prevent complications in a group of men with pneumoconiosis whose X-rays were initially negative for tuberculosis. If it could be proved that isoniazid materially decreased the incidence of complicated pneumoconiosis an important step toward the alleviation of the most disabling aspects of coal dust disease would be possible. Because of a relatively high incidence of tuberculosis among Yugoslav miners there was an opportunity for a study economically not feasible in the United States of America. The data available in Yugoslavia indicate that it might be anticipated that for a group of X-ray category 2 and 3 of pneumoconiosis cases (1958 ILO classification) a great percent would develop complications within a five-year period. If the prophylactical administration of isoniazid for a one-year period is capable of reducing this incidence by as little as 25%, a study of 400 pneumoconiotics over a five-year period, divided at random into two groups, one given isoniazid and the other a placebo, would produce a statistically significant difference in the incidence of complicated pneumoconiosis. To accommodate dropouts this number was increased to 250 in each of the two groups.

II. Description of Research Plan

A group of 529 male workers from mines in Serbia, Crna Gora, Macdeonia, Bosnia, and Herzegovina who had pneumoconiosis of categories 2 and 3 without radiographic evidence of tuberculosis, and who were tuberculin positive was studied. Initially tuberculin tests and 35 cm X 35 cm chest X-rays were done on the entire group. The group was then stratified by age and X-ray classification, and within these categories, divided at random into two groups with 269 miners in one group and 260 in the other one. One group was administered isoniazid in a dosage of 5 mg. per kg. of body weight per day for a one-year period. In the same period the second group was administered a placebo indistinguishable (without chemical analysis) from the isoniazid tablets.

In order to choose the necessary number of cases with pure noncomplicated pneumoconiosis of categories 2 and 3 a great number of cardlists of inpatients and outpatients was searched (about 3500). Workers examined in the field and in mines periodically according to law were listed, too. Then a tour of mines was made in order to check whether the workers eligible for the experiment were alive and where they were living. The exact addresses were taken, the last diagnosis checked, and in the case of complicated pneumoconiosis they were eliminated. At last, the miners choosen in the abovementioned manner were X-rayed so that a firm choice of patients with pneumoconiosis of categories 2 and 3 without radiographic evidence of tuberculosis would definitely and undoubtly be selected. At the same time the tuberculin test was made by PPD RTV_{23} according to Mantou. The X-raying and tuberculin testing were performed under the same condition, i.e., the X-raying was done using the same type of roentgen-apparatus, with the same elements, by one and the same operator. The tuberculin test was also performed by the same person, while the skin reaction was interpreted by the same doctor. Patients with a induration diameter of at least 5 mm were considered to have a positive tuberculin reaction. Other skin changes (erythema, bulla, vesicula, etc.) were recorded. X-ray films were read independently by three doctors who neither had any data about the X-rayed person, about his previous rentgenogram nor had they knowledge of whether the patient was receiving isoniazid or placebo. In order to be able to select 500 persons necessary for the experiment more than 1000 persons had to be examined. From the stratified sample the group for isoniazid was chosen at random. The average age of the treated group is 44.4 years and of the control 47.0 years.

After that, points for giving tablets were established. For nine mines (metal and coal) 22 points were organized taking care of the vicinity of the working place or domicile of ex-miners, or the possibility of engaging medical staff from health services to give tablets on the spot. The distribution of the daily dose of the drugs was performed by medical technicians or nurses from the health service in the mine or domicile. In one case it was a person from the safety service in the mine. This person did this at the entrance of the mine thus being able to gather people from both shifts. On the day of rest it was the responsibility of the patients to take the drugs at home by themselves. The persons distributing the drugs were controlled by the physician once or twice a week, and by the Principal Investigator or his Deputy once monthly. It was proved that the taking of drugs was regular owing not only to the efforts of the engaged persons, but more to the previous successful informing of the pneumoconiotics of the significance of taking the drugs as a prophylactic procedure. Besides, there were no troubles in the digestive system except in sporadic cases, therefore, the success was nearly complete.

