7. Final Discussion

In the experiments on rats the lung, the trachea and their subcellular fractions were investigated to analyze the trace elements present and to obtain information on their chemical forms and on their possible sites of action within the different cell compartments. In addition some other important tissues such as liver, kidney and heart were included. Those organs are constantly exposed to many oxidants and xenobiotics and, as the tissues of the respiratory tract, require a specific antioxidant system.

The use of the different microtechniques of element analysis such as INAA and GFAAS allowed the quantitative determination of several trace elements. INAA was used to determine the concentration of arsenic, chromium, cobalt, iron, manganese, rubidium, selenium and zinc in the rat lung and trachea and their distribution among the subcellular compartments of these tissues. With manganese the limit of detection was not low enough to analyze its concentrations in the subcellular fractions. As INAA is no suited to the determination of copper and nickel, GFAAS was applied in the analysis of the latter two elements.

In this way the distribution of these trace elements was studied in the tissues of selenium-deficient and selenium-sufficient rats. All elements were found to be distributed inhomogeneously among the body compartments (blood, lung, trachea, kidney, liver and heart) and subcellular fractions of the lung and trachea. As expected, the selenium concentration found in the tissues and subcellular fractions of the lung and trachea of selenium-deficient animals was distinctly lower than in selenium-sufficient animals. Except for the manganese concentration in the blood and the arsenic concentration in the heart ($p<0.01$), no significant differences between those two groups were observed. It is therefore very likely that pathological changes found in selenium deficiency are not related to secondary effects caused by changes in the tissue concentrations of these elements.

Of special interest were the findings that the lung had the highest concentration of arsenic which exceeded that in the liver by a factor of 3 and that both arsenic and nickel were present in relatively high amounts in the lung, trachea and their subcellular compartments (mitochondria and cytosol).

First information on the presence of protein-bound forms of arsenic, cadmium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium and zinc in the lung and trachea cytosol of the rat was obtained by chromatographic separation of the cytosolic proteins and determination of the distribution of these elements among the separated proteins. Copper, iron, manganese, selenium and zinc are known to be essential constituents of redox-active

enzymes, but there is the possibility that in the lung these elements may also be contained in further proteins not yet identified. It can be assumed that with the exception of rubidium all elements investigated are attached to proteins. Interestingly, arsenic and nickel were also found to be protein-bound. So far nothing is known about interactions between these elements and lung proteins. Arsenic and selenium were chosen for more detailed studies on their interaction with proteins and on the distribution of these trace element-containing compounds.

The experiments on selenium were carried out on selenium-sufficient and selenium-deficient rats and on human and rat epithelial cell lines.

The effects of the selenium status on the distribution of ^{75}Se among the body compartments were studied in rats fed for several generations with a selenium-deficient diet or a selenium-sufficient diet. Remarkable differences in the ^{75}Se distribution were observed between the two groups of animals, which are caused by a tissue-specific hierarchy. The content of the tracer found in the plasma, liver, kidney, spleen, testis and thyroid was high in the selenium-deficient rats. Considerably more ^{75}Se was found in the selenium-deficient tissues.

The labeled tissues of lung and trachea were homogenized and than partitioned by differential centrifugation into the nuclear, mitochondrial, microsomal and cytosolic fraction. The remarkable differences in the ^{75}Se distribution were observed in the homogenates and subcellular fractions obtained from selenium-deficient and selenium-sufficient animals. In the lung and trachea samples of the selenium-sufficient animals the highest specific activity was found in the microsomal and cytosolic fraction, respectively, and the lowest in the nuclei in both tissues. In the samples of the deficient animals the levels were increased in all fractions.

In the second part of the experiments the effects of the selenium status on the expression of the selenium-containing proteins was investigated in the lung and trachea obtained from labeled selenium-sufficient and selenium-deficient animals, and also the intracellular distribution of the selenoproteins in these fractions. In this experiment ^{75}Se with high a specific activity was used. The proteins of the homogenates and subcellular fractions of the two tissues were separated by means of SDS PAGE and the selenium present in the proteins was identified by autoradiography. Eleven selenoproteins have been found in this way. Their relative molecular masses were: 10 kDa, 12 kDa, 15 kDa, 16 kDa, 18 kDa, 20 kDa, 23 kDa, 25 kDa, 53 – 54 kDa, 57 – 60 kDa, and 74 kDa. The results confirm the previous hypothesis that the distribution of selenium among the different binding forms is strongly dependent on the selenium status. After administration of labeled selenite to the selenium-deficient rats only a small percentage of the amount retained in the lung and trachea was found in the 25 kDa

