RADIOACTIVATION ANALYSIS IN BIOCHEMISTRY AND MEDICINE

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L'analyse chimique est un auxiliaire essentiel de la recherche biochimique. Depuis quelques années, de nouvelles techniques d'analyse telles que l'emploi des traceurs radioactifs, l'échange d'ions et la chromatographie donnent de bons résultats. L'analyse par radioactivation déjà appliquée avec succès—quoique de manière assez restreinte—en biochimie humaine et animale, est probablement appelée à jouer un rôle de plus en plus utile dans l'une et l'autre sciences. Les avantages de la méthode: simplicité, rapidité, sensibilité et précision, ont été mis à profit de façon limitée en odontologie, en médecine légale et dans la recherche sur le cancer. Dans ces domaines de la recherche clinique, comme dans d'autres, un grand nombre de problèmes intéressants paraissent pouvoir être étudiés par cette méthode.

Jusqu'à maintenant, en biochimie, l'analyse par activation est surtout appliquée aux échantillons de tissus, en vue de la recherche non seulement d'éléments à l'état de traces, mais aussi de constituants plus courants (tels que le sodium et le potassium), lorsque les autres méthodes manquent de sensibilité ou sont incommodes. Le métabolisme de certains éléments, comme le strontium, ne peut être que partiellement déterminé au moyen des traceurs radioactifs ou des procédés classiques de la biochimie. Les méthodes par activation permettent d'étudier la répartition de l'élément normal dans les tissus et les excreta.

La combinaison de l'activation neutronique et de la chromatographie sur papier a l'avantage d'augmenter la sélectivité et la sensibilité. La plupart des applications de l'analyse par radioactivation en biochimie supposent l'utilisation d'un réacteur nucléaire, mais des mesures ordinaires, rapides et exactes, du taux de sodium sanguin peuvent être faites à l'aide d'une source portative de neutrons, facilement utilisable dans les hôpitaux.

L'auteur examine en détail les problèmes suivants, qui semblent pouvoir faire l'objet d'autres recherches par les techniques de radioactivation:

(1) mesure de ^{127}I lié aux protéïnes dans le sang humain;

(2) métabolisme du nickel chez l'homme;

(3) métabolisme des éléments à l'état de traces dans les tumeurs;

(4) rôle du manganèse dans la formation des os;

(5) rôle du vanadium dans la calcification des dents et la prévention des caries;

(6) mise en évidence de nouveaux éléments à l'état de traces, dans la nutrition humaine;

(7) étude des intoxications chroniques causées par des métaux industriels.

" All science is measurement ", said Helmholtz. This dictum might not be wholly acceptable in clinical science, but it is beyond dispute that new chemical and physical techniques for the measurement of bodily functions have contributed much to the spectacular advance of medicine during the last half-century. Radioactivation analysis is a refined, yet simple, method of investigation which has already proved serviceable to a limited extent in medicine and biochemistry. As its possibilities in these fields become more widely known, its applications will rapidly increase, particularly in the many hospitals and research laboratories already equipped for work with radioactive materials.

The place of radioactivation technique in biochemistry was reviewed extensively[1] in 1957. The present survey necessarily covers some of the same ground, but work done during the last couple of years will be noted particularly, and reference will be made to a number of problems on which future research seems likely to be rewarding.

TISSUE ANALYSIS

Some elements present in human and animal tissues are very difficult to analyse, even with the most sensitive spectrographic or colorimetric techniques. *Table 1* shows figures quoted by Koch *et al.*[2] to illustrate the range of values reported by different authorities after analysis of normal human tissue.

Table 1. Analysis of normal human tissue

Element	*Tissue*	*Concentration*	*Ref.*	*Method*
Cr	Lung ash	2500 μg/g 45 μg/g	3 2	Colorimetry Colorimetry
Co	Blood	0·17–1·48 μg/100 ml 0·35–6·30 μg/100 ml	4 2	Spectrography Radioactivation
Co	Spleen	0·47 μg/g 0·03 μg/g	5 2	Colorimetry Radioactivation
Cu	Liver	5 μg/g 11 μg/g 25 μg/g	6 2 7	Colorimetry Spectrography Colorimetry

For other elements, as will be noted later, similar uncertainties exist.

It is fortunate that the four substances, hydrogen, carbon, nitrogen and oxygen which constitute 96 per cent of living matter do not have any useful radioactive isotopes made by neutron bombardment of the stable elements. But for this dispensation (which is so often regretted in radioactive tracer work) the analysis of tissue by neutron activation would be virtually impossible. As it is, the task is sometimes hard enough because of the wide range of induced activities among the elements to be identified and estimated in normal or pathological tissue samples.

The contribution of an individual element to the total induced activity may be isolated in two main ways:

(1) analysis of the emitted radiation by coincidence counting, by spectroscopy or by counting through suitably chosen filters (really a simple form of spectroscopy); and
(2) chemical separation followed by radioactive assay.

These techniques are particularly important in the medical and biochemical applications of radioactivation analysis, where the samples under investigation may contain a dozen or more radioactive nuclides in appreciable amounts after irradiation.

