EUR 474.e

EUROPEAN ATOMIC ENERGY COMMUNITY - EURATOM

STUDIES ON THE DISTRIBUTION OF TRACE ELEMENTS IN A MOLLUSK FROM A FRESHWATER ENVIRONMENT, BY ACTIVATION ANALYSIS

by

F. GIRARDI and Margaret MERLINI

1963

Joint Nuclear Research Center
Ispra Establishment - Italy
Biology and Nuclear Chemistry Services

Paper presented at the Symposium on Radioactivation Analysis and its Application to the Biological Sciences
Saclay (France), 26 - 28 September 1963

LEGAL NOTICE

This document was prepared under the sponsorship of the Commission of the European Atomic Energy Community (EURATOM).

Neither the EURATOM Commission, its contractors nor any person acting on their behalf :

1° — Make any warranty or representation, express or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this document, or that the use of any information, apparatus, method, or process disclosed in this document may not infringe privately owned rights; or

2° — Assume any liability with respect to the use of, or for damages resulting from the use of any information, apparatus, method or process disclosed in this document.

The authors' names are listed in alphabetical order.

This report can be obtained, at the price of Belgian Francs 40,—, from : PRESSES ACADEMIQUES EUROPEENNES 98, Chaussée de Charleroi, Brussels 6.

Please remit payments :

— to BANQUE DE LA SOCIETE GENERALE (Agence Ma Campagne) - Brussels - account No. 964.558,

— to BELGIAN AMERICAN BANK AND TRUST COMPANY - New York - account No. 121.86,

— to LLOYDS BANK (Foreign) Ltd. - 10 Moorgate - London E.C.2,

giving the reference : "EUR 474.e - Studies on the distribution of trace elements in a mollusk from a freshwater environment, by activation analysis".

Printed by Guyot s.a.,
Brussels, December 1963.

EUR 474.e

STUDIES ON THE DISTRIBUTION OF TRACE ELEMENTS IN A MOLLUSK FROM A FRESHWATER ENVIRONMENT, BY ACTIVATION ANALYSIS by F. GIRARDI and Margaret MERLINI.

European Atomic Energy Community - EURATOM
Joint Nuclear Research Center - Ispra Establishment (Italy)
Biology and Nuclear Chemistry Services
Paper presented at the Symposium on Radioactivation Analysis and its Application to the Biological Sciences
Saclay (France), 26-28 September 1963
Brussels, December 1963 - pages 26 - figures 3.

A semi-automated system, for the determination of trace elements in biological materials, by means of activation analysis, is described. The system is based on the chemical separation of elements in the irradiated specimens by chromatographic procedures; automatic gamma spectrometry, with the use of sample changers; and automatic treatment of the experimental data by computer techniques.

EUR 474.e

STUDIES ON THE DISTRIBUTION OF TRACE ELEMENTS IN A MOLLUSK FROM A FRESHWATER ENVIRONMENT, BY ACTIVATION ANALYSIS by F. GIRARDI and Margaret MERLINI.

European Atomic Energy Community - EURATOM
Joint Nuclear Research Center - Ispra Establishment (Italy)
Biology and Nuclear Chemistry Services
Paper presented at the Symposium on Radioactivation Analysis and its Application to the Biological Sciences
Saclay (France), 26-28 September 1963
Brussels, December 1963 - pages 26 - figures 3.

A semi-automated system, for the determination of trace elements in biological materials, by means of activation analysis, is described. The system is based on the chemical separation of elements in the irradiated specimens by chromatographic procedures; automatic gamma spectrometry, with the use of sample changers; and automatic treatment of the experimental data by computer techniques.

Although the system has not yet been perfected, it appears that approximately 100 samples per month with 12 to 15 elements per sample, can be determined. It is possible, therefore, to use this system for an accurate and more thorough investigation of well-chosen, biologically significant, elements.

The biological results obtained on *Unio*, a bivalve from Lake Maggiore, revealed the presence of elements never before determined in a mollusk from a freshwater environment. This fact points up the necessity of an adequate and accurate tool for the quantitative determination of the elementary composition of organisms and their environment. The tool which appears to us to satisfy all the necessary conditions, is activation analysis.

Although the system has not yet been perfected, it appears that approximately 100 samples per month with 12 to 15 elements per sample, can be determined. It is possible, therefore, to use this system for an accurate and more thorough investigation of well-chosen, biologically significant, elements.

The biological results obtained on *Unio*, a bivalve from Lake Maggiore, revealed the presence of elements never before determined in a mollusk from a freshwater environment. This fact points up the necessity of an adequate and accurate tool for the quantitative determination of the elementary composition of organisms and their environment. The tool which appears to us to satisfy all the necessary conditions, is activation analysis.

EUR 474.e

EUROPEAN ATOMIC ENERGY COMMUNITY - EURATOM

STUDIES ON THE DISTRIBUTION OF TRACE ELEMENTS IN A MOLLUSK FROM A FRESHWATER ENVIRONMENT, BY ACTIVATION ANALYSIS

by

F. GIRARDI and Margaret MERLINI

1963

Joint Nuclear Research Center
Ispra Establishment - Italy
Biology and Nuclear Chemistry Services

Paper presented at the Symposium on Radioactivation Analysis and its Application to the Biological Sciences
Saclay (France), 26 - 28 September 1963

CONTENTS

KEY TO THE FIGURES

KEY TO THE TABLES

STUDIES ON THE DISTRIBUTION OF TRACE ELEMENTS IN A MOLLUSK FROM A FRESHWATER ENVIRONMENT, BY ACTIVATION ANALYSIS

SUMMARY

A semi-automated system, for the determination of trace elements in biological materials, by means of activation analysis, is described. The system is based on the chemical separation of elements in the irradiated specimens by chromatographic procedures, automatic gamma spectrometry, with the use of sample changers; and automatic treatment of the experimental data by computer techniques.

Although the system has not yet been perfected, it appears that approximately 100 samples per month with 12 to 15 elements per sample, can be determined. It is possible, therefore, to use this system for an accurate and more thorough investigation of well-chosen, biologically significant, elements.

The biological results obtained on *Unio*, a bivalve from Lake Maggiore, revealed the presence of elements never before determined in a mollusk from a freshwater environment. This fact points up the necessity of an adequate and accurate tool for the quantitative determination of the elementary composition of organisms and their environment. The tool which appears to us to satisfy all the necessary conditions, is activation analysis.

1 - INTRODUCTION

Discovery and work done on trace elements in the field of biology date back at least one hundred years. Most of the early interest was confined to a few elements such as copper, zinc, and manganese in the blood of Mollusca (Erman, 1816; Harless, 1847; Underwood, 1956). But, as spectrochemical techniques became available, and small quantities of elements could be determined more readily, new scientific horizons were opened, particularly in the field of animal and plant nutrition.

The atomic era gave further impetus to these investigations by presenting the scientist with a new implement for studying trace elements, the radioisotopes. In addition, the testing of nuclear weapons made imperative the examination of many elements which, in the past, had received little or no attention. It became evident that the radioisotopes of certain elements could be accumulated to dangerous levels in animals and plants and subsequently in man, through the food chain.

As a result of the improvement of techniques in general, and the appearance of newer methods, there has been a gradual shift in the approach of the biologist to problems connected with trace elements. The interest which was once purely chemical, gave way to a more biological and recently, to a biogeochemical one. This is especially true for those engaged in the study of the ultimate composition of marine components. It was felt that the cycle of trace elements in marine organisms was a basic problem which should be solved as soon as possible because of the many questions arising from the disposal and dispersal of radioactive wastes in the oceans.

There is a mass of early data on the elementary composition of organisms from aqueous environments, much of which is fragmentary and of questionable value due to the methods employed (Vinogradov, 1953). And there are the investigations of the past 5 or 6 years on the

elemental composition of components of marine environments in which the techniques used (spectrographic analysis and activation analysis) have given more accurate information (Goldberg and Arrhenius, 1958; Nicholls, Curl and Bowen, 1959; Fukai and Meinke, 1962; Rona, Hood, Muse and Buglio, 1962; Pilkey and Goodell, 1963).

Unfortunately, the freshwaters have not received the same measure of attention allotted to the oceans, and as a result, tenable data are lacking. Furthermore, there has been no systematic study of an environment and its biota to better understand the ecological and biogeochemical relationships.

One of the reasons for the paucity of information on trace elements in all biological materials, up to now, has been the lack of a sufficiently sensitive method which could easily bear the weight of hundreds of analyses of different types of biological materials, in order to arrive at data which are statistically and biologically significant. Activation analysis appears to us to be a suitable means for solving these problems for the following reasons :

1. Its sensitivity is the highest available for a great number of elements. U, Th, Zn, Co, Sb, Hg, Cr, Cs, Rb, I, Br, Sr, Ba, Sc, and rare earths, among the more significant in biological studies, have detection limits well below the part per million, even when done on samples of less than 100 mg. As a result, it is possible to determine them in a single animal and even in individual organs and tissues.

2. The matrix of biological samples (C, H, O, N) does not activate to a great extent. It may also be considered transparent to thermal neutrons, so that the activity of the other elements is completely independent from variations in matrix composition. It is, therefore, possible to devise a single analytical scheme that can be used for a large variety of biological samples without any modification, and without the danger that the presence of different matrices might affect the accuracy of the results.

3. Due to the intrinsic characteristics of the method there is very little possibility for accidental contamination.

4. The method can be simplified and the procedure made automatic. This would facilitate a program of study of the elemental composition of an environment and its components, which would be too long if carried out according to the traditional scheme (irradiation of sample and various standards, chemical separation, a comparison of the activity of the sample with the standard, etc.).

The work presented here shows our attempt to develop a system which could produce biologically and statistically significant data at a reasonable rate of speed and with the minimum of personnel and time. Naturally, the elements that may be identified and measured depend on the type of chemical separation used. In this study we have used a scheme that we had already worked out for another problem (Girardi and Pietra, 1963) and then modified for the biological samples. Since the main problem here was primarily one of general methodology, no attempt was made to include other elements, even though of greater biological significance, into this original scheme.

2 - THE ANALYTICAL METHOD

In setting up the analytical method we felt that as many operations as possible, of the ones commonly employed in semi-routine analyses, should be avoided. This is necessary in an automated system which must be capable of analyzing a high number of samples in a short time, and with a reasonable amount of personnel and means. To reduce the work as much as possible, the following were taken into consideration :

1. Irradiation of the standards, necessary for analyses using the relative method, should be kept to a minimum or eliminated. The standards occupy precious space in the irradiation capsules, and thus, decrease the number of samples which can be analyzed. Measurement of the standards requires a considerable part of an operator's time for he must prepare the counting sources. The counting takes up the analyzer's time, and as a result, decreases still further the efficiency of the method.

2. Direct gamma spectrometry of irradiated specimens does not give sufficient information. In all of the samples we have irradiated, the gamma spectrum, after decay of the short-lived elements (less than 15 hours), is principally Zn^{65}. A high bremsstrahlung component is also evident, which impedes the detection of gamma emitters. A chemical separation is, therefore, necessary.

Radiochemical techniques, such as precipitation, which require the addition of carriers and measurement of the chemical separation yields, are not acceptable since they require a great deal of the operator's time.

3. Counting during the day requires an analyzer and operator, full-time. Automation of the operations to take advantage of the nocturnal hours is imperative.

4. Evaluation of photopeak surfaces and the calculation of weights and parts per million by hand are time-consuming operations that should be kept to a minimum.

These drawbacks have been surmounted as follows :

1) The analyses are not done with the relative method, but with a single-standard method, previously worked out. According to this method, the samples to be measured are irradiated together with a flux monitor. The activity of this monitor, together with the data concerning the irradiation, decay and activity measurement, permit us to compare the activity in the sample with that of a series of standards accurately measured once, to be used thereafter.

2) The chemical separations are set up to use the minimum amount of personnel. Only two operations are done : elution through chromatographic columns and evaporation of solutions. The use of chromatographic separations permits the operator to do a large number of samples simultaneously. One operator without any particular training in radiochemistry can do the separations on 6 samples in one day without difficulty. The separations are done with recoveries higher than 95 %. In this way, the measurement of chemical separation yields is not required.

3) The counting and calculation of weights and parts per million from the experimental data are rendered automatic through the use of an automatic sample changer, by recording the spectrographic data on punched tape, and by having the calculations done by an electronic computer, IBM 7090.

2.1 — Description of the Method

2.1.1 — *Preparation and irradiation of sample.* The bivalve, *Unio mancus elongatus*, Pfeiffer, was collected in February 1963 from a zone in Lake Maggiore near Angera, about 10 km south of Ispra. Animals of approximately the same size were separated into groups of three and frozen so that determinations could be done on mollusks from same population, collected at the same time.

The animals were washed externally to remove superficial detritus, measured, opened, and dissected into shell and soft parts, which were weighed. These parts were dried at 100° C and subsequently ground in a mechanical agate mortar. A weighed amount (circa 100 mg) was

then sealed in a quartz vial. Three vials and a neutron flux monitor (an aluminium alloy wire containing 1 % cobalt) were enclosed in the irradiation container and irradiated for 72 hours in a flux of 2×10^{13} neutrons/cm²/sec. The analysis scheme is shown in figure 1.

2.1.2 *Chemical separation.* The irradiated sample is dissolved in concentrated HNO_3 which is then eliminated by the addition of concentrated HCl and boiling. The solution is brought to dryness and 4 M HCl is added before passage through a column of Dowex 1 × 8 chloride form resin, and a column of cellulose impregnated with di (2-ethylhexyl) orthophosphoric acid (HDEHP), (fig. 2). Iron, Zn, Cd, Ag, Au, and sometimes traces of Th are retained on the resin, while Sc and the greater part of Th are retained on the cellulose column. The first are eluted in 4 fractions, as indicated in figure 2. The cellulose column is washed with 10 M HCl, and Sc and Th are counted directly from the cellulose column. The eluted solution is dried, water is added and the resulting solution is passed on a column of alumina in chloride form in order to eliminate the sulphates and phosphates. These last give, in fact, a beta activity so high that its bremsstrahlung disturbs the next measurements by gamma spectrometry. The eluted solution is directly analyzed for the determination of Cr, Co, and Sr.

2.1.3 *Measurement of the activity.* The measurement of the activity is done by gamma ray spectrometry directly from the separated fractions, the volume of which (20 ml) is sufficiently reduced so as to make unnecessary a subsequent concentration to increase the sensitivity of the determination. Mercury-gold and Sc-Th are measured directly from the resin and from the cellulose, respectively, after transfer to a container similar to that used for the solutions.

The spectrometer is made up of an integral line Harshaw probe (3 × 3 inch diameter) whose output pulses are analyzed by a Laben 256 channel pulse height analyzer. The data are recorded on punched tape at a rate of 20 channels per second. An automatic sample changer allows for the counting of a series of 8 samples without any assistance. Since the counting time is the same for all 8 samples, the sample changer was made in such a way that one can choose, for the various samples, the distance between detector and sample according to 5 different positions. In this way the different activities of the samples can be compensated for by changing the distance between detector and sample; and large variations in the counting rates are avoided. The measurements are done during the day, at present. An automatic 100-position sample changer is now being designed so that all the separated fractions of 12 samples can be counted during the night.

2.1.4 *Calculation of data.* The information on the punched tape is transferred to punched cards by means of an IBM 1620 computer. In addition, the following information is transferred to punched cards previously prepared by the operator :

a) Irradiation data :

1) Reference number
2) Irradiation time
3) The time the sample came out of the reactor

b) Irradiated samples :

1) Reference number of the irradiation
2) Identification number of the samples
3) Weight of samples

c) Measured spectra :

1) Reference number of the irradiation
2) Identification number of the sample
3) The moment the measurement was initiated
4) Length of time measured
5) Reference number of the spectrum
6) Elements to be determined.

For each element that is analyzed there is, in the computer library, a punched card containing the following information which is necessary for doing the calculations :

1) Reference name of the element
2) Channel number of the maximum of the photopeak to be measured
3) Half-width of the photopeak
4) Decay constant
5) Activity measured in the photopeak with these reference conditions : neutron flux : 10^{13} neutrons/cm^2 - second; irradiation time : saturation; decay time : zero.

The scheme for the calculations is shown in figure 3.

Using the information contained in the library and the given instructions, the computer chooses the zone of the spectrum in which the desired photoelectric peak of the element to be determined is present, and then evaluates the photopeak surface. It calculates the value of this surface area for the conditions to which the specific activity is referred in the library, compares it with the photopeak reference activity, and then calculates the element weight and the parts per million. The calculation is completed by an evaluation of the statistical error made in such a determination. The machine-time required to do one determination is about 0.5 second.

3 - DISCUSSION OF METHOD

Our experience with the performance of the method, particularly as far as automation is concerned, is far from complete. Nevertheless, a few things can be pointed out :

3.1 — Possibilities of the Method

Although the method has been tried out on a small scale, it appears that approximately one hundred specimens could be run per month with one operator working half-time for the collection and preparation of samples, and another working full-time for the separations, counting, and the checking of the data. At this speed, we feel that a comprehensive study of some well chosen trace elements in a freshwater environment is feasible.

3.2 — Sampling

Some kind of sampling is necessary to reduce the quantity of material to be irradiated to less than 100 mg per sample. Only in this way is it possible to irradiate six or more samples simultaneously without doing the chemical separation step in a high radiation field. The homogenization of the sample attained with a mechanic mortar is good, but the mortar must be cleaned very well after each operation to avoid cross-contamination. This operation is time consuming.

3.3 — Chemical Separations

The chemical separation step done according to the scheme presented, is acceptable from the standpoint of the time required, the purity of the separated fractions, and the chemical yields attained. Obviously, the scheme must be revised to include elements of greater biological significance, which heretofore were not considered in the setting-up of the method. Dissolution of the samples in a closed system should also be studied in order to avoid the loss of volatile elements, such as bromine, for example.

3.4 — Counting

The automatic counting problem has been resolved satisfactorily. The number of samples to be counted needs to be increased to one hundred to take full advantage of the nocturnal period for the automated counting and leave the analyzer free during the day for other work.

3.5 — Automated Data Handling

The use of the electronic computer for the evaluation of the photopeak surfaces and the calculations of weights needs further study, although the results obtained up to now are rather encouraging. The results are still checked by hand, since the reliability of the results given by the computer is far from the 100 % necessary for an automated system. Errors are primarily due to the fact that the program is not completely satisfactory, particularly when the statistics are poor due to a low counting rate.

4 - BIOLOGIGAL RESULTS

With the chemical scheme elaborated, the following elements were determined in the shell and soft tissues of the freshwater bivalve, *Unio mancus elongatus* (Pfeiffer) from Lake Maggiore : scandium, gold, zinc, cadmium, chromium, iron, mercury, cobalt, thorium, strontium, silver, and manganese.

4.1 — Gold, scandium, thorium, cobalt, mercury, silver, chromium, and cadmium

Of all the elements measured, these were often at the limit of the sensitivity of the technique, under the conditions employed, falling well below one part per million (ppm). Exceptions to this are the chromium and cadmium contents in the tissues, which invariably showed a higher quantity of all the elements when compared with the shell on an equal weight basis (table 1).

Gold. As early as 1897 gold was detected by Liversidge (quoted in Vinogradov), 1953 in the shell of a marine oyster. Lately, Fukai and Meinke (1962), by means of activation analysis, calculated 0.0057 ppm of gold (dry matter) in the soft tissues of the marine little-neck clam. No information is available on the content of gold in freshwater animals. We found an average of 0.047 ug of gold in the combined parts of *Unio*, however, the variation was so great that we consider this figure purely indicative.

Scandium. As far as it could be ascertained, there are no data on the presence of scandium in freshwater or its biota. Nor does there appear to be any physiological role for this element in higher animals and plants. Its marine abundance is given as 0.00004 ppm (Goldberg, 1957). In *Unio* there is more than 100 times that amount in both the shells and the soft tissues.

Thorium. Of the naturally occurring radioactive elements, the uranium and thorium series have been studied in inland waters. Although uranium has been demonstrated in freshwater algae, there is no information on its content in animals. Thorium does not appear to be concentrated by plants and Hutchinson (1957) feels that thorium and its isotope ionium, are probably present only in suspension in lakes. This might account for the fact that, when it was possible to make a comparison, the soft tissues contained more of the element than the shell.

Cobalt. This is one trace element which has been recognized as important and necessary in many aspects of human and higher animal physiology. The quantity of Cobalt has been calculated to be between 0.00002 to 0.00004 ppm in natural water (Hutchinson, 1957). It appears that Co, like ferrous iron, can be added to the hypolimnion (deep layer of a body of water) from reduced mud. In *Unio* Co is decidedly higher in the soft parts than in the shell, which is in keeping with what is known concerning its function in the synthesis of vitamin B^{12} and its role in nutrition.

Mercury. Our data indicate that the soft tissues of *Unio* contain slightly more of this element than the shell. Aside from that, little can be added since there is no information on the content of mercury in freshwaters. Goldberg (1957) reported 0.0003 ppm of mercury in the marine hydrosphere. A comparison of this figure with our determinations reveals the fact that *Unio* contains at least 1 000 times as much.

Silver. Hutchinson (1957) suggests that the cycle of silver in the biosphere is similar to that of copper, for the ease with which it adsorbs on organic matter. He quotes 28 mg/m^3 of silver in the water of Lake Michigan, or 0.028 ppm. Despite the dispersion found in the amount calculated for *Unio*, we can say that there is at least 100 times more silver in the soft tissues alone.

Chromium. This element is not considered "essential" in man or the higher animals although it has been detected in human blood. A range of 0 to 0.040 ppm has been given for Lake Michigan (Hutchinson, 1957) and the soft tissues of *Unio* far exceed this value. However, they do not contain as much chromium as the soft tissues of a snail from the Columbia River which was reported as 20 ppm (Davis, Perkins, Palmer, Hanson, and Cline, 1958).

Cadmium. Although no details were given, cadmium was reported to have been found in the protein of the marine mollusk, *Pecten* (Vinogradov, 1953). The soft parts of *Unio* averaged 21 ppm, the highest for this group of elements which, in general, is found in minute quantities. One interesting observation was that cadmium occurs in the liver of the albacore and in the integument of the tuna (Goldberg, 1962).

4.2 — Manganese, strontium, iron, and zinc

These were among the first elements demonstrated in both marine and freshwater mollusks because their elevated quantity facilitated qualitative determinations (Vinogradov, 1953). We have verified the older observations in the literature (table 2).

In the whole animal, the manganese content is 3 times superior to Sr, Fe, and Zn. In the soft tissues it represents 1 % of its dry weight; and in the shell it averages 0.06 % (table 3). A consideration of the distribution of these elements in each part of the animal, points up the following :

1. Mn predominates in both the shell and the soft tissues.
2. In the shell, the quantity of Mn is followed by Sr, Fe, and lastly, Zn.
3. In the soft tissues the rank order is : Mn, Zn, Fe, and Sr (table 3).

Since Mn predominates, it appeared interesting to see the relationship of the other elements (Fe, Zn, and Sr) to it. Therefore, the atom ratios between each of these elements and Mn were calculated with the following results :

	Shell	Soft Tissues
Fe : Mn	405	330
Zn : Mn	59	347
Sr : Mn	487	45

That is, for every 1 000 atoms of Mn in the shell there are 405 atoms of Fe, 59 atoms of Zn and 487 atoms of Sr. In the soft tissues there are 330 atoms of Fe, 347 atoms of Zn, and 45 atoms of Sr for every 1 000 atoms of Mn. The higher atom ratio of Sr/Mn and Fe/Mn in the shell as compared with the soft parts, might be due to the fact that both Fe and Sr (as well as Mn) are sufficiently closely related to Ca geochemically so as to permit their presence in the crystal structure. It is known, in any case, that Sr fits well in the crystal lattice of aragonite and X-ray diffraction powder patterns done on shells of *Unio*, showed that the crystals are all aragonitic (Merlini, unpublished data).

Nelson (1962) showed that the Sr/Ca ratio in the shells of freshwater mollusks is almost directly related to the ratio of these elements in the water. An estimate was made of the atom ratio of the Fe to Mn in the water and it was found to be 492 atoms of Fe for every 1 000 atoms of Mn which approximates that found in the animal. Instead, the atom ratio of Sr to Mn in the animal does not follow that found for the water which was calculated to be 122 atoms of Sr for every 1 000 atoms of Mn.

4.3 — Concentration Ratio

The quantity of Sr, Fe, and Mn in the water of Lake Maggiore has been recently determined as follows : Sr = 0.39 ppm; Mn = 0.2 ppm; Fe = 0.1 ppm. Although the water samples were not taken at the same time nor at the same locus as the animals used in this study, we felt that we could get an idea of the order of magnitude of the concentration ability of *Unio* for these elements.

Clearly, this bivalve is a strong accumulator of Mn and Fe, especially in the soft tissues (tables 4). Despite the fact that there is almost twice the amount of Sr in the water than Mn, and almost 4 times the quantity of Fe, Sr is not concentrated to nearly the same extent as the other 2 elements by either part of the animal. Nevertheless, it is interesting that more Sr is retained in the shell than Fe; whereas the opposite is true for the soft tissues (table 3).

4.4 — Element to Element Relationships

It is presumed that if the quantity of one element is directly related to that of another element, then they both follow the same water-to-animal cycle (Pilkey and Goodell, 1963). Therefore, the correlation coefficients were calculated for element versus element in the soft tissues, and the same in the shell, also, the elements in the soft tissues versus those in the shell. Certain elements could not be used since they had not been quantitatively estimated for each animal. Among the elements which remained, we found the following inter-dependencies :

Soft Tissues	p-Values	Shell	p-Values	Soft Tissues/Shell	p-Values
Zn - Co	0.001	Zn - Au	0.05	Au - Sr	0.01
Zn - Mn	0.001	Zn - Mn	0.05	Fe - Au	0.01
Zn - Sc	0.01			Sc - Sc	0.05
Co - Sr	0.01			Sr - Fe	0.05
Co - Mn	0.01			Mn - Fe	0.1
Sr - Mn	0.01				
Sc - Co	0.05				
Zn - Sr	0.1				
Fe - Cd	0.1				
Fe - Mn	0.1				

5 - DISCUSSION OF BIOLOGICAL RESULTS

In taking into consideration the presence of the various elements determined in the mollusk, *Unio*, one of the first problems which presents itself is the mode of accumulation. The principle processes of uptake are :

1. Through direct transfer of ions or dissolved matter from the hydrosphere to animal.
2. The uptake of seston with adsorbed surface ions.
3. The uptake of elements as particulate matter.

In the first case, Fretter (1953) and Korringa (1952) postulated that the mucus which covers the gill plates picks up the ions and transports them with food into the digestive system. According to Korringa, polyvalent ions like Zn, Hg, Mn, and Cu are accumulated because of the electrical properties of the mucous sheets as well as the ions. Different studies confirm this postulation. Among them, Fretter (1953) reported the rapid uptake of strontium ions from the surrounding water by the nudibranch *Acanthodoris pilosa*, which then passed through the general surface of the body and some into the gut. Rao and Goldberg (1954) reported similar experiments with pelecypods using Ca^{45} and further demonstrated, by means of radioautographs, that the initial transfer of the radiocalcium from the sea water to the bivalve occurred at the mucous surface of the gills.

Unio is an animal which spends its entire juvenal and adulthood partially or wholly imbeded in the lake sediment, be it mud, sand, or mud and rock. It is, therefore, greatly influenced by its immediate surroundings. It feeds off plankton by filtering large quantities of water through its branchial cavity. The water filtration serves a dual purpose in this case : for alimentation and for aeration. The oxygen-carbon dioxide exchange takes place between the water and the blood in the manifold gill plates. It is easy, therefore, to follow the same line of thought as that put forth by Korringa : water, food, and ions are brought into the animal by the siphons, trapped by the mucus, and then some ions are transferred into the digestive apparatus with the food, while others pass through the body. Special parts of the mantle, in secreting the shell, pass on certain elements to the matrix or mineral part of the shell. Others go through the digestive system where they form strong organic complexes and remain in the internal organs (such as Mn, Zn, Fe, Co, etc.), or they are eventually expelled through excurrent

siphons. Ingested seston with adsorbed ions, or elements as particulate matter could be dissociated in the digestive tract. Goldberg (1957) stated that since there are many naturally occurring glycoproteins in the gut of many invertebrates, it is likely that specific enzymes, capable of breaking down the mucus, could liberate the ions for use or for discard. In *Unio* digestion is accomplished in the stomach with the aid of digestive juices. The digestive glands are found around the stomach (Murray and Leonard, 1962).

The ultimate role of the various elements in *Unio* remains to be clarified. It is often supposed that if an organism accumulates certain elements and incorporates them in its cells, then these elements serve a "purpose" (physiological), albeit unknown. Unfortunately, little can now be said about the physiological role of the elements found in *Unio*, above that which has already been discussed. One is tempted, though, to counterbalance this outlook with a less teleological viewpoint :

1) Accumulation of certain elements may simply be a matter of an inefficient mechanism for their excretion.

2) There may be no mechanism for control of absorption of certain elements.

3) In the case of elements which show irregularity in replicate analyses, one can think of the presence of micro- or macrodetritus, parasites, or all three which might contribute to the presence and quantity of particular elements.

But aside from these considerations, much can be learned and should be done about a systematic study of the ultimate composition of animals and plants in a particular environment. For instance, Chave (1954) showed, after an extensive investigation of the biogeochemistry of magnesium, that the most important factor governing the distribution of magnesium in calcareous skeletons is the mineralogy of the carbonate. The calcitic forms contained larger amounts of Mg than the aragonitic ones and the phylogenetic level of the organisms, the temperature of the water in which they lived, were the main factors exerting on the Ca/Mg ratio. Salinity, age, sample size or depth at which the organisms lived, had no apparent influence. Nelson (1962) reported that the Sr/Ca in the shells of freshwater mollusks is almost directly related to that in the water, which in turn, is related to the salinity and the source. Temperature, in this case, was minor.

As more atomic installations are created, the problem of radioactive waste disposal becomes more acute. The oceans and rivers have already become trashcans for such waste, thus posing the serious problem of contamination of our food and water sources. A knowledge of the elementary composition of the biota and the hydrosphere would be the basis for the prediction and understanding of the passage of radioelements through the environment and the food chain, to man.

6 - CONCLUSIONS

A semi-automated system, for the determination of trace elements in biological materials, by means of activation analysis, has been elaborated. The system is based on the chemical separation of elements in the irradiated specimens by chromatographic procedures; automatic gamma spectrometry, with the use of sample changers; and automatic treatment of the experimental data by computer techniques.

Thirty samples were analyzed to test, on a small scale, the possibility of the system and to see the statistical and biological significance of the data obtainable before undertaking any major biological program. Twelve elements were determined in each sample by adapting to these biological samples, a chemical separation scheme which had previously been used for other materials.

Although the system has not yet been perfected, it appears that approximately 100 samples per month with 12 to 15 elements per sample, can be determined. We have, therefore, shown the feasibility of using this system for an accurate and more thorough investigation of well chosen, biologically significant, elements.

The biological results obtained on *Unio*, a bivalve from Lake Maggiore, revealed the presence of elements never before determined in a mollusk from a freshwater environment. This fact points up the necessity of an adequate and accurate tool for the quantitative determination of the elementary composition of organisms and their environment. The tool which appears to us to satisfy all the necessary conditions, is activation analysis.

ACKNOWLEDGEMENTS

The authors are happy to thank Mr. G. Bertolini (Nuclear Chemistry) and Mr. Ph. Bourdeau (Biology) for their suggestions and discussion of the manuscript; Mr. K. Weber (Metallurgy) for the X-ray diffraction patterns of the shells; Mr. P. Ihm and Mr. Anselmi (C.E.T.I.S.) for their assistance with the mathematical calculations done at C.E.T.I.S.

Fig. 1 — Flow sheet of the analytical method

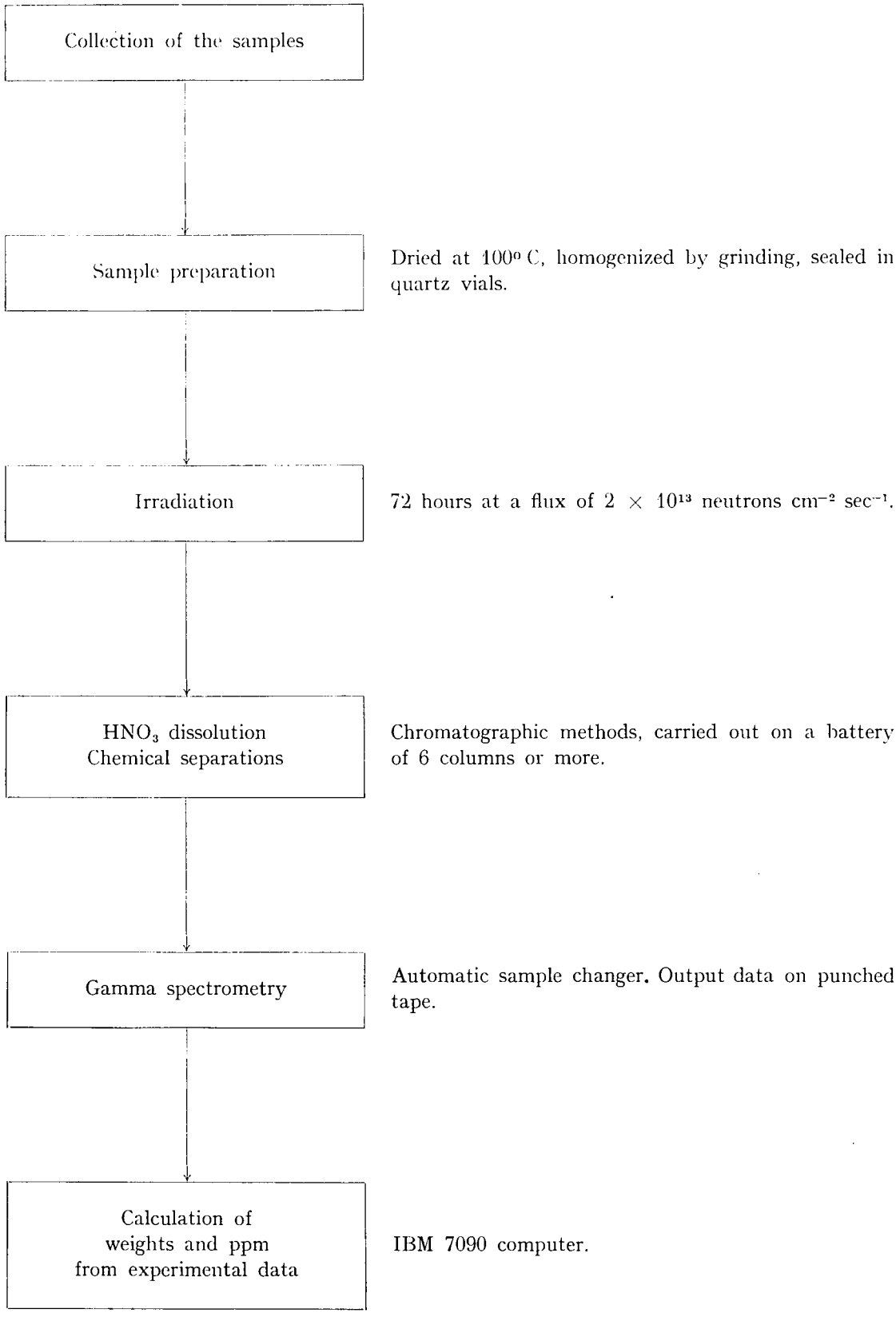

Fig. 2 — Flow sheet of the chemical separations