protein identified, as glutathione peroxidase (Gpx1), and instead other selenoproteins such as the 20 kDa protein (PHGpx), the 15 kDa protein (Sel15) and the 12 kDa protein were preferentially supplied. In the lung and trachea of the selenium-sufficient animals, however, the majority of the tracer was contained in the glutathione peroxidase. In the homogenates and cytosols of the lung and trachea from rats fed either a selenium-deficient or a selenium-sufficient diet the activity of the glutathione peroxidase - Gpx1 (EC 1.11.1.9) was measured. There were distinct decreases in the Gpx1 activity in the cytosols and homogenates of the lung and trachea in the deficient rats as compared with the selenium-sufficient animals. The activity of the enzyme was ten - fold lower in both lung and trachea fractions of the deficient animals.

The phospholipid hydroperoxide glutathione peroxidase (PHGpx) is a monomer with a molecular mass of 19.7 kDa, found in many tissues in both cytosolic and membrane-associated forms. It can directly reduce phospholipid hydroperoxides. The results of several studies suggested that the enzyme may have an important function in the redox regulation of a variety of processes such as inflammation [49]. A 15 kDa protein was found in several mammalian tissues [57, 58]. Its function and its biological significance are not yet known but its further investigation is of special interest with regard to the decreased incidence of lung and prostate cancer with selenium supplementation. Krykov et al. in their studies showed that a 12 kDa selenoprotein also called SelR or SelX is a zinc-containing protein [64]. The presence of selenium and zinc, known to have antioxidant affects, suggests a role of the 12 kDa selenoprotein in the protection against oxidative stress and in the redox regulation of cellular processes. However, the function of this protein is not yet known.

In further experiments the selenium-containing proteins in the lung and trachea were more closely investigated by means of two-dimensional electrophoresis. This electrophoretic technique has a better resolution, as the proteins are first separated via their pI and then via their molecular masses. For these experiments only selenium-deficient animals were taken, because of the much stronger retention of the tracer. In this way it was possible to compare the distribution of the selenium-containing protein among the tissues of the respiratory tract. In the homogenates and subcellular fractions of the lung and trachea more than 30 selenium-containing proteins could be distinguished, with molecular masses in the range of 10 – 80 kDa and pI values of 3 -10. Some of them such as Gpx1, Gpx3, Gpx4, Trx1, SelP, SelT and Sel15 could be identified by means of immunoassays and localized in the cellular compartments. The immunoblot of SelP was also positive in the homogenate of the human lung epithelial cells. The distribution pattern of the selenium-containing proteins was very similar in the

lung and trachea samples. They differed only in the intensity of the tracer. The differences were observed in the distribution of the proteins among subcellular fractions. In the mitochondria of the lung three bands with molecular masses in the range of 53-40 kDa were observed. The band at 18 kDa was absent in both cytosolic fractions.

Another part of the experiments was concerned with the identification and characterization of the selenium-containing proteins in cultured cells of the lung and trachea. For this purpose the lung epithelial cell line A549 (human), the lung epithelial cell line CCL (rat) and the tracheal fibroblast cell line CRL7422 (rat) were chosen. The lung epithelium is constantly exposed to high concentrations of oxygen and oxidants and is thus a primary target for reactive oxygen species (ROS) [131]. Therefore, lung epithelial cells are fortified with high intracellular and extra-cellular levels of antioxidants [132]. However, massive amounts of ROS are generated in conditions such as inflammation or exposure to cigarette smoke, air pollutants, and drugs [131, 132]. Consequently, the oxidation-reduction state of the cell is altered, an oxidant-antioxidant imbalance results and the overall tissue integrity is threatened [133]. These cells have developed sensitive and effective antioxidant defenses, in which selenium may play an important role. Another function of the lung epithelium is the formation of a restrictive barrier between the lumen and the underlying sub-mucosa. In asthma and chronic obstructive pulmonary disease, this epithelial barrier is disturbed, possibly contributing to the initiation and exacerbation of the inflammatory process that occurs in these diseases [134]. Many of the inflammatory diseases can initiate a repair process, characterized by accumulation of the fibroblasts and myofibroblasts and the extra-cellular connective tissue [131].