MINE	No. Persons In Experiment	INH	Placebo	No. of Points	Start
Stolice	38	22	16	3	Oct. 1966
Zajaca	68	26	42	3	Oct. 1966
Sase	24	12	12	1	Nov. 1966
Lece	51	35	16	3	Dec. 1966
Vrska Cuka	55	30	25	3	Sept. 1967
Bor	31	20	11	2	Sept. 1967
S. Stijena	50	20	30	2	Nov. 1967
Kisnica	42	20	22	1	Nov. 1967
Zletovo	170	84	86	4	Dec. 1967
Total	525	269	260	22	

DISTRIBUTION OF PNEUMOCONIOTICS BY AGE

AGE	Pneumoconiosis Category (ILO 1958)							
	Cat. 2		Cat. 3		All Men			
	INH	Placebo	INH	Placebo	INH	%	Placebo	%
21-30	3	3	-	-	3	1.1	3	1.2
31-40	74	54	29	9	103	37.5	63	25.1
41-50	64	49	28	45	92	33.4	94	37.4
51-60	42	40	17	20	59	21.5	60	23.9
60	10	19	8	12	18	6.5	31	12.4
Total	193	165	82	86	275	100.0	251	100.0

MINE	Start		First	Control	
	INH	Placebo	Progr.	Tb	Died
Stolice	22	16			
Zajaca	26	42			1
Sase	12	12	1		1
Lece	35	16	1		
Vrska Cuka	30	25	1		
Bor	20	11			
S. Stijena	20	30	1	1	
Kisnica	20	22		(2) 1	
Zletovo	84	86	3	2	1

After a one-year period X-ray examinations as well as tuberculin tests in both the experimental and control group were performed. This was done under the same conditions as in the start, i.e., with the same type of roentgen-apparatus, with the same elements, and same persons. The films were read independently by the same doctors and again they did not have any data about the X-rayed persons. The film readings were done on three occasions.

Changes which indicated a possible tuberculous infection were observed in four cases. In seven persons a progression of pneumoconiosis was found, and in two of them there were signs of a tuberculous infection. Three of the pneumoconiotics died; one was a victim of an accident, one committed suicide and one died. We are carrying on an inquiry regarding this last person in order to find out the cause of his death.

The first control was completed at all points at the end of 1968 because the last group of persons could start to take isoniazid or placebo at the end of 1967. There were many reasons why the points could not be established simultaneously in all places: in two miners we had to wait for the roentgen-apparatus to be installed which had to be of the same type used in the other places; small mines with a small number of non-complicated pneumoconiosis; different systems of medical care, i.e., a different health service organization from state to state, even from area to area which required many contacts with a greater number of physicians; health services; mine boards, etc. We were aware that the success of this work would depend on the good knowledge of people and medical personnel of the aims of the experiment and on their acceptance of this work. Besides, the main reason for a successive start was our plan to have by all means the same technical personnel for each part of the work. Some times there were difficulties with road communications in the mine area or settlements (during wintertime) where miners and ex-miners live.

The distribution of the drugs itself was conducted in blind so that neither the physicians nor the patients or distributors knew what the patients received. The labels on the bottles were identical, with the only difference that on the labels of one sort of pills a small misprint was made.

It was anticipated by the contract that this would be the last year of activity on the project. Meanwhile, because of reasons mentioned above this activity has not been finished and no final report of the obtained results is ready.

Namely, since the points could not be established simultaneously the last group of miners started to take isoniazid or placebo at the end of 1967. This means that the last control (examinations) will have to be done at the end of 1972. The difficulties which arose in organizing of this complex experiment were reported in previous reports. Then an extension of the time-limit for completing the experiment was provided.

It was observed that it was a pity we had no financial means for an every year control which would help to keep better contacts with the persons participating in the experiment. Therefore, since a long interval was made between the previous and next control, it should surely be expected that a number of persons would be dropped from the control. For that reason it should be necessary to undertake a new action to get people acquainted with this work in order to provide a regathering of the miners for the next control. In the meantime a reorganization of the health services has taken place, some physicians have left the mines, many managing bodies have changed. All that, requires a re-engagement and intensified efforts of the Institute team in performing the project. In spite of this situation we had regular contacts with the people in the experiment through letters or in person, we were interested in their social problems, state of health, and helping them to realize benefits according to the law, etc.

III. Further Activity

If it would be possible to realize an extension of the work by introducing respiratory function tests, gas analyses, allergen tests, bacteriologic and cytologic sputum analyses, the time-limit would be prolonged. New financial sources would be needed for that. If it would not be possible, the further course of activity would be as follows: radiography, tomography wherever needed, sputum analyses in the cases where evidence of tuberculosis developed. All results would be elaborated statistically.

This report represents only a preliminary communication. Any suggestions and proposals regarding the further activity would be greatfully accepted.

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DISCUSSION AFTER DR. POPOVIC'S PAPER:

Dr. Lainhart:

Did you have any reaction to either drug -- either the isoniazid or placebo on the part of the individuals taking part in this study? Did any of them have gastroenteritis or actually have to stop either drug?

