CONFERENCES AND SYMPOSIA

NUCLEAR ACTIVATION TECHNIQUES IN THE LIFE SCIENCES

REPORT ON THE SYMPOSIUM
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INTRODUCTION

It is a well-known fact for all biological systems that, in addition to the bulk nutrients, very small amounts of certain compounds and elements are essential for the proper development and well-being of the organism. The group of compounds known as the vitamins is, of course, a well-known example of this. In contrast to the vitamins, which are almost exclusively organic in nature, certain inorganic elements in 'trace' quantities have also been shown to be essential for well-being, although in many cases it is not known exactly how they function. Unfortunately, not all such trace elements play a beneficial role; indeed, small quantities of some elements can cause serious damage to both animals and plants.

To study such effects, both beneficial and harmful, it is clearly necessary to make use of very sensitive methods of analysis. One such technique that is widely used at the present time is nuclear activation, although, of course, it is not the only available trace analysis technique and is not in all cases the best. However, the combination of the great importance of trace elements for man and his environment and the widespread use of nuclear activation techniques to study these problems led to the holding in May 1967 of the first IAEA Symposium on "Nuclear Activation Techniques in the Life Sciences" in Amsterdam, the Netherlands. Five years later this, the second symposium of the same title, was held in Bled, Yugoslavia, to provide a forum for reporting and discussing the developments and improvements that had occurred in the intervening years.

The present meeting was attended by 99 participants representing 23 countries and 3 international organizations. A total of 49 papers were presented, and sessions were devoted to the following topics: (a) general analytical methods; (b) toxicology and public health; (c) animal and plant studies; (d) medicine, in vitro studies; (e) medicine, in vivo studies.

GENERAL

Reviews of current thinking on the role of essential trace elements in man and animals were presented by H.J.M. Bowen (UK) and K. Schwarz (USA).

Biochemical studies of trace elements have implicated quite a large number of elements as being involved in the life cycle. Of the well-established trace elements such as copper, iron, iodine, molybdenum and zinc, many well-defined compounds are known. For instance, over 50 iron-proteins and at least 30 proteins and enzymes containing copper have been identified. In contrast, a number of other elements such as arsenic, boron, bromine, chromium, cobalt, manganese, nickel, selenium, silicon, tin and

vanadium are also known to be essential, or are strongly suspected of being such, but little has been established about the chemical form of their existence in living matter. The difficulty in establishing what is an essential trace element and what is not is not a simple analytical matter. Analyses of animal tissue or plant material by sensitive means reveal the presence of many elements in small traces, which derive from the diet and the environment but which as far as is known are not all essential. In man and animals it was established by 1957 that iron, iodine, copper, manganese, zinc, cobalt and molybdenum were essential, and since then a further 6 have been proved as necessary. These are selenium, chromium, tin, vanadium, fluorine and silicon. When one considers that only 0.1 microgram of certain selenium compounds is sufficient to protect a rat against selenium deficiency diseases it is obvious that special research techniques must be used. It is clear that man is a bad experimental animal in this case. At the present time some of the most important and rewarding research is being done with rats kept in plastic cages with filtered air to breathe and with specially purified foods to eat.

The occurrence of mineral elements in plants was reviewed in a paper by P. Peterson (UK). Plants also require trace elements, such as cobalt, copper and zinc, but far less is known about botanical needs in general and many other elements not yet identified may be essential. Plants have the ability in some cases to adapt to the environment and certain species grow in mineral-rich soils where other plants cannot survive. These plants have value as indicators in mineral prospecting. Other species accumulate non-essential elements from the soil to a remarkable degree and plant ashes containing over 2% chromium have been recorded, the highest being for an Australian shrub in which up to 25% was found. Accumulation of dangerous elements, of course, could give rise to concentrations that are dangerous to man and animals.

The toxicological effects of trace elements on man and animals, reviewed in a paper by J. Parizek (ČSSR), show some interesting actions and interactions. The essential trace element selenium is poisonous if too much is given and in areas where this element is plentiful cattle grazing on the vegetation are very much affected. Cadmium, a non-essential element as far as is known, causes damage to the reproductive organs, while mercury destroys the kidneys. Remarkable protective actions of one trace element for another have also been discovered. Selenium protects animals against the damage caused by mercury and cadmium if selenium compounds are administered shortly after the intake of the metals. On the other hand, if they are administered in the reverse order, the effects of selenium poisoning are greatly increased. Other similar cases are also known and it is clear that intricate biochemical research must be undertaken to find the mechanisms involved. It should be pointed out, however, that neutron activation is a method of elemental analysis that gives no information about the chemical form of the trace element. Activation analysis can therefore be a good biochemical tool only when it is used in conjunction with other analytical techniques.