The human lung epithelial cells A549, rat hepatocytes HepG2, and human tracheal fibroblasts CRL7422 cells were treated with a selenite solution. The cytosolic proteins and the proteins present in the culture medium were separated by HPLC according to their native molecular masses and the elements bound to them were detected by ICP-MS. The chromatographic selenium profiles obtained from cytosols of the selenium-treated cells differed from those of non-treated cells. In the cytosols of the cells after selenium addition two peaks were observed at retention times of about 730 s (60 kDa) and about 2300 s (<3 kDa). The intensity of the peaks differed between the cell types. The peak with a molecular mass of 60 kDa had the highest intensity in the lung epithelial cells and the peak at <3 kDa in the liver cells. In the cell media a different scenario was observed. The 60 kDa fraction (630 s) was strongly increased in the liver cells but not in the lung cells. The application of selenium influenced the expression of selenium-containing proteins in the cells. Of special interest is the selenium-containing species which appeared in the low molecular mass range. Its intensity was found to

be high in the hepatocytes. This small species might be of importance in the selenium transport. Until now the studies on the selenium transport have mainly been focused on the selenoprotein P. Selenium addition affected the expression of other trace element-containing proteins. The comparison of the chromatographic profiles of copper, zinc, nickel and manganese from the cytosols of treated and untreated cells showed significant changes in the profile intensity. The intensity of the profiles decreased in the cytosols of the treated cells, which indicates that the retention of these elements in the cells was lower after selenium addition. In the case of copper, zinc and manganese there were no changes in the profile characteristics but significant differences in the profile characteristics of nickel and lead were observed. Selenium interacts with those elements either by an effect on their retention or an effect on the expression of the cytosolic proteins binding these trace elements.

The lung epithelial cells and tracheal fibroblasts are morphologically different cell types of the respiratory tract. It was interesting to investigate, if they differ in the expression pattern of the selenium-containing proteins and also if there are differences between selenium-containing proteins in the respiratory system of the rat and human. The samples obtained from those cells were separated by SDS-PAGE or 2D-electrophoresis and the selenium-containing proteins were made visible by autoradiography. In the homogenates and cellular fractions of both cell types several selenium-containing proteins were found with molecular masses and pI values corresponding to those found in the lung and trachea samples obtained from rats. This indicates that in the tissues of the respiratory tract of the rat and human the same selenium-containing proteins are expressed. The proteins present in both cell types differed in the intensity of labeling. In the lung epithelia the bands at 57 kDa were most strongly labeled, while in the tracheal fibroblasts the bands at 57 kDa, 20 kDa, 16 kDa and 15 kDa had the highest tracer content.

Several selenium-containing proteins with molecular masses below 10 could be distinguished in the lung and trachea samples. The finding that selenium is present in the mammalian organism in the form of several small proteins is of great interest with regard to the metabolism and function of this element. Small proteins can pass the cell membrane quite easily. The newly found small proteins may play an important function in the transport in those tissues. By adding a protease inhibitors to the sample and by sample treatment below 4 °C it was excluded that the small proteins are a fragments of the larger compounds produced in vitro by accidental proteolytic decomposition. After re-electrophoresis of the large selenium-containing proteins no small selenium compounds have been observed in the gel.

Selenium protects mammals from oxidative stress. The element was shown to play a role in the prevention and reduction of lung cancer, but the possible involvement of the selenoproteins in those mechanisms has not been elucidated [94]. The dietary selenium intake can strongly influence the selenoprotein levels. Most of the attention in this field has been focused on glutathione peroxidase. However, the role of selenium in the form of this enzyme could explain several, but not all the effects of the element deficiency. About two thirds of the selenium present in the organism is not bound to this enzyme but is present in other compounds [140]. The fact that with insufficient selenium intake the element is mainly used for restoring the level of the other proteins, indicates their importance. This is supported by the previous finding of Behne et al, which shows that the level of the 25 kDa protein is mainly built up during the later phases of repletion, after the pools of the other selenoproteins have been refilled [130]. The chemopreventive effect of selenium is probably mediated through its function in more than one component. Information on the presence of more than 30 selenium-containing proteins in the lung and trachea was obtained after labeling of rats in vivo with ^{75}Se and identification of the selenium-containing proteins from the tracer distribution after electrophoretic separation. Those compounds differed in their distribution among the subcellular compartments, which suggest that they may be involved in different cellular processes.

Further studies are needed to explain the role of selenium-containing proteins in the lung and trachea and especially the biological significance of the 15 kDa and 12 kDa selenoproteins.