Dr. Popovic:

Yes, they had some troubles but only sporadic ones. We explained to them to have some cakes or some bread, not to take isoniazid on an empty stomach. Of course, they did not know which tablets they received and after that troubles disappeared. That meant, we practically succeeded in regular taking of the drugs. We don't know for the present time if the seven cases of PMF and four cases of suspicious TB received izoniazid or placebo? We will know it in five years.

ROUND TABLE DISCUSSIONS OF MAJOR AREAS

Dr. Djuric: On the first day we reviewed the work done in Poland and Yugoslavia in the field of occupational health and toxicology, and really we can say that it is in two fields. One is exposure and intoxication with heavy metals, that means mercury, cadmium and lead. The other is such as carbon disulphide exposure to organic solvents. All of the reviews were very interesting and we obtained highly valuable data. It is necessary to think about further programs in this field. So I would say some words later about our plans, but perhaps our Polish friends have some suggestions and proposals?

Dr. Piotrowski: I would like only to mention with respect to the program with heavy metals that we are planning for the future, we will be interested chiefly in the mechanism of binding of these metals inside of the body and the mechanism and forms eventually of the excreted material. There is a very great need for knowledge on these problems. The techniques which we will apply enables us to solve such problems. There will be included some programs that were discussed by Dr. Bessey. For instance, work on metal binding substances, will start with metallothionein. We will work on the problems of the biosynthesis and distribution of this compound in the body and eventually whether or not the complex is identical with that found by other techniques, for instance, morphological techniques. So our efforts involve mostly animal experiments. I would like to underline that our laboratory is concerned with animal experiments and little with human exposure and human problems. Thank you.

Dr. Urbanowicz: We have been working for many years on toxicity of non-ferrous metals. We will extend our investigation in this field and will concentrate on three problems. The first problem is the biological response to non-ferrous metals in the presence of exposure to other metals. So we are interested in the multimetal exposure. The second problem will be the relationship of the metal toxicity to the chemical composition of the metal compound involved. The third problem will be the influence of environmental factors on the toxicity of metals.

Dr. Djuric: In connection with the work of our Polish friends I have a proposal. Namely, it is obvious that they are doing mostly experimental work on animals because they do not have factories in which workers are exposed to cadmium and mercury. To remind you, in Yugoslavia we have big mercury mines in Slovenia in which our group on radioactivity is doing very nice work. It would be, perhaps good to start some collaboration with our Polish friends so that they can come from time to time to study together with us on these problems of miners and workers exposed to mercury. We have also in Sabac a factory in which a group of workers is exposed to cadmium. Perhaps, it would be good that the group for studying cadmium would come and study the human exposure too, which is low but interesting. For example, I think this idea about ALAD hydrogenase is very interesting one and should be studied in human exposure. So it would be good to find some way for collaboration between our group in Belgrade and Polish groups, working in the same field.

Dr. Urbanowicz: I think that it is an excellent idea and in reverse we shall be very glad to give all facilities which we can give to our Yugoslav friends if they would try some experiments in our laboratories on animals. We shall be very glad to check our experiments in the Yugoslav mines and factories.

Dr. Piotrowski: This idea of collaboration, I think is a very fine one and interesting with respect to possibilities of seeing groups exposed at very different levels. Namely, for instance, we do have in Poland factories with mercury exposure, but I would say that the level of exposure is low, and all the questions could not be answered with human beings with such low levels of exposure. If you, for example, have in Yugoslavia another factory or mine in which the concentration and doses are much greater, then it seems possible to study some problems that usually are not seen in human beings. And I would like only to mention that with respect to the identification of human beings with mercury exposure we already started investigation in which we are following the dynamics of the rhythm of excretion of mercury with differences introduced by the weekly rhythm of exposure. The question arises, how the mercury excretion will be different with limited exposure and with high exposure. It would be very desirable to get access to a factory or mine with an extremely high level of exposure.

Dr. Bessey: I wonder on the mercury exposure if there is an area that has to do with possible exposures that should be considered. I am sure, in the USA, ten yars ago, if one asked about exposures to mercury that would lead to definite symptoms, in general the answers would be that it is not likely. Occasionally one found a case. But the recent increased concern about mercury comes from it's industrial use and it's conversion to organic forms that get in the food chain. There is an increased interest in mercury in several places now in the USA due to the finding of exposures that heretofore were not thought to exist. For instance, metallic mercury is used in a lot of places in instrumentation. It is almost impossible to work with metallic mercury and not have some spillage. This spillage gets in places that are not very accessable and one may not be aware over period of time of the exposure. Now, in several places, appreciable vapor mercury concentrations have been found; such places and rooms usually do not have a good ventilation. So I think it probably will turn out that in the last five years in many places where it was supposed there was no exposures to mercury that a problem really exists.