Radioactivation analysis of the blood

The analysis of blood is a problem which illustrates the difficulties very clearly. Spencer *et al.*[8, 9] have experiments in progress using a 5-watt pool-type nuclear reactor giving a thermal neutron flux of $2{\cdot}5 \times 10^8$ neutrons cm^{-2} sec^{-1}. They calculate that irradiation of 10 ml of serum for one hour in this modest neutron flux should produce measurable activities in twelve elements (Br, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, P and Zn) (*cf. Table 2*). The estimation of sodium by this method is very easy. If the irradiated sample is left to decay for 12 h, 99·6 per cent of the residual γ-ray activity will be due to ^{24}Na. The concentration of sodium in the original specimen may then be found by a simple counting arrangement. Most of the other nuclides noted in *Table 2* decay more quickly than does the ^{24}Na and must therefore be separated promptly if their activities are to be measured.

Table 2. Activities induced in 10 ml of human blood serum after exposure for 1 h to a thermal neutron flux of $2{\cdot}5 \times 10^8$ neutrons cm^{-2} sec^{-1}

Isotope	$t_{\frac{1}{2}}$(h)	*Disintegrations/min**
^{80m}Br	4·6	2350
^{80}Br	0·3	43,500
^{82}Br	35·9	290
^{49}Ca	0·14	444
^{38}Cl	0·62	800,000
^{60m}Co	0·18	30
^{64}Cu	12·8	222
^{66}Cu	0·085	950
^{128}I	0·42	1440
^{42}K	12·5	1909
^{27}Mg	0·16	123
^{56}Mn	2·58	30
^{24}Na	15·0	320,000
^{32}P	348	35
^{69}Zn	0·87	134

* Immediately after neutron activation as specified above.

Chemical treatment of irradiated samples

Complete recovery of the desired element may not be possible, but the accuracy of the determination need not suffer. A few milligrams of the element concerned is added in suitable form as a stable carrier to the irradiated specimen. The proportion of the carrier which survives the chemical separation is readily found by conventional methods and an appropriate correction made to the counting rate observed in the assay of the radioactive sample.

Rapid chemical separation of copper and zinc from irradiated blood was achieved by Bowen[10]. ^{64}Cu($t_{\frac{1}{2}} = 12{\cdot}8$ h) was isolated with a yield of about 70 per cent and without radioactive impurities; the chemical procedures for eight samples occupied only two hours. Similar results were obtained for ^{69}Zn.

Estimation of gold in tissue

Tobias *et al.*[11] separated ^{198}Au from irradiated blood by precipitation with a stable carrier and subsequent electroplating onto a platinum

planchet. Gold is not usually considered as a constituent of human tissue and the total amount in the body is of the order of 50 μg. Tobias *et al.* were able to estimate the gold content of red cells, white cells and plasma in a 10 ml sample of blood.

Muller[12] used an ingenious technique to estimate radiation dosage in treatment with ^{198}Au. This material, in colloidal form, is used for the relief of disseminated cancer, as for example in the pleural or peritoneal cavity. Muller allowed the ^{198}Au to decay ($t_{\frac{1}{2}} = 2{\cdot}7$ d) after injection, until negligible activity remained. Small samples (1 g or less) of tissue were then removed, ashed and subjected to neutron activation. The ^{198}Au produced by this second irradiation was estimated by analysis of the complex decay curves obtained by scintillation counting over a period of two or three weeks.

Estimation of sodium, potassium and phosphorus

Tissues other than blood have also been examined by radioactivation. Reiffel *et al.*[13] were able to estimate sodium, potassium and phosphorus in muscle, using samples of a few milligrams. The induced activities in sodium and potassium were identified by counting with an end-window Geiger tube and absorbers of appropriate thickness. Phosphorus was estimated after several days' decay had reduced the other two activities to insignificant levels. An accuracy of about 5 per cent was obtained by these simple arrangements.

Druyan *et al.*[14] have recently used neutron activation analysis in the estimation of bone sodium. This estimation is usually a difficult problem, because the ratio of calcium to sodium in bone (40 to 1) makes flame photometry almost impossible. Under neutron bombardment the only calcium isotope formed in appreciable amount is ^{49}Ca, with $t_{\frac{1}{2}} = 8$ min. If counting is delayed for two hours after irradiation, this isotope, originally quite abundant, does not interfere to a noticeable extent. Gamma-ray spectrometry and half-life measurements provide further means of ensuring that only the ^{24}Na activity is counted. No chemical treatment of the irradiated bone is necessary.

