Summary

In studies carried out on rats fed either a selenium-deficient or a selenium-sufficient diet, element analytical methods have been combined with biochemical separation procedures. Subcellular separation of the tissues into a nuclear, mitochondrial, microsomal and cytosolic fraction has been achieved by differential ultracentrifugation. Instrumental neutron activation analysis (INAA) has been used to determine the concentration of several trace elements such as selenium, arsenic, iron, manganese, zinc, cobalt, rubidium and chromium in the lung, trachea, and their subcellular fractions. Atom absorption spectrometry (AAS) has been employed for the determination of copper and nickel in the mitochondrial and cytosolic fractions of the lung and trachea. As was to be expected, the selenium concentrations in the lungs and trachea of the deficient animals were lower than those in the rats fed the selenium-adequate diet. With the other elements no significant differences between the two groups were observed. All elements investigated were distributed inhomogeneously among the subcellular fractions. With most of these elements the highest concentrations were found in the mitochondria and microsomes. Chromium was enriched in the nuclei, and arsenic and rubidium were mainly present in the cytosol.

After fractionation of the cytosolic proteins by size exclusion chromatography (SEC), inductively coupled plasma mass spectrometry (ICP-MS) has been employed to determine the trace element-containing proteins on-line in the eluate. The separation methods permit the investigation of the native metal composition of the trace element-containing proteins. First information on the presence of protein-bound forms of arsenic, cadmium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, silver and zinc in the rat lung and trachea cytosol was obtained by chromatographic separation of the cytosolic proteins and determination of the distribution of these elements among the separated proteins. It could be assumed that all the elements investigated with the exception of rubidium are attached to proteins. Copper, iron, manganese, selenium and zinc are known to be essential constituents of redox-active enzymes, but there is also the possibility that in the lung these elements may also be contained in further proteins not yet identified. Interestingly, arsenic and nickel were found to be protein-bound. So far nothing is known about interactions between these elements and lung proteins. Further studies were therefore carried out to investigate more closely the biological functions of these elements and especially the role of arsenic in the lung and trachea and also in the other tissues. Arsenic and selenium were then chosen for more detailed studies.

In order to obtain some information on the selenium-containing proteins and also on their sites of action and on their possible functions in the tissues of the respiratory tract and their subcellular fractions, radiotracer techniques have been combined with biochemical separation procedures.

The selenium-containing proteins in the subcellular compartments have been determined by labeling of rats in vivo with ^{75}Se, gel electrophoretic separation of the proteins and autoradiographic detection of the tracer. In the second part of experiments the effects of selenium status on the expression of the selenium-containing proteins in the lung and trachea of the sufficient and deficient animals were investigated. In this way about 24 Se-containing proteins could be distinguished in the tissues and heir subcellular fractions. The molecular masses of the subunits were in the range between 10-30 kDa and between 50-80 kDa, with pI value between 3 and 10. In all subcellular fractions the selenium-containing proteins at about 60-55 kDa, 20-25 kDa and 15 kDa were predominantly labeled. The results of comparison experiments confirm the previous hypothesis that the distribution of the element among the different binding forms is strongly dependent on the selenium status. After administration of the 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 and instead other selenoproteins were preferentially supplied, whereas in the lung and trachea of selenium-sufficient animals the majority of the tracer was contained in this protein and also in the 23 kDa and 20 kDa and 15 kDa. These findings indicate that the small amount of selenium administered to selenium-deficient animals is mainly used to restore the level of other selenium-containing compounds.

In order to obtain more information on the sites of action and possible functions of arsenic, the distribution of the As-tracer in the body compartments and their subcellular fractions was investigated. In this case the rats were labeled *in vivo* with ^{73}As. The comparison of the ^{73}As retention pattern in the body compartments of rats fed either a selenium-deficient or a selenium-sufficient diet showed that the administration of the element is strongly dependent on the selenium status. Much more arsenic remained in the tissues of selenium-deficient animals. The ^{73}As content differed between the tissues.

The rats fed the selenium-sufficient diet could metabolize arsenic faster and its retention in the tissues was lower than in the tissues of the selenium-deficient animals. The tracer content was also determined in the subcellular fractions of many tissues. Arsenic was incorporated mainly into the nuclei, but was also found in the cytosolic and mitochondrial fractions.
The arsenic-containing proteins present in homogenates and subcellular compartments have been investigated by gel electrophoretic separation of the proteins and autoradiographic detection of the tracer. It could be shown for the first time that there were ^{73}As-containing proteins in those tissues. Their relative molecular masses were: >250 kDa, 75 kDa, 50 kDa, 37 kDa, 29 – 30 kDa, 25 kDa, 16 kDa, and 15 kDa. The evaluation of the autoradiograms shows that there were differences between the distribution of the arsenic-binding proteins present in the tissues and among their cellular compartments. This indicates that the arsenic compounds may be involved in different intracellular processes.