Dr. Cralley: I have had about 29 years of experience measuring environmental exposures and studying their relationship with biologic responses. I can add more to what Dr. Bessey said, and perhaps, give additional perspectiveness. We have known for some time much of the nature of mercury exposure in the industrial environment and its source. As an example, it has been a problem in many of the laboratores where the mercury vapor concentrations were high. In many instances the only way to control contamination was to tear out and replace floors and other areas. There is no simple way to control exposures. Mercury must be handled with care. Uncontrolled disposal of mercury wastes, or its unsuspected presence can pose subsequent problems.

Dr. Valic: The idea of collaborative research is, of course, very welcome. But there may be a problem in connection with the proposal put forth by Dr. Djuric about the collaborative research in Idria which is the mining area and where the Hg smelter is located. We have been working there for the last 20 years, and we have published already a number of papers on that and on related subjects in connection with mercury exposure. Now I only want to draw your attention to the fact that there is not a high mercury exposure in the Idria mine. Mercury is mined in the form of cinabar. The solubility of cinabar is very low. As a matter of fact we have no mercury intoxication in the region of Idria any longer. Now, the smelter was a source of high exposures. The old smelter has been replaced by new smelter, and there are practially no exposures or the exposures are very mild. If you expect in Idria high levels of exposure then you will be disappointed. Now, coming back to the problem mentioned here already to study the excretion or say biotransformation of mercury in people exposed to different mercury levels, that is exactly the project we are working on in Zagreb. It is a joint project between the Institute for Medical Research and the Andrija Stampar School of Public Health and the US National Air Pollution Control Center. We are studying mild changes in the organism of four population samples exposed to four different mean levels of mercury (1. occupationally exposed; 2. living around industry processing mercury, traffic officers, custom officers; 3. urban population; 4. rural population.)

Dr. Henschel: I think Dr. Valic made a statement, which, to me is very important. This is the fact that the exposures in the future will not be massive and acute but more chronic and very low level exposures. That means they are much lower than we have been thinking about in the past. The little amount every day that the individuals are taking in may well become the toxicological problems of the future, rather than the mass exposures of the past. Except for instance of accidents the high exposure has been controlled. But it is the little amount that we have neither measured nor taken into consideration, that over long periods of time may in the end, actually mean bigger total body exposures than one would get with the large acute exposures.

Dr. Piotrowski: I think that it is not the proper time to discuss the details of the proposed collaborative studies. I mean maybe you do not have in Yugoslavia high exposures, for instance, to mercury. But you certainly have, as I know from earlier conferences, for instance, a factory with high exposures of carbon disulphide. You may be looking for factories with very low exposures to check something and we may have such factories in our country. As I understand the proposal of Dr. Djuric we are looking for some kind of collaboration by which new techniques and methodology could be tested and applied in places which would be not limited to ones own country; by which we could, eventually, in some way manage to go to these other places. I think we can not, at this meeting, present more detailed proposals, as the idea seems very interesting. I think that if you Dr. Henschel could consider the possibilities of such collaboration within the framework of our contracts, it would increase our operative system of the contract and the possibilities we have.

Dr. Djuric: Just that was my idea, if you have some new methods or results, which you wish to evaluate, it could be good to try them here in Yugoslavia. Dr. Urbanowicz said that he has to go to Finland to find people exposed to cadmium. OK, it is fine, but it's also good to come to us. On the other hand we would like to check exposure of our people, because we have not worked on cadmium and it will be a help to us if somebody would come and together with us, perform such a study. Now, I would like to say something about our studies under project 02-001-3. From the review, you learned that we developed very broad studies on carbon disulphide exposure and intoxication and on lead exposure and intoxication mostly among an exposed population around the lead mine and smelting plant in Mezice. On carbon dispulphide we have pulbished about 45 papers and I can say that this study is so broad now that it can not be included in a single project. So we have submitted a special proposal to study the use of electroencephalography in diagnosis of CS_2 intoxication. We are also proposing another proposal for study on vascular changes in chronic, long term, low level exposures to carbon dispulphide. We would like to include in this study also a study on possible vascular changes in population exposed to lead. This study could include ophthomological examinations, biochemical research and all kind of blood vessels investigations. I would like to point out some very interesting facts obtained in our research until now. It is obvious that long exposure to carbon disulphide causes vascular changes in relatively young workers after some years of exposure. It is important to find out how long it takes to develop these vascular changes. The second point is the mechanism of these vascular changes; for example, in a preliminary investigation on carbohydrate metabolism and CS_2 exposure we found some changes occur which are similar to those in diabetes mellitus. The second thing is that we hope to finish the study in Slovenia on the population exposed to lead in next one-two years. Then we will shift our study to the Trepca mine and to start a similar, but more complicated investigation on lead and other heavy metals (As, Bi, Cd) present in the ore and environment of Trepca mine and smelting plant.