Arsenic is an element widely distributed in our environment. Inorganic arsenicals are best known for their toxicity and on the other hand for their pharmacological effects. Several biochemical changes accompanying arsenic deficiency have been described, but the fundamental mode and sites of action of the element are not yet known. Many questions about its toxicity mechanisms but also its essentiality are still open. In earlier experiments arsenic was found to be present in tissues and subcellular fractions of the rat in quite high concentration. In this work it was found to be incorporated into several cytosolic proteins of the lung and trachea. In vitro studies on the different cell lines indicated that the element was metabolized by the cells.

In the experiments on arsenic human cell lines A549 (human lung epithelium), CRL (human tracheal fibroblasts) and HepG2 (human hepatocytes) were exposed to an arsenic solution in a concentration that was found not to be non-toxic. The cytosolic fractions of these cells and the culture media were analyzed by HPLC and ICP-MS. The combination of these two methods

allowed the separation of the proteins present in the cytosols and media and the on-line detection of the elements bound to them. In this way the metabolic pathway of arsenic in those cells and also the effects of arsenic addition on the expression of other trace element-containing proteins (such as Cu or Zn) could be examined. In the chromatographic arsenic profiles of the cell cytosols after treatment with arsenic three arsenic-containing peaks were detected, while in the profiles of the untreated cells no arsenic-containing fractions were observed. The peaks represented proteins with molecular masses of: I > 80 kDa, II ~ 8 kDa, III < 3 kDa. The arsenic-binding proteins of fraction III was also expressed in the culture media while in the pure media and the media after cell growth of untreated cells no arsenic-containing proteins were found. The fraction III may contain methylated arsenic metabolites, which were excreted by the cells. Changes in the metabolism of other trace metals after treatment with arsenic were also observed. In this case the chromatographic element profiles from the cytosols of the cells and culture media treated and non-treated with arsenic were monitored. The copper profile changed significantly in the trachea fibroblasts cells. In all cultured media of the treated cells the concentration of copper, zinc, iron and manganese was reduced after cell growth.

Several arsenic-containing species were detected in the cells after arsenic treatment. Therefore, further studies were carried out to investigate more closely the biological functions of arsenic and especially the role of arsenic-containing proteins in several tissues. As arsenic is present in the organism in vary small amounts, methods with extremely low limits of detection are needed in the investigation of the arsenic-containing proteins. For this purpose tracer methods are very suitable, in which animals are labeled in vivo with ^{73}As. With a half life of 80.3 days and gamma rays in the energy of range 53 keV, this radionuclide is very well suited to tracer experiments. A small problem may occur in the measurement of the activity by means of the scintillation detector, due to the high self-absorption of the low energy gamma rays. However, the measurement of the samples with the same geometry allowed to avoid this problem. Because of the low detection limits of the tracer method (below the femtogram range), the small amount of the arsenic were used in experiments. The tracer activity reflected therefore the distribution of the native arsenic.

Human populations in various countries (India, Bangladesh, Thailand, Taiwan, China) are chronically exposed to arsenic through consumption of water containing arsenicals. It results in a variety of in-health effects, which depend strongly on the dose of exposure, age, health-status parameters, nutrition and vary greatly among individuals. Experimental data suggest that animals are not as sensitive to inorganic arsenic compounds as humans, and that this

difference is not due entirely to differences in the gastro-intestinal absorption [82]. There is strong evidence of that arsenic is carcinogen in humans, but not so much in animals, a unique scenario not found for other carcinogens. At present there are no recognized models for the study of arsenic-induced carcinogenesis. However, it is convincingly established that arsenicosis is mediated through modification of gene expression, and cell proliferation due to oxidative stress and other uncharacterized or poorly defined physiological aberrations or modifications. The elucidation of such interaction could lead to the identification of sensitive subpopulations, provide cues for preventive or mitigation measures for arsenic intoxication, and suggest possible mechanisms of toxicity.

The epidemiological studies in developing countries revealed that under-nourishment and deficiency in dietary protein (animal food is a major source of dietary selenium) in particular, were significantly associated with increased prevalence of arsenic carcinogenesis. Moreover, patients with chronic arsenicosis had lower selenium levels than the non-affected controls in a Taiwan population [141]. These observations suggested that a lower selenium intake is associated with enhanced arsenic toxicity, and a possible relationship between arsenic sensitivity and selenium nutrition. While many experimental studies have shown an interaction between selenium and arsenic, most of these studies have only examined the interactions between the two elements at acutely high doses of arsenic. Furthermore only very limited data are available regarding the interaction between dietary selenium and ingested arsenic at non-toxic concentrations. In the few existing studies employing the dietary selenium-deficiency model, it has been shown that changes in the dietary level of selenium affect the kinetics of ingested arsenic in rats. In this remarkable differences in the ^{73}As distribution were observed between the tissues of selenium-deficient and selenium-sufficient rats, with a much higher percentage of the tracer dose being retained in the tissues of the selenium-deficient animals. Based on these results, it is hypothesized that selenium deficiency causes an enhanced accumulation of arsenic.