GENERAL ANALYTICAL METHODS

Although the interpretation of data in relationship to biological systems was an important theme of the Symposium, the manner in which the data were acquired was also an important consideration. As in all analytical

things, the data can be no better than the sample. Although contamination after nuclear activation analysis is of relatively little importance, contamination during the collection of the sample prior to irradiation is of great importance, particularly when one is interested in measuring parts per 10^9. In a very interesting and relevant paper Versieck and Speecke (Belgium) investigated the contamination of specimens caused by normal surgical instruments used to take samples. Their technique was to use previously neutron-irradiated instruments so that any radioactivity measured in the sample could only have arisen from the knives or needles employed. Under some but not all circumstances very serious contamination can arise from the act of sampling, causing the presence of some elements in amounts far exceeding their natural levels.

A further possible cause of false results in sample preparation arises from losses during ashing of the sample prior to irradiation. This is frequently done to get rid of the organic matter. Experiments to investigate such losses by tracer methods are always somewhat suspect as it is difficult to be sure that the tracer and the traced are in exactly the same condition. In a paper by Hislop and Williams (UK) this difficulty was avoided by irradiating a sample of bone with high-energy gamma rays (to produce ^{203}Pb by the ^{204}Pb (γ, n) ^{203}Pb reaction) and following quantitatively the 279-keV gamma ray of ^{203}Pb after various treatments. Ashing at 600°C was shown to be safe but losses occurred at 710°C. Moreover the behaviour of tracer lead was not the same as that naturally incorporated into bone. As other elements were also activated, their behaviour could also be followed and noticeable losses of sodium and potassium but not calcium also occurred. Levstek et al. (Yugoslavia) found losses of vanadium of over 50% during ashing of samples at 500°C when investigating methods of analysis of this element.

One of the difficulties encountered when analysing biological material is the normal presence of sodium, potassium, chlorine, bromine and phosphorus, whose induced activities tend to swamp those of elements of lower abundance. This also interferes with the potential multi-element capability of modern neutron activation analysis using Ge(Li) detectors. Plantin (Sweden) has used a chemical separation scheme based on ion exchangers, and computer data interpretation to overcome these difficulties.

Bagliano et al. (Italy) compared neutron activation analysis and oscillographic polarography (using a double electrolytic cell) for the analysis of some aquatic plant samples and showed that the latter method had a better precision and was more accurate for some elements (Fe, Co, Ni and Zn), but was much slower in performance. Only 12 samples could be analysed per month with a full-time operator but neutron activation analysis was more useful for routine work when about 25 samples per month were handled by a part-time operator.

Other papers in this session dealt with measurements by prompt atomic and nuclear reactions, spectrum analysis with a small computer, general methods for the determination of vanadium and lead, and the reliability of neutron activation analysis as tested by standard intercomparison materials.

TOXICOLOGY AND PUBLIC HEALTH

Some of the toxicological problems of trace elements touched upon in the paper by Parizek (ČSSR), mentioned above, were exemplified in this

session by reports dealing with mercury hazards in dental practice, the possible effects on man of contamination from hard metal dust, welding fumes and smoke, and by studies of pollution in air and water.

Dentists and their assistants work, as is well known, with mercury and its compounds and it might be expected that their bodies would show higher mercury contents than the normal populace. Lenihan et al. (UK) observed that this is in general true but enormous variations occur among the group, which can be attributed to local variations of conditions such as surgery layout and ventilation.

Industrial atmospheres are more varied than that of a dentist's surgery and three papers described investigations into aerial conditions in factories. Brune et al. (Sweden) observed that the lungs of workers in a factory dealing with heavy alloys (tungsten based) contained appreciably more tungsten than controls. Dams et al. (Belgium) studied the segregation of elements in different sized air-borne particulates in the production of nodular cast iron. Hewitt and Hicks (UK) examined some metabolic relations for trace elements in blood and body tissues from animals exposed to welding fumes.