Dr. Bessey: Let us shift the discussion we have just been having a little to the next subject, chemical agents, and their toxicity. But I would like to continue the converation along the same lines, but raise questions in a little different direction. One area is that mentioned by Dr. Henschel already and that is the great number of new substances which will appear in the future. But a big and difficult problem is the one of what are the health consequences of long time exposure to low levels toxic chemicals. Because as Dr. Henschel has pointed out, accidental exposures and even exposures at relatively high levels we seem to be winning because we can do something about decreasing the high levels. But when we consider this problem of protection from the environment of the future you find that among the problem about which we need be concerned is the low level exposures over long periods of time. This raises a whole new set of problems, certainly biologically it does. It is not difficult to see the problem associated with a substance which may cause death even after short exposures. But, what about the problem of large numbers of people exposed over long periods to low concentrations of a variety of substances. What about the influence of an agent like carbon monoxide at a level that produces a low concentration of carboxyhemoglobin that may exist for years. Does this make any difference to health? As far as we know one does not lose his life from low carbon monoxide intoxication, but what about the heavy cigarette smokers or garage workers, are there health effects. Not many people are concerned about the influence on other aspects of health, development, of the degenerative diseases of various sorts, perhaps very subtle things that we now do not recognize which may be associated with chronic low level exposures. Then, of course, this raises the phenomenon in the area of carcinogenesis of a process that may develop from exposure to low levels of substances for long periods of time. So, I think this is one of the areas, looking to the future, we must be concerned about. And it will take new kinds of people and new kinds of techniques. It is an area that we don't know very much about. We do not know what we are looking for, so that it seems to me like this is an area of the future that we need to be thinking very much about. Most certainly, we have to know more about mechanisms of action, the kind of problems that Dr. Piotrowski and others here have been working on. Because it is from this source that may come some leads on what it is we are looking for.

Dr. Henschel: I would like to add two more areas to those Dr. Bessey has already mentioned. One of these is the effect of small doses of chemical agents at the gene level. The effects will be seen in the future generation. I am sure that a lot of these agents even at low concentrations have their effect at the intracellular level rather than at the whole organisms level. And the other area, that seems to me of great importance is the interaction between a chemical agents, multi-exposures to a number of chemicals. We know that some are antagonistic, and for some the effects will be potentiated. We also must consider the effects of these low level exposures to toxic chemical agents and the interaction with drugs, because a large percentage of our working population probably is taking some type of drug therapy. We know practically nothing about the interaction between therapeutic drugs that the individuals are taking and the action of a toxic chemical agent, when they are put together. We do know for instance, that barbiturates may change completely the level of activity of some of the drugs and some cases may completely change their action as for example the anticoagulants. Just a single dose can completely upset the therapeutic regimen that the physician has the individual on. I think these are some other areas that have to be considered for the future.

Dr. Urbanowicz: Poland is a country which is becoming very rapidly industrialized, and I think it is a very good place for studies on the effect of low level exposure. We just built big plants, for instance, in the non-ferrous metal industries. Around these installations we are building new cities, into which 20 or 30 thousand people are shifted from the country. These people, in most cases, have not been exposed to toxic agents earlier. I think we have very good possibilities to study these low levels of toxic agents in connection with industrialization of our country. We have new factories, then we have various level of exposure. Just beside the factory there, are new houses built, in which people are living who have never been previously exposed. These are excellent groups which can be compared.

Dr. Piotrowski: I would like to expand on remarks of Dr. Urbanowicz. Namely, from the two subjects mentioned by Dr. Bessey, mechanism of action of toxic agents and, certainly of levels, the lowest levels at which some toxic latent or delayed action may be possible. I think that the second one is more difficult and one has to have at ones disposal certain circumstances in order to be able to perform such investigation. I am talking about a kind of study that might be called a prospective one. Because, in order to find these relationships which we are talking about, we have probably to take into account many years of observations. Second, we have to limit our studies to an exact group of people whose exposure history we know. I mean, in the new factories worker turnover is much faster moving, changing places and the work they do, and so on. And therefore at the beginning much larger groups should be included into the observation, and some of them, in still decreasing number, should be observed for years. I join Dr. Urbanowicz in the opinion that our country would be appropriate for such kind of long term studies if there would be an interest in such kind of solutions to the toxicological problems.