A significant function of selenium is to protect the cell from the oxidative stress and free radical formation that occurs during exercise. Selenium can be considered the "rate-limiting" substrate in the glutathione (GSH) system. Without selenium the peroxidase enzyme cannot be formed and consequently the antioxidant protection by the Gpx system is compromised. In the case of selenium deficiency the activity of Gpx (and of other selenoenzymes) decreases. This leads to an increase in the ratio of reduced glutathione to oxidized glutathione. Therefore in selenium deficiency more free sulfhydryl groups exist. This fact may explain the

high retention of arsenic in the selenium-deficient tissues, as arsenic is known for its high affinity to the sulfhydryl group.

The distribution pattern of the orally administered arsenic in the rat tissues differed from that after i.p. injection. It can be explained by the difference in the arsenic validity in the organism after i.p or oral administration. In the tissues obtained from animals after oral administration clearly less ^{73}As was found. Once absorbed, arsenic is bound to haemoglobin, leucocytes, and plasma proteins. It is cleared from the intravascular space within 24 hours, and distributed in most tissues [70, 71]. Arsenic compounds, well absorbed by the gastro-intestinal tract or after intra-peritoneal injection, were rapidly distributed to organs or tissues rich in proteins containing sulfhydryl groups, and accumulate mainly in liver, kidneys, spleen, lung and adrenal gland [73]. These tissues were most strongly labeled. In the case of i.p. arsenic administration the element is directly absorbed by the organs in the abdominal cavity and by the vessel system, with blood faster reached also other organs. The pathway of arsenic from the gastro-intestinal tract to blood is more complicated, depends on many factors (stomach and intestinal linings act as a barrier for many large or charged compounds). For this reason it takes longer to reach the tissues. On that account, 48 hours after i.p. administration arsenic was mostly found in the pancreas, diaphragm, thymus, blood and spleen, whereas in the case of the tissues of the animals after oral arsenic administration the blood had the highest tracer content.

It has been reported that when arsenic is administrated to cells it initially binds to cellular proteins before reduction or methylation can occur. Therefore, the binding of arsenic to cellular proteins is a key determinate in the arsenic metabolism. Although various studies have attempted to isolate arsenic-binding proteins, non proteins from mammalian tissues have been identified and demonstrated to bind with arsenic. It is of great interest to analyze the arsenic-containing proteins present in the tissues of the rat and to get information on their subcellular distribution and their biological effects.

In order to obtain some information on the sites of action of arsenic and thus on its possible functions, the subcellular distribution of the tracer was investigated. The tissues with the highest arsenic retention after administration of ^{73}As i.p. were taken for this investigation. Pancreas, thymus, spleen, liver, diaphragm, and lung were homogenized and than partitioned by differential centrifugation. In the homogenate and subcellular fractions of these tissues differences in the ^{73}As distribution were observed. The retention of the tracer was highest in the cellular fraction of the thymus and pancreas, whereas in the cellular compartments of the liver, lung and diaphragm the retention was similar. The lowest tracer activity was found in

kidney compartment. The tracer was found to be incorporated inhomogenously within the different cell compartments of the different tissues. It was mostly found in the nuclei, but also in the cytosolic and mitochondrial fractions.

In a further experiment the distribution of the arsenic-containing proteins in the homogenates of several rat tissues such as spleen, adrenal gland, spermatic ducts, diaphragm, liver, thymus, trachea, brain, heart, lung, pancreas, testis, epididymis, small intestine and kidney was studied. The proteins present in these tissues were separated by means of SDS-PAGE and the arsenic-containing proteins then identified by autoradiography.