The air outside factories is usually considered to be cleaner than inside and this has also been investigated. A preliminary study was made by Lyon et al. (USA) of the fate of trace inorganic elements in a large coal-fired steam plant producing 990 MW of electricity. When one considers that 500 million tons of coal were burnt in the USA in 1970 and that although elements such as arsenic, mercury, cadmium, zinc etc., which are known to be toxic, are present only in fractions of micrograms per gram, the total amounts of these elements are considerable. The report presented no real conclusions but outlined some of the difficulties involved in the investigations, which should undoubtedly be continued. Air pollution studies in cities, in particular Guilford, Surrey, UK, and Mexico City, Mexico, were also reported. Although nothing drastic was reported, such investigations provide valuable reference data for future use.

The concentrations of some trace elements in the aquatic environment were considered in a study of 7 of them in the water, detritus, algae and fish of the Sava River. Drašković et al. (Yugoslavia) concluded that suspended material, plankton and detritus play an important role in the transport of trace elements. More detailed studies, but confined to one element, mercury, were reported by de Goeij et al. (Netherlands). These workers used an elegant semiautomatic radiochemical separation procedure for the analysis of mercury in Rhine sediments, marine organisms collected from the Netherlands Wadden Sea, preserved fish and fish products, and in the tissues of various fish-eating birds and mammals.

In the final paper of this session Das and van der Sloot (Netherlands) described methods for the determination of cadmium and mercury in biological samples and tapwater by neutron activation analysis and isotope dilution, and presented some typical results.

ANIMAL AND PLANT STUDIES

During the past half century animal and plant studies of various kinds have been amongst the most important sources of information on the biological role of trace elements and have led to profound economic benefits in regions of the world where diseases in plants and livestock arise from

naturally occurring trace element imbalances. Activation analysis, however, has not yet figured largely in many of these investigations and only four papers dealing with applications of activation analysis were presented in this session.

A paper by Pethes et al. (Hungary) described the use of liver biopsy samples for the detection of imbalances caused by trace element supplementation in intensive livestock farming. Liver biopses of 40-50 mg wet weight were analysed non-destructively by activation analysis for Co, Cr, Cu, Fe, Rb, Sb, Zn and other elements with the aid of a Ge(Li) detector. For the quantitative determination of copper the Ge(Li) spectrometer was combined with a NaI(Tl) detector to measure the 511-keV annihilation radiation from ^{64}Cu by a gamma-gamma coincidence technique.

Another paper dealing with animal studies was presented by Mazière et al. (France) on selenium metabolism in rats. Several batches of rats restricted in movement and subjected to varied diets of selenium were given a tracer dose of ^{75}Se by injection or orally. The radioactivity of the whole body and the specific radioactivity of the blood and plasma selenium were kept under observation for 1 to 3 months, depending on the batch concerned. Determinations of stable selenium were carried out by means of destructive or non-destructive radioactivation, depending on the type of sample. The application of the occupancy principle to these data enabled the absorption coefficient and total body content of selenium to be determined. The validity of the method was verified, after sacrificing the animals, by analysing homogenates of whole rats and various organs.

For projects requiring the analysis of large numbers of samples, automated methods are potentially of great interest and value. As part of a collaborative program between France and the USSR, an automated unit for the routine determination of P, N, K and Ca in plants by neutron activation analysis was designed and constructed for installation at the Dokuchaev Soil Institute, Moscow, USSR. Methodological and technical studies, and the construction and testing of the system, were all carried out at Grenoble and were described by Vernin (France). The system comprises an on-line computer, a 14-MeV neutron generator, a pneumatic sample transfer system, 2 NaI(Tl) detectors and various auxiliary equipment. Up to 500 samples per day can be analysed for the four elements of interest by fast neutron activation analysis.

In both marine and terrestrial biocycles, humic acids are thought to play an important role in the transport of trace elements. A paper by Huljev and Strohal (Hungary) described the analysis of humic acids isolated from North Adriatic marine sediments. These were hydrolysed into their main components and analysed by instrumental activation analysis for a group of nine trace elements. The results obtained indicated that a number of trace elements can bind to humic acids under natural conditions. Both chelation and surface absorption mechanism are involved, but only the former can be studied effectively by the techniques described.

MEDICINE, IN VITRO STUDIES

Trace element concentrations in autopsy and biopsy samples of human tissues, in food and in excreta have been a subject of intense interest for many years, particularly in connection with studies of the possible role of

trace elements in various human diseases. Activation analysis, on account of its high sensitivity and comparative freedom from problems due to reagent contamination, has made important contributions to this field.

In cystic fibrosis, probably the most serious non-tubercular disease found in children today, concentrations of sodium in finger nail clippings and sweat are usually significantly raised and are an important diagnostic indicator of the disease. Fite et al. (USA) have analysed nail clippings from over 2000 subjects by activation analysis and have shown that, while measurements of sodium in nail clippings are of undoubted clinical interest, there are nevertheless too many 'false positives' for the technique to be considered entirely satisfactory. Other elements, particularly Cu, also appear to act as diagnostic indicators of cystic fibrosis. Cu levels, however, appear to be completely normal at birth (at which time it is most important to diagnose the condition) and only tend to become elevated when infants with the disease reach an age of 4 to 6 months.

Significant changes in trace element concentrations in connection with various other diseases were also reported. Larsen et al. (Denmark) described changes in the tissue levels of As, Mn and Se in patients with chronic renal insufficiency. Kasperek et al. (Federal Republic of Germany) studied 10 trace elements in blood serum from 184 normal individuals and 286 patients with various diseases and suggested that ratios of trace element concentrations might be more useful than the individual concentrations as indicators of disease. Masironi (World Health Organization) described a co-ordinated research program involving laboratories in several different countries, which, it is hoped, will throw new light on the possible role of trace elements in cardiovascular diseases.

Metabolic investigations of various kinds, involving dynamic studies of stable trace elements, were described by several speakers. Behne and Diel (Federal Republic of Germany) investigated the effect of oral glucose and intravenous insulin on trace elements in blood serum and noticed significant changes in the concentrations of such elements as Cr, Co, Se and Zn. These authors went on further to suggest that such phenomena could possibly lead to Cr and Co deficiencies in insulin-requiring diabetics. Kasperek et al. (Federal Republic of Germany) reported that serum zinc levels show a marked response to food intake. After a large breakfast, for example, the serum zinc concentration drops over a period of 3 to 6 hours to less than 70% of its initial fasting value; the addition of vitamin C to the breakfast significantly diminishes the degree of this response. These results point to the importance of collecting blood samples for analysis under carefully controlled conditions.

Other papers on dynamic studies described the use of activable stable tracer isotopes such as ^{58}Fe and ^{50}Cr for studies of iron utilization in pregnant women and ^{46}Ca for a study of Ca absorption in the neonate. By such means it is feasible to undertake tracer studies without subjecting the patient to any form of radiation exposure.

Perhaps the most elegant paper on in vitro studies was one dealing with molecular biology. Sabbioni et al. (EURATOM) have developed a simple activation analysis method for determining phosphorus with a sensitivity of 0.1 ng: the activity measurements are made by Čerenkov counting. By combining this method with conventional biochemical procedures, their technique permits the determination of monomeric residues in nucleic acid samples, the measurement of chain length of polynucleotides and the study

of radiation damage in nucleic acids. For all of these studies only a few micrograms of the nucleotide material are required.

The proper application of in vitro analysis procedures calls for careful attention to standardization and quality control. Johansen and Steinnes (Norway) demonstrated that high precision can be obtained in the analysis of a standard reference serum. A standard deviation of about 0.7% was reported for Na and Cl and 1.5% for K. For these measurements activation analysis was used as an independent check on results obtained by the more conventional flame photometric methods. Such precision, however, is not always required. Schicha et al. (Germany) found that trace element concentrations in adjacent regions of an organ (e.g. liver, lung, heart, kidney) may differ by factors between 1.6 and 4.5 for essential elements and even more for non-essential elements. In such cases variations due to sampling are likely to be much greater than the analytical errors.

Other papers on in vitro analysis dealt with Se and Cr in blood and foodstuffs, F in teeth, the metabolism of Br in nursing mothers and a rapid method for the determination of protein-bound iodine. This last method, described by Lubkowitz (Venezuela) was interesting in that it permitted the determination of up to 84 samples per day and overcame the need for complicated calculations and decay corrections.

MEDICINE, IN VIVO STUDIES

Since the feasibility of determining levels of elements in living subjects after carefully controlled exposure to neutrons was first demonstrated by Anderson in 1964 rapid improvements in the technique have been made. It is currently possible to make absolute measurements by in vivo activation analysis of total-body Ca, Na and Cl as well as relative measurements of N, P and other elements. Partial-body in vivo activation analysis has also been established as a feasible technique for most of the same elements and for I in the thyroid. In all of these measurements the dose delivered to the living subject is, in general, similar to that of comparable X-ray and radioisotope examinations.

For absolute determinations of body constituents a high uniformity of neutron flux in all regions of the body has to be achieved. In this respect, the most successful technique at the present time appears to be that described by Anderson et al. (UK). A scanning procedure was used whereby the subject, lying in a rectangular box constructed of paraffin wax and packed around with sealed polyethylene bags filled with water, is drawn past a collimated source of 14-MeV neutrons. The maximum observed variation of neutron flux was about 3% of the mean. This apparatus has been used for the determination of whole-body sodium in man. Further development, however, is required to make it suitable for the determination of whole-body sodium in sick patients.

A paper by Boddy et al. (UK) reviewed some of the advantages and disadvantages of different sources of neutrons for in vivo activation analysis, such as from reactors, radioactive (α, n) sources, ^{252}Cf and neutron generators. Except when neutron beams are available from already-existing installations such as reactors and particle accelerators, the most attractive alternative sources specifically for in vivo activation analysis appear to be radioactive (α, n) sources and sealed-tube neutron generators. For limited applications in partial-body in vivo activation analysis, ^{252}Cf sources have

highly favourable characteristics. A scanning geometry irradiation facility described by Boddy uses two opposed 14-MeV neutron generators, which permit simultaneous bilateral irradiation of the subject. By adopting a similar scanning geometry for the whole-body counter and by making the speed of the scan identical with that used for the irradiation, radioactive decay along the length of the body is automatically compensated for all radioisotopes, irrespective of half-life.

An alternative neutron irradiation facility described by Cohn et al. (USA) utilizes 14 encapsulated (α, n) sources and permits the determination of whole-body calcium, sodium, chlorine and phosphorus. These sources have the advantage of producing considerably more ^{49}Ca, ^{24}Na and ^{38}Cl than do 14-MeV neutrons, per unit absorbed dose delivered to the patient. They are not, however, so suitable for the determination of phosphorus and cannot be used for nitrogen.

An interesting variation on these techniques of in vivo activation analysis is achieved by the use of a pulsed neutron source. Biggin et al. (UK) used a pulsed beam of cyclotron-produced neutrons of average energy 2.5 MeV and measured the prompt gamma rays produced by the $^{14}N(n, \gamma)^{15}N$ reaction. By making use of the fact that fast neutrons take a finite time to slow down in the body, they were able to measure the capture gamma rays for several microseconds after the end of each pulse and thereby achieved a much better signal to noise ratio. The technique as described is not yet suitable for absolute measurements, but in serial studies it gives a precision of about 1% for whole-body nitrogen at a dose to the subject of 100 mrem.

Clinical applications of in vivo activation analysis were mentioned by all the speakers in this session but were dealt with in greatest detail by Nelp et al. (USA), particularly with respect to whole-body calcium. 114 patients and 8 normal subjects have been studied by this group using cyclotron-produced neutrons of 4-12 MeV; typically in this work the patients were exposed to a uniform whole-body radiation dose of 200 mrad. It was reported that serial balance studies (e.g. in end-stage renal disease) can be carried out much more conveniently by in vivo neutron activation analysis than by conventional methods. Various possible methods of treating osteoporosis are being investigated with the aid of this technique.

CONCLUSION

The symposium provided a valuable review of the wide variety of activation analysis procedures now applied in the life sciences, many of which are already in use on a routine basis for large numbers of samples. Although the techniques described were, in some cases, sufficiently complex to require the assistance of an expert analyst, it was particularly recognized that the successful exploitation of nuclear activation techniques in the life sciences now, more than ever, also calls for the closest collaboration of clinicians, biochemists, epidemiologists, physiologists and experts from other life sciences disciplines.

The complete proceedings of the symposium will be published shortly in Vienna by the International Atomic Energy Agency.

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