Dr. Cralley: As we compare study data from different countries I think we need to bear in mind that there is going to be certain apparent differences. These can result from differences in study groups, procedures, and unrelated additive stress. We must keep rather detailed information on major background areas that may distinguish one study from another. For example, temperature can make a important difference and the rate of metabolism in cold countries versus that of a very warm climate and although we have the same level of exposure there may be a different biologic response. A second area which may be important is the individual work requirements of the job. We can not compare exposures on jobs where one is a sedentary job against one which is classified as very heavy work. We know these will give very different responses. A study of such factors may help us explain later on, otherwise apparent discrepancies of findings.

Dr. Henschel: I would stress the point Dr. Cralley made because, particularly in the case of airborne contaminants, in individuals pulmonary ventilation may increase from 6 to 8 liters a minute at rest or a very light work, up to 125 liters a minute at extremely heavy physical work. This means that the actual lung exposure to the material - the amount the individual takes into his lungs may be increased by the factor of 10 to 15 just depending upon the level at which the individual works. We also know that the inspiratory flow rate, that is the flow rate of air into the lungs through the bronchial system, will change the level at which materials are deposited in the lung. We know that these things occur but in most of our standards these factors are not properly taken into consideration. I think that we should know specifically what the demands of the job are, so that we can get some idea of the metabolic as well as the ventilatory aspects that are involved.

Dr. Bessey: Because this flow-dose-time problem requires other approaches, I would like to mention a couple of aspects that bear on this. One, we have talked about mechanism of action. Another is the enormous number of materials involved. For example, there is something like 5000 new organic compounds produced every year, many of which eventually get involved in low level long time exposures. Just testing for toxicity is no longer an adequate way of getting at such problems. It seems likely in the future that the development of certain principals in this area, that will give you better predictive levels, that will allow you to make a good guess based on chemical structure and what you know biologically in reference to new compounds. It will allow one to consider certain things collectively instead of individually, because the job will be enormous if you are thinking of approaching it on an individual substance basis. Now one area, in connection with this problem, might be called comparative toxicology. At the present time, even in doing testing, as you all know, choice of animals are based on convenience, and not on what one is trying to test. It is the problem of toxicity of an agent on animals with man being the species we are eventually interested in. It makes very little sense to be testing the toxicity of material on the rat in which this material may have a type of metabolism which can not at all be compared with man. To pick a very good example, of course, would be the 2-napthalamines carcinogenic agents. You all know that these do not cause cancer in rats at all. They are effective for dog and that is because the active carcinogenic agent is not 2-napthalamine, it is a phosphate compound. Rats do not convert the 2-napthalamine into the phosphate. Man does, the dog does. So that in this case, the dog would be the proper animal of choice. The problem is we do not know enough comparative metabolism of toxic agents to know what the animal of choice should be. Now it is possible that in the next 10, 15, 25 years we could accumulate enough comparative information on the metabolism of toxic agents by various species so that one could then make a selection of the proper of model, relative to man's problem. So here is an area that's hardly been touched. I can not think of a single agent in which we know enough about the metabolic pathways.

Dr. Bessey: Could I make a general comment that refers to both the chemical and physical agents. I am sure that the many of you by now must think that Dr. Henschel and I are raising questions for which the final solution is to put man in complete isolation in order to protect him from some of the things we have mentioned. And this is probably true, but I think we are very rapidly approaching the stage where some cost benefit decisions have to be made. But in order to make a decision about what to do, we need to know where the risk starts and not just completely remove all risks. It takes time to collect the information about risks, years and even if we start now, we will probably not have this kind of information for a long time. Decisions will have to be made. So scientists must be looking forward to the kind of demand that will be made on them. It will also be important to know when there are no risks involved so we need no longer consider those substances.

Dr. Cralley: I would like to stress again Dr. Henschel's discussion of the importance of the interaction of certain physical agents and on the chemical agents. There is the vast spectrum of ultra-violet light as it relates to the many active chemicals. We know many carcinogenes are not effective unless ultra-violet light is present. We know that in the case of celery rot disease the dermitites occurs only in the presence of sun light. There is a large area of known disease interrelationships but there is a much larger area about which little is known, there is much more opportunity for research then we realize.

Dr. Henschel: The importance of some of the physical agents is reaching the point where demands are being placed for standards for permissible exposure limits. Noise, of course, the demands have reached the points where exposure standards have been formulated. The need has also been raised for standards for industrial heat exposures and vibration. We can agree pretty well on noise standards that will make sense. In general the same is true for heat. But at the moment I don't believe that we can make any sense out of trying to develop a standard for vibration, because really we know little about the health effects of vibration.

Dr. Cralley: I think we have covered the broad blood scope of this area already. It is important to keep in mind that whenever we take environmental measurements there must be a purpose and a plan if the objectives are to be achieved. There can be three major purposes for environmental sampling. One is for regulation compliance, another is for the surveillance and effectiveness of control procedures already instituted, and the third is to provide epidemiologic data to determine the dose-response relationship. My comments are related to the later, the dose-response relationship. I think it is very important to describe the exposure of a worker in as great detail as possible, for a number of reasons. These data are essential for the design of proper laboratory research. As for example, a great deal of research is under way now on the effect of exposure to asbestos. In the laboratory, the toxicologist in preparing the fibers for dosing purposes, ground-up fibers from the relatively pure asbestos. Now some new information will come from this, but this type of asbestos is not at all what the workers are exposed to. If we are going to get answers from animal exposures on the response of the worker to his workroom exposure, we have to duplicate the environments of the worker in the exposures of animals. The second point is that we must have data characterizing the respirable dust. We know from years and years of past research that the total dust will be quite different than the respirable dust. Also, we know that the respirable dust will change it's characteristics as the material being processed pass through different operations from the raw materials to the finish product. This would present a different set of circumstances as far as the characteristics of the respirable dust of these workers are concerned. And if we are not careful to characterize these exposures we will have research from different groups having different response finding and apparently the same exposure agents. I don't think we need get into a detailed discussion how to take samples or where to take environmental samples. It is important though to have statistical validity for the data derived from the samples. The selecting of the methods of analysis is very important; it is quite possible in some of the laboratories that although the method is good, it is to insensitive for our purposes. For example, measurements in parts per thousand may be sufficient in some instances where parts per million are needed in other instances. So it is very important to select the method of analysis that will get the concentration down to the range in which we are interested.

Dr. Lainhart: Perhaps we have said many of the things already that I really would like to cover. I think that there are several major points and perhaps, this is from an epidemiologic point of view that I am speaking. When we speak about occupational diseases, we talk about an individual who is exposed for perhaps 1/3 of his life. So it seems to me, we have to look in a broader sense at what are exposures that may play a role in terms of a man's, or a woman's total life. This is really related to such things as; how we measure, what we measure and the very difficult problem that we have touched on many times in this conference already, and who we measure including control groups. The problems that I see of trying to define, first of all what is an occupational disease and then what effect that has on a person, is all tied up with these other things. So this gets down to looking at something which has been called the total man; how do these 8 hours a day effect man's life. What effect does this have on other chronic diseases? I go back to my favorite subject of coal workers pneumoconiosis; how do we define this disease? We have had conferences on black lung or CWP, and we had each discipline define this in a different way. The toxicologist talks about the dust and coal mine dust, the physiologist talks about the effects that this has on pulmonary function or, perhaps on the function of the lung in addition to just getting air in and out. The pathologist looks at this as spots on the lung of certain character, certain size or certain morphologic changes. And then the work physiologist begins to look at this in terms of how much work can man do. So I think that, at least, I would like to be able to begin to think about an overall view of what a worker is and the effect of his work on his life expectancy or health expectancy. Now, maybe these are such broad ideas that we are not smart enought to be able to talk of them or even think about them yet. This seems to me to be the future and this gets into such things as the chronic low level exposure to any substance. There is much discussion in our country, for example, at this moment, about carbon monoxide. Does this, in fact, produce a chronic disease? We all know the acute effects of carbon monoxide, but does the policeman on the corner have any long term chronic effect due to the passing of automobiles and buses constantly with obviously a level of carbon monoxide. So, I think that these kinds of things are the things that future of occupational health research will show. Now this gets into the specific areas of how does this effect the man, what are the metabolic parameters and how are these effected then by other things? It has been said, for example, that a certain enzyme system may be involved in heavy metal poisoning; are these effected by anything else that the man does? We have been attempting, for example, in the asbestos investigations to look at the man's hobbies, his part-time work at home, in the evening in his workshop in the basement where he uses paints, solvents, mechanical gadgets of all types, saws, drills, etc; what effect does this have on the man, if any? Maybe, we have talked about unknowns here that have no effect, but somehow I feel that this is where we are going.