After evaluation of the autoradiograms several arsenic-binding proteins could be distinguished. The proteins labeled with ^{73}As had relative molecular masses of >250 kDa, 75 kDa, 50 kDa, 37 kDa, 29 – 30 kDa, 25 kDa, 16 kDa and 15 kDa. There were remarkable differences observed in the characteristics of the arsenic-containing proteins between the tissues after SDS-PAGE. The ^{73}As-binding bands with molecular masses of >250 kDa, 75 kDa, and 50 kDa were detected in all homogenates. The band at 37 kDa was present in the blood, spleen, adrenal gland, liver, thymus, heart, kidney and pancreas. The homogenates of the adrenal gland, kidney, and thymus had labeled bands at 30 -29 kDa. The band at 25 kDa was found in the blood and in the homogenates of the spleen, thymus, heart, kidney, and pancreas. In the pancreas, thymus spleen and adrenal gland homogenates a further ^{73}As-containing protein was detected at 16 kDa. The very strongly labeled band at 15 kDa was found in the blood and in the homogenates of the spleen, heart and kidney. In this way also the arsenic-binding proteins present in the subcellular fractions of the thymus, spleen, pancreas, diaphragm, lung and liver were investigated. The data suggested that the distribution pattern of arsenic-binding protein differed among the cellular compartments of different tissues of the rat. The differences found in the distribution of the arsenic-binding proteins among the tissues and their cellular compartments suggest that the arsenic compounds may be involved in different intracellular processes.

Arsenic-binding proteins were investigated in the cultured cells of the respiratory tract (A549 human epithelial cells and CCL rat epithelial cells) after in vivo labeling with ^{73}As. After evaluation of the autoradiograms six proteins containing ^{73}As were found. Their relative molecular masses were: >250 kDa, 75 kDa, 50 kDa, 37 kDa and 18 kDa. In the media after cell growth one ^{73}As-containing band with a molecular mass of about 75 kDa was detected.

In the homogenate of the CCL cells six bands at > 250 kDa, 75 kDa, 50 kDa, 37 kDa and 18 kDa could be seen, while in the pellet only bands at > 250 kDa, 75 kDa, 50 kDa and 37 kDa were detected. In the cytosols two weakly labeled bands at about 50 kDa and 18 kDa were

present. The homogenate and the pellet of the A549 cells contained four radioactive bands at > 250 kDa, 75 kDa, 50 kDa and a very blurred one at about 12 kDa, whereas in the cytosols the tracer concentration was too low to detect ^{73}As-labeled bands. The arsenic-binding proteins found in the rat lung epithelium corresponded to those found in the lung samples of the rat. Moreover, the distribution pattern of arsenic-binding proteins was almost the same between the human and rat epithelial lung cells. This suggested very similar metabolic pathways of arsenic in these cell types.

For further characterization of the arsenic-containing proteins present in the homogenates of the lung epithelial cells and the homogenates of the rat tissues, two-dimensional gel electrophoresis was applied. This method has stronger denaturing effects (8 M urea) than SDS-PAGE and it may eliminate all arsenic not firmly bound to proteins. In the human and rat lung epithelial cells after 2D electrophoresis several spots with molecular masses between 80 and 10 kDa, and pI values between 3 - 10 were found. Also in the homogenates of the tissues obtained from rat several ^{73}As-labeled spots could be distinguished. It was possible to localize two spots in the 2D gel of the thymus homogenate, labeled with arsenic. The spots with pI values of 7.98 and 9.04 were cut out and after trypsin digestion, their peptides were used for MALDI-MS analysis. The two arsenic-containing spots found in the gel after 2D electrophoresis were identified as beta 1 globin and hemoglobin. So far nothing is known about the role of these proteins in arsenic metabolism. Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells in mammals and other animals. The molecule consists of globin, the apoprotein and four heme groups, with an iron atom in each of them. The high binding affinity of the globin and hemoglobin for arsenicals is probably due to the large number of cysteine residues. The rat hemoglobin is a tetramer, consisting of two α chains and two β chains. The rat hemoglobin has three cysteines in each α chain (Cys104, Cys111, and Cys13) and two cysteines in each β chain (Cys93 and Cys125). However, arsenic exists in several forms in the living organism, and it is not clear which arsenic species binds to these proteins.

The differences found in the distribution of the arsenic-binding proteins in the tissues and among their cellular compartments indicate that the arsenic compounds may be involved in different intracellular processes. So far nothing is known about these proteins, their functions, their structure and the way in which arsenic is incorporated into them. All elements which are incorporated non-covalently into proteins, are lost during the electrophoresis because it is a denaturing separation method, which causes that the proteins to lose their native structure. ^{73}As that remained in the proteins after SDS-PAGE indicated that this metalloid must be

firmly bound, either covalently or as arsenosugar or one of the chemically active methylated compounds of trivalent arsenic.

The finding that arsenic is bound to proteins is of the grate interest. It does not explain the biological function of this metal and its role in the living organism but it may help to explain its essentiality in the living organisms. Further studies in this area are therefore needed.