TRACE ELEMENT ANALYSIS

The estimation of trace elements is a problem in which progress is often halted for lack of sufficiently sensitive analytical techniques. Radioactivation analysis offers interesting prospects here. Firstly, there may be new trace elements awaiting recognition. It is not known, for example, whether aluminium, chromium, cobalt and nickel are of any importance in human nutrition. The concentrations present in the body are near—or beyond—the limits of reliable measurement by chemical, colorimetric or spectrographic methods. Consequently there is much uncertainty about the excretion and absorption of these elements, and even about their presence in normal tissue. All four elements can be detected with good sensitivity by neutron activation. Cobalt has already been the subject of such a study[2] but more remains to be done for, as Underwood[15] points out: " no serious attempts to demonstrate an essential function for nickel, with the aid of modern experimental techniques, have yet been undertaken " and

" the occurrence of aluminium in animal tissues, blood and urine has been the subject of considerable controversy, arising largely from difficulties and errors in the analytical, especially the spectrographic, techniques employed ".

Cancer research

Tobias *et al.*[16], using radioactivation techniques, have demonstrated an increased uptake of cobalt by tumour cell nuclei in mice. Further experiments on the metabolism of trace elements in human cancer might be rewarding, particularly in view of the importance of certain of these elements in hormone or enzyme systems. The concentrations to be measured are so low and the amounts of material available so small (as, for example, in biopsy specimens) that radioactivation is the only method of analysis likely to succeed.

Rare earths in tissue

Little, if anything, is known about the biochemistry of the rare earths. Many of these elements are very suitable for radioactivation analysis, because of their large neutron capture cross-sections. Chemical separation is difficult but ion exchange has been used successfully by Brooksbank *et al.*[17] These authors found three rare earths—Er, Tm and Yb—to be present in animal bone at levels higher than 1 part/million.

Some of the rare earths, along with other uncommon elements, occur among the fission products which will be manufactured to an increasing extent through the development of atomic energy, and may sometimes be important as environmental hazards. The metabolism of these elements in plants, animals and marine organisms should obviously be studied, with particular attention to the possibility of concentration processes in food chains. In some instances, radioactivation of the naturally occurring elements, or of added stable tracers, provides a good method of making such experiments before the radioactive fission products are present in measurable quantities.

RADIOACTIVATION ANALYSIS FOR ARSENIC

Recent investigations with arsenic have demonstrated the advantages of radioactivation analysis and have suggested topics for further research or enquiry.

Arsenic and lung cancer

As Holland *et al.*[18] point out: " arsenic is the only component in cigarette smoke that is known to be carcinogenic in man ". The arsenic enters the leaf of the tobacco plant from sprays or dusts used as insecticides. Bailey *et al.*[19] analysed cigarettes from many countries, and found concentrations (expressed as As_2O_3) between 0·0 (Turkey) and 81·0 parts/million (Denmark). In one British brand the level fell from 25–100 parts/million in 1948 to 1–2 parts/million in 1956. The British manufacturers report[20] that the average arsenic content of their cigarettes in 1956 was 7 parts/million and that the decline is continuing. In the U.S.A.[18] the arsenic content of five leading brands in 1957 ranged between 42·5 and 52·0 parts/million;

the corresponding limits in 1933 were 7·5 and 30 parts/million. The proportion of the arsenic in a cigarette which is inhaled has been variously estimated at 5 per cent[20] and 11 per cent[18].

Since the daily intake of arsenic may be substantially greater in smokers than in non-smokers, it is reasonable to expect a measurable difference in excretion rates. Small samples of hair from 1000 people were recently subjected to radioactivation analysis[21] in an attempt to demonstrate such an effect. Arsenic levels were found to be the same in smokers as in non-smokers, though considerably higher in men than in women (*Figure 1*); the explanation for this unexpected finding is not known. It would be valuable to know whether the rate of arsenic excretion in the urine shows any correlation with cigarette consumption. Arsenic has been estimated in urine with excellent sensitivity by radioactivation analysis[22]. Large-scale investigations could be made reasonably quickly with small samples. In the measurements just mentioned, the range of arsenic levels found was very wide—from 0·01 to 0·33 parts/million. It is tempting to speculate whether this spread of values includes a variation related to smoking habits.

Arsenical poisoning in industry

Arsenic, for centuries a favourite instrument of homicide, is now dangerous mainly as an industrial poison. Doig[23] describes three cases of arsenical poisoning in tank cleaners, employed in removing scale from lead vessels used to store sulphuric acid. Arsenic (from pyrites) is a common contaminant of commercial sulphuric acid. The scale in the storage tanks may contain as much as 45 per cent of As_2O_3, some of which is converted into arsine—one of the most toxic gases known. In one of Doig's cases, the diagnosis of arsine poisoning was not made until several months after the victim's death, because no one in the factory associated his illness (haemolytic anaemia) with his occupation. In another case, samples of hair and nail were found by radioactivation analysis to be rich in arsenic.

Arsenical poisoning is an occupational risk in the manufacture of insecticides, weed-killers and sheep-dip. Protective clothing is not always used and other simple hygienic measures are often ignored. Some interesting measurements were made[21] on a worker from a sheep-dip factory who was admitted to hospital with a squamous carcinoma of the scrotum, attributed to chronic irritation by arsenical dust. Samples of beard hair were taken with an electric razor at intervals after the patient's admission and subjected to radioactivation analysis to measure their arsenic content. The following results were obtained:

Weeks after admission	*Arsenic content* (parts/million)
0	3·12
1	1·79
3	0·84
4	0·94

The hazards associated with the industrial uses of arsenic are not always recognized by workers and managers. In certain factories, regular inspection of hair and nail samples by radioactivation analysis would be

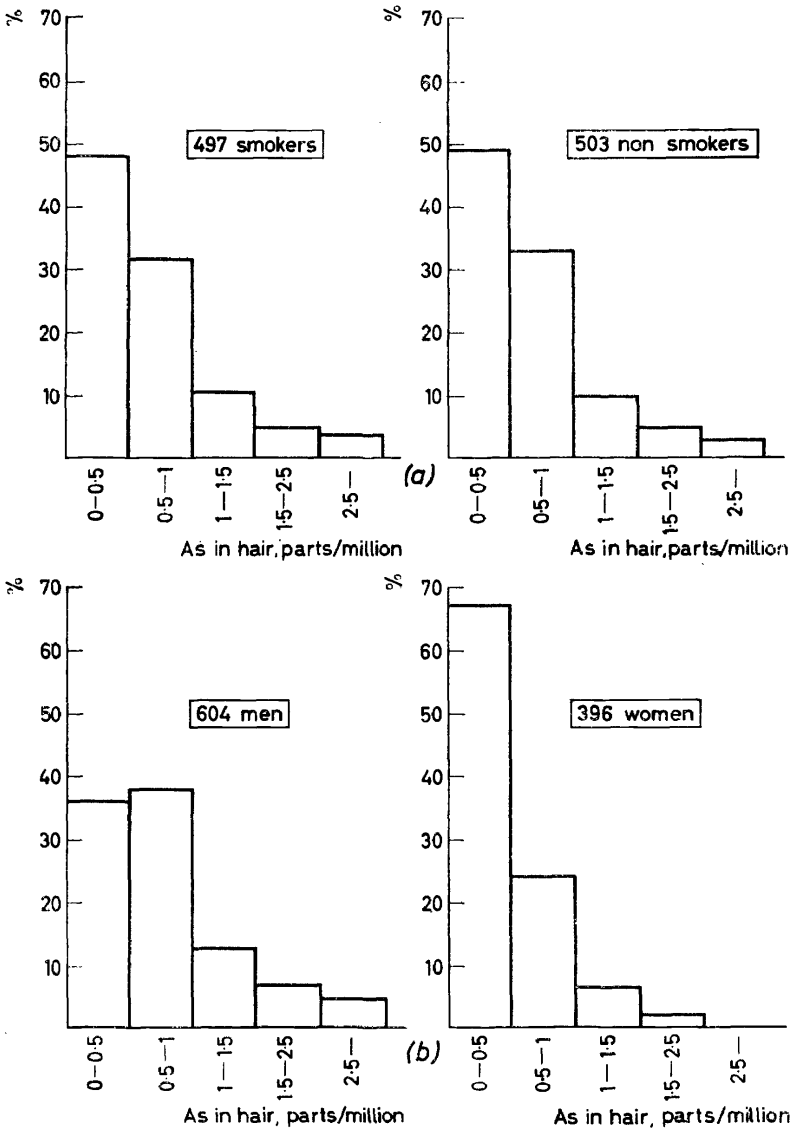

Figure 1. Arsenic levels in hair: comparison between (*a*) smokers and non-smokers, (*b*) men and women

a useful practice, providing early warning of undue contamination which might have serious consequences if not arrested. Tests of this kind are, of course, impracticable by conventional methods of analysis.

RADIOACTIVATION ANALYSIS IN DENTAL SCIENCE

Many problems in dental science await attack by activation techniques. Trace elements have an important role in the formation, preservation and

decay of teeth. Some are absorbed from cleaning or restorative materials; others occur naturally. Experimental work in human subjects is very difficult because many of the elements concerned are present at such low concentrations that a single tooth does not provide an adequate sample for analysis by chemical, colorimetric or spectrographic methods. Consequently it is often necessary to use pooled material from many different subjects, with inevitable loss of precision. The analysis of teeth is important because of the belief that susceptibility to dental caries is affected by nutritional factors during the period of enamel mineralization. At present this hypothesis can be tested only by large-scale experiments such as fluoridation of water supplies and subsequent dental examination of large numbers of children. Useful investigations could be made in a less controversial way with the help of radioactivation analysis.

Vanadium in teeth

It is known, for example, that vanadium is isomorphous with phosphorus and can replace it in the apatite lattice from which teeth are largely built. Rygh[24] found the presence of vanadium in the diet of rats and guinea pigs to have a significant effect on the mineralization of teeth during the first weeks of life. Animals restricted to vanadium-free diets showed the greatest incidence of caries. Geyer[25] found that incipient caries, produced by adjustment of the diet in Syrian hamsters, could be halted by the addition of vanadium to the food. As yet there is no convincing evidence for the presence of vanadium in normal human teeth. Detection of this element by radioactivation analysis should be possible with excellent sensitivity.

Arsenic in teeth

Arsenic is a known impurity in dental cements containing zinc. It has been suggested[26] that toxic effects might follow absorption from fillings. The amount of arsenic in a single tooth is too small to be measured conveniently by normal analytical methods. Radioactivation analysis was used by Nixon[27] on 26 sound permanent teeth and 50 extracted teeth previously filled with zinc phosphate cements. A tissue sample of about 20 mg was taken from each tooth in a region close to the filled cavity. After irradiation in the Harwell reactor the samples were treated chemically for the isolation of arsenic.

The mean arsenic concentrations were:

normal teeth 0·060 parts/million As element

filled teeth 0·065 parts/million As element

It may be concluded that no appreciable amount of arsenic is absorbed from impurities in the filling material.

Mercury in teeth

Nixon[27] has indicated another investigation in which radioactivation analysis will be of advantage. Mercury amalgam, once believed to be a dangerous material for the filling of teeth is now generally considered

harmless, but misgivings are still expressed[28] about its possible toxicity—not unreasonably, since the amount of mercury in a typical filling is large compared with the normal daily intake of this element.

Tracer experiments have been made on extracted teeth but are clearly objectionable in living subjects. Radioactivation analysis using ^{197m}Hg ($t_{\frac{1}{2}} = 23$ h) which can be made at very high specific activity would provide the desired information without much difficulty. Particular attention should be given to the possibility of mercury absorption in children, where the greater patency of the dentinal tubules provides a more ready path for transfer than in adults.

Manganese and bone formation

Manganese is now known to be an essential trace element in many plants, but its place in human nutrition remains obscure. The concentrations normally present are so low as to be beyond the reach of conventional analytical methods. Consequently the reported values are too meagre or too discordant to be of much value. *In vitro* experiments show that several enzymes, including bone phosphatase, are activated by manganese. Dietary deficiency of this element prevents normal bone formation in rabbits, rats and mice. The resulting deformities are accompanied, in rabbits, by a pronounced depression of bone phosphatase activity.

Dietary deficiency of this element is the cause of perosis, a serious bone disease in poultry. The disease is accompanied (and, in fact, preceded) by a marked depression of blood and bone phosphatase activity. It has been suggested[29] that slipped epiphysis, a deformity sometimes found in children, may be related to perosis. There is no evidence that manganese is essential for human nutrition. It is, however, so important to plants and animals that further study with the refined analytical methods now available is desirable. Detection by radioactivation in tissue samples is difficult because of the longer-lived and more abundant activities contributed by sodium and potassium. This problem has been tackled successfully by Borg[30] and Bowen[31].

COMBINATION OF RADIOACTIVATION WITH OTHER TECHNIQUES

The usefulness of isotopic tracers can be significantly enhanced by combination with radioactivation analysis. A small dose of a radioactive nuclide, administered to a patient or an animal, may often be used to establish the size of the exchangeable reserve of the corresponding stable element. Sometimes it is possible to study the turnover rate of an element in a biochemical reaction. There are occasions when such tests cannot be done without giving an unjustifiably high dose of radioactivity, because no isotope of suitable half-life and specific activity is available. Unless the added radioactive tracer equilibrates quickly, measurements of its storage and excretion will not be applicable to the stable element already present in the body. On the other hand, the estimation of many stable trace elements in the body or in excreta is difficult or impossible because the conventional methods of analysis are lacking in sensitivity.

Protein-bound iodine

Some of these difficulties are encountered in the radio-iodine test used for the study of thyroid function. The rate of iodine turnover in the thyroid can be estimated by measuring the radioactivity in the protein fraction of the circulating plasma; this radioactivity is mainly due to freshly-made thyroxine and tri-iodothyronine. The significance of the turnover rate cannot be fully assessed without a knowledge of the size of the stable thyroxine pool. Measurement of this quantity by estimating the ^{127}I in the plasma proteins is a task of remarkable difficulty. The concentrations to be measured are about 5 μg/100 ml of plasma in the human subject. At these levels fortuitous contamination is hard to avoid.

The estimation of protein-bound ^{127}I by radioactivation is an attractive technique which does not appear to be widely known, though it is mentioned in a recent report from Oak Ridge[32]. Proximity to a nuclear reactor is essential, since ^{128}I has a half-life of only 25 min. In Britain, and perhaps in other countries, a postal service for the estimation of protein-bound ^{127}I by radioactivation would be fully occupied and would be generally preferred to the present chemical methods.

Strontium in human bone

Strontium is another element whose behaviour can be elucidated more fully by radioactivation than by tracer tests. Here the commonly available radioactive isotopes are too dangerous for administration to human subjects at the levels needed for tracer tests. The stable element is, however, present in human and animal tissues in amounts suitable for estimation by radioactivation[33] using the short-lived $^{87}Sr(t_{\frac{1}{2}} = 2{\cdot}7$ h).

Activation chromatography

The combination of radioactivation and chromatography has been reviewed recently by Benson[34]. Many elements can be estimated rapidly and accurately by neutron irradiation of paper chromatograms, followed by inspection with a Geiger counter. Since no chemical manipulation is needed after the neutron activation, short-lived isotopes can be used. Benson measured cobalt and vanadium in tissue ash, from the activities of $^{60m}Co(t_{\frac{1}{2}} = 11$ min) and ^{52}V ($t_{\frac{1}{2}} = 3{\cdot}8$ min) on activated chromatograms. He also describes methods for the analysis of phosphorus-containing intermediates in carbohydrate and lipid metabolism. Such investigations would otherwise be very difficult in large animals, where it is not practicable to administer ^{32}P in sufficient quantities for the analysis of phosphatides by the normal methods of radiochromatography.

SCOPE OF ACTIVATION ANALYSIS

Except for a few elements at either end of the periodic table, the technique of activation analysis is almost universally applicable. Its ultimate sensitivity is usually far beyond the level required in practice. Figures published by the Oak Ridge laboratory of the United States Atomic Energy Commission[32] show the concentrations of several elements found in biological material examined there, along with the corresponding limits of

sensitivity attainable with no great difficulty in that laboratory. Even these figures, which are given in *Table 3*, can be improved by a factor of 10 or more by using a more powerful reactor or more delicate methods of radioactive assay. Many other elements, not shown in this compilation, could be studied by activation analysis if the need arose and the necessary modest effort was applied.

Table 3. Radioactivation analysis of tissue[32]

Element	*Concentrations observed in tissue* (parts/million)	*Limit of sensitivity* (parts/million)
Antimony	0·5–10	0·005
Arsenic	0·005–1	0·001
Bromine	1–10	0·001
Cadmium	1–5	0·01
Caesium	1–10	0·05
Cobalt	0·1–1	0·05
Copper	0·1–700	0·01
Iodine	1–10	0·05
Nickel	0·5–2	0·1
Potassium	100–1000	0·02
Rubidium	10–100	0·1
Selenium	0·001–10	0·001
Silver	0·1–1	0·1
Sodium	10–10,000	0·007
Strontium	6·5–30	0·5
Tellurium	10–100	0·2
Zinc	1–1000	0·02
Zirconium	1–10	0·2

Sources for radioactivation

The nuclear reactor is the most convenient instrument for radioactivation of biological material, but other possibilities have been discussed[1]. Charged particles from high-energy accelerators have a limited usefulness because of their short range and consequent intense heating effect. For some elements, including phosphorus and iron, radioactivation analysis with charged particles has very good sensitivity[35].

The electron linear accelerator, when supplied with a suitable target and moderator, makes a good neutron source. MacGregor[36] estimates a total neutron production of 3×10^{13} neutrons sec^{-1} from a 20 MeV, 12 kW electron linear accelerator operated with a water-moderated uranium target. The corresponding thermal neutron flux is 2×10^{11} neutrons cm^{-2} sec^{-1}. A linear accelerator working at 25 MeV, 10 kW will make millicurie amounts of about fifty isotopes, using (γ,n) reactions. Many of these isotopes are not available from a nuclear reactor.

Electron linear accelerators are likely to be used to an increasing extent in radiation therapy. Their possibilities for radioactivation analysis for medical purposes should not be overlooked.

When radioactive isotopes were first made by artificial means, it was often predicted that they would be widely useful in the treatment of diseases which had so far resisted the skill of the physician. These hopes have not been fully consummated, but it is becoming evident that the subtler

applications of induced radioactivity will be a fruitful field of study for practitioners of medicine and the sciences ancillary to it. Like the thermometer, the spectroscope and the oscillograph, the Geiger counter may almost be as useful in the relief of suffering as in the advancement of knowledge. In this process the technique of radioactivation analysis has a modest but distinctive contribution to offer.

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DISCUSSION

G. W. LEDDICOTTE (*U.S.A.*): I should like to refer to some routine analyses which I and my collaborators have carried out. In 1953, we demonstrated the presence of at least ten of the rare earths in a specimen of bone. The analysis was not carried further because of the difficulty in differentiating one type of rare earth from another. At the time, it was considered that a procedure devised by Boyd *et al.* was the most suitable for rare earth analysis and a paper on the subject was included in the *Journal of Physical Chemistry* in 1953. The technique, unfortunately, took some 72 h, during which period some of the sensitivity was lost. Nevertheless, in a sample of a hard tissue such as bone, it was possible to determine erbium, for example, in the 10^{-10} g range. The largest concentration of any rare earth in the system was 2·2 parts/million. Rare earths were also prominent in monazite ores and sands. Hickory leaves contained a few hundred parts/million of the rare earths and variations in the leaves were more or less related to monazites in various parts of North Carolina. Some of the trees were more selective for rare earths.

It was found feasible to make an analysis of cadmium in bone and a content of about 0·1 parts/million was determined. It was also feasible to determine 1 part/million of iodine during work on proteins.

Marked differences in the selenium content of human bodies as between the Dakotas and other parts of the United States have been discovered. The effects on liver tissues of the presence or absence of selenium in diets is being studied at Washington, and radioactivation analysis techniques are proving extremely important in that work.

J. H. MÜLLER (*Switzerland*): I should like to refer to work done with the object of evolving a procedure suitable for routine assessments of gold deposits in tissue specimens when ^{198}Au is used in larger doses for cancer therapy*.

The intracavity interstitial injections of radioactive colloids, especially colloidal ^{198}Au, are now of internationally acknowledged importance in cancer therapy. The essential advantages are the good anatomical localization of innumerable small radioactive foci dispersed within the required sites, together with the induction of vital reactive, mainly histiocytic phenomena, of which the most remarkable is the resulting concentration of the radioactive colloidal material within the whole tributary lymphatic apparatus of the region treated. This is of paramount importance in view of the frequent and dangerous lymphatic disseminations of the cancer cells. These therapeutic methods might be described as paraselective and, as such, within the realm of the scientific ideal of selectivity.

Qualitative data and quantitative approximations about the concentrations of radioactive particles within the lymphnodes and other anatomical structures could previously be obtained by the physical means available, *i.e.*, external counting and scanning, counting of ashed active samples, calculations and autoradiography. If there is sufficient residual activity, autoradiography provides very important evidence, but it is not adequate for making precise assessments of radiation dosage.

When colloidal ^{198}Au is used for clinical and therapeutic applications it is very difficult to work on active samples because, by the time the samples are available, the residual activity is usually minimal or has already decayed to zero. Neutron activation analysis was accordingly employed for the first time a few years ago and has yielded quantitative data of sufficient accuracy. The particular problems involved do not require extremely high precision; it is sufficient to have well-ascertained orders of magnitude which permit a reliable survey of the radiation dosimetry involved. Chemical separation is unnecessary, so that it is also possible to repeat the activation analysis—a factor of advantage in certain cases. Quantitative assessment of the gold content in samples examined is obtained solely by nuclear physical methods. Well-defined small samples of the tissues to be examined are collected—by surgery or, in exceptional cases, during autopsy—some weeks or months after ^{198}Au treatment. After the primary activity has decayed, the samples are ashed and reactivated—formerly in the B.E.P.O. reactor at Harwell, and more recently in the swimming-pool reactor at Wüzenlingen, Switzerland—together with exactly calibrated gold samples, at a site where the neutron flux is homogeneous.

* *See* J. H. Müller: "Neutron activation analysis for medical isotope dosimetry with special consideration of colloidal Au^{198}", *Radioisotopes Sci. Research, Proc. Intern. Conf., Paris, Sept., 1957*, Vol. III, pp. 667–683; Pergamon Press (1958).

For the quantitative determination of the gold content of the samples we have hitherto mostly worked from the complex decay curves obtained by scintillation counting of the reactivated samples.

Gamma scintillation spectrometry is also being used now. It is more practical as it permits a rapid and reliable survey of numerous specimens within a relatively short time. For preliminary studies we were kindly loaned a 50-channel analyser belonging to Reactor Ltd., Wüzenlingen. The procedure will soon become routine in our own laboratory where a single-channel discriminator, quite adequate for this particular work, will be used.

Results to date indicate that the definition of the average beta radiation dosage within the structures investigated is sufficiently precise. The mean values are, for example, 7000 rads within the abdominal and mediastinal lymphnodes, after intraperitoneal application of 150 mc of colloidal ^{198}Au, and 8000–16,000 rads within the axillary lymphnodes after extended colloidal ^{198}Au infiltration of the whole breast region in cases of breast cancer, performed two to three weeks before operation. For the procedure, the colloidal ^{198}Au is used in amounts of 90–130 mc diluted in 120–180 ml of physiological saline for multiple interstitial injections, aiming at a diffuse imbibition of the whole mammary, para-mammary and retro-mammary regions from the sternum to the axilla. The procedure results in gamma irradiation throughout the large area infiltrated. It is of the same order of magnitude as in conventional pre-operative Roentgen therapy but, in addition, considerable amounts of radioactive material are concentrated within the retrosternal, the axillary and even the supraclavicular lymphnodes.

I started this method of pre-operative interstitial irradiation of breast cancer some six years ago. It has given extremely encouraging preliminary clinical results and is now being used on a steadily increasing scale.

W. W. MEINKE (*U.S.A.*): Our group has also been interested in analysing marine biological ashes. Dr R. Fukai of the Tokai Regional Fisheries Laboratory in Tokio has assisted in this work and a review of trace analyses in seaweed, molluscs, crustacea, fish and sea-water will be published*. This report also contains comparisons of the sensitivity of activation analysis with that of conventional methods. *Figure 2* shows this relation of sensitivities expressed in terms of the sensitivity index, pS(= −log (sensitivity in g)).

In experimental work on these samples using the Michigan reactor ^{52}V ($t_{\frac{1}{2}}$ = 3·8 min), ^{188m}Re($t_{\frac{1}{2}}$ = 20 min), and ^{101}Tc($t_{\frac{1}{2}}$ = 14 min) (from ^{101}Mo) have been separated by rapid radiochemical procedures and their spectra measured. Longer-lived radioisotopes of gold, arsenic and tungsten have also been determined. The approximate experimental sensitivities determined for these elements at a flux of 10^{12} neutrons cm^{-2} sec^{-1} are as follows:

Vanadium	^{52}V	2×10^{-9} g
Arsenic	^{76}As	5×10^{-8} g
Molybdenum	^{101}Tc	5×10^{-7} g
Tungsten	^{187}W	5×10^{-9} g
Rhenium	^{186}Re	1×10^{-9} g
Gold	^{198}Au	5×10^{-10} g

* *Limnology and Oceanography*, **4**, 4, 398 (1959).

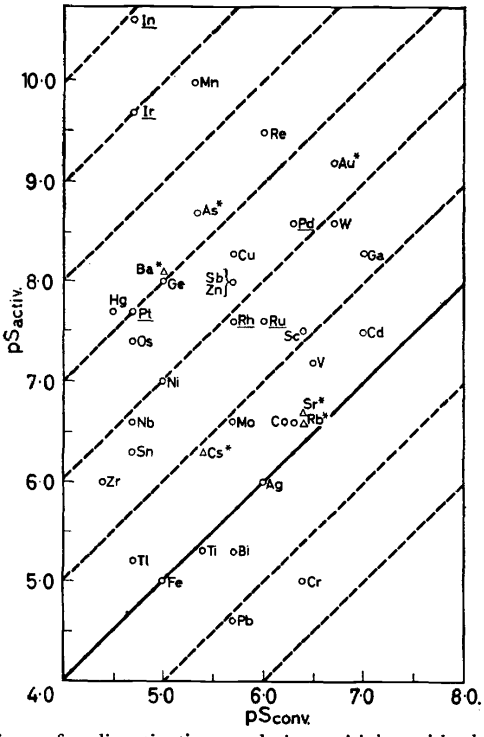

Figure 2. Comparison of radioactivation analysis sensitivity with that obtained in conventional methods. Elements whose symbols are underlined have not previously been reported in samples of the types mentioned. Activation analysis has been reported for those marked with an asterisk

The abundance of the elements in the samples in most cases did not approach these sensitivities. A short preliminary summary of the analyses has recently been submitted to *Nature**. Work has also been done by Dr D. Kaiser on the determination of cobalt in rat kidney tissues after injection of vitamin B_{12}. A rapid chemical separation isolates $^{60m}Co(t_{\frac{1}{2}} = 10{\cdot}5$ min), giving a sensitivity of about 5×10^{-8} g†. An extension of this has been a similar determination of copper in rat kidney tissue at the 10^{-6} to 10^{-7} g level by using a rapid chemical procedure and measuring $^{66}Cu(t_{\frac{1}{2}} = 5$ min).

A compendium of radiochemical information entitled *Source Material for Radiochemistry* is now available free of charge from the Division of Physical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D.C. It contains several hundred items and lists the authors and titles of source documents, a brief abstract, address where the item is obtainable and its price.

L. C. KELLERSOHN (*France*): I agree with Dr Lenihan that the use of a tracer such as iodine-131 does not provide a complete picture of the thyroid function; it is also necessary to know the iodine-127 content of the blood, especially the plasmatic protein iodine. The chemical determination of blood iodine is complex, delicate and inclined to be imprecise.

* R. Fukai and W. W. Meinke *Nature* **184**, 815 (1959).
† D. G. Kaiser and W. W. Meinke, *Talanta* **3**, 255 (1960).

In the Frédéric-Joliot Section, Biological Service, French Atomic Energy Commissariat, we have recently attempted to devise an activation analysis dosage technique for plasmatic protein iodine. The elements which cause difficulty are mainly sodium, chlorine and bromine. But these elements do not occur in the blood organically bound. We have therefore tried to separate the blood protein iodine and mineral iodine before or after irradiation by passing them several times over ion-exchange resins or by precipitation of the protein and repeated washing. Irradiation was carried out for one hour at 2×10^{11} neutrons cm^{-2} sec^{-1}. Measurements on the samples and the standards were made by means of a 100-channel, magnetic memory analyser. The 428 keV spectrum of iodine-128 has been obtained in the sample but it does not permit precise quantitative measurement. It is hoped to reduce the Compton continuum from the spectra of sodium, chlorine and bromine by using an NaI (Tl) and anthracene crystal double-counting head.

P. LEVEQUE (*France*): In France monoenergetic photons from a 124 MeV betatron have been used for determining nitrogen, oxygen and carbon by the (γ,n) reaction. It has not proved possible to determine trace quantities of these elements. For the determination of larger quantities, however, the method is perfectly satisfactory. There is the additional advantage that the reactions are threshold. On the betatron, it has been possible to vary the maximum energy so that nitrogen (threshold 11 MeV), oxygen (threshold 16 MeV), and carbon (threshold 19 MeV) could be determined successively.

J. H. MÜLLER (*Switzerland*): Similar work has been done with a 31 MeV betatron at Zurich.