Dr. Henschel: The aspect of performance which we are usually concerned with is what the individual is capable of doing or is his capability to do things changed as a result of his occupational environment? One aspect of the overall performance, frequently overlooked is the individual's capability to receive information from his environment, to process the information in his nervous system and then to make proper judgements or make the proper response; this is preception-processing-response system that we must not overlook. Maybe many of the things that we take for granted, the high incidence of automobile accidents, or other accidents may be related to a breakdown in the system. Maybe low level chronic exposure to some of the toxic substance may be a factor in the breakdown. Do chronic exposures in any way influence ones ability to make proper judgement at the proper time, with the proper responses within a minimum time. On this whole aspect, really very little has ever been done. In todays' complicated society with its complex demands the ability to receive information, process it and make rational judgements is of utmost importance.

Dr. Rafalski: According to my experience, the occupational disease patterns we have been talking about, we don't find in our industry among workers as the heavy occupational diseases such as lead intoxication. So, one can say that the tendency is rather to the non-specific symptoms related to the whole exposure in plants. We can look, for instance, in the pattern of our absenteeism diseases, and we see cases of lung symptoms when we compared two plants. In one plant we found more non-specific diseases. So, the pattern of occupational diseases is going to be changed. That is one thing, another is how to measure those non-specific diseases and in connection with the exposure in any plant is the question. Secondly, about the criteria, when we are talking about exposure in industry in occupational and non-occupational exposure, we should define the criteria. Dr. Lainhart mentioned life expectancy and morbidity and mortality, and I say that is the proper way to make an epidemiological study and maybe, in the epidemiological study, we will find the criteria; the long-term criteria to the exposure in occupational and non-occupational circumstances.

Dr. Szymczykiewicz: It seems to me that in the epidemiological study the sickness absenteeism should be parallely studied. According to my experience, in the data analyses of total specific absenteeism we can find much interesting information supporting the main investigated problem. It seems to me that this is a very important test.

Dr. Lainhart: I think it is pertinent to the discussion to talk about how we measure a population. We have talked a little bit in the Conference about standards of pulmonary function, for example, in terms of the "normal" man, or women. Right now we are almost at the point where we have to develop our own standards of normality. I would hope that such Conferences, as this and other International Organizations, can begin to develop world standards for pulmonary function, work physiology, levels of a particular material in the blood or urine. Each of us almost has to develop his own standards and this means then, you do the studies in Poland or in Yugoslavia or we do them in the USA and we can not compare our standard measurements, the amount of toxicity, or whatever else we measure. We need to develop new standardized techniques for measuring man and his functions.

Dr. Potkonjak: Normal values, in general, is the problem for all dealing with the problems of lung function. There are many and many normal values. I don't believe that it is possible to find normals who could represent all places in any country or the world over. There is the problem of the typology of the population. For example, in our country normal values are quite different for the people living in Vojvodina or for the people living in Dalmacia. It is not possible to apply the same values to all. It is one thing I wanted to express. The other is the definition of the occupational health, what Dr. Lainhart initiated. As for myself, I believe that the main point for these different definitions is the etiology. There from comes the problem; is the occupational disease reserved only for employed persons or otherwise, is it possible that unemployed persons can be diseased by the occupational disease. Silicosis, for example, was considered as an occupational disease, but we know that in particular circumstances some children develop silicosis. Is this an occupational disease or not? On the other side, influenza is considered as a common disease, which everybody can catch, but if I had been exposed in my working place, I am not sure that my influenza is not an occupational disease. That is why I think that occupational diseases have to be considered, first of all from the point of view of etiology.

Dr. Lainhart: I think that there is a philosophic difference here between the way medicine is practiced in various countries. In our country, for example, traditionally we have private medicine and occupational health or occupational medicine has been set aside and not really included as a part of general medical knowledge. When I was at the Medical School, for example, I am sure I heard of silicosis, but probably no other occupational disease. I don't know about medical education in either of your countries, but it seems to me that in a country such as yours, where you have more complete medical programs than we have in the States, that occupational becomes a part of the total health problem, rather than being separated and considered only by a few people on conferences like this and not by the general medical community. So this would speak to your problem that, regardless of where or how the exposure occurs, we are the ones who perhaps know more about etiologic agents and therefore, can contribute to the total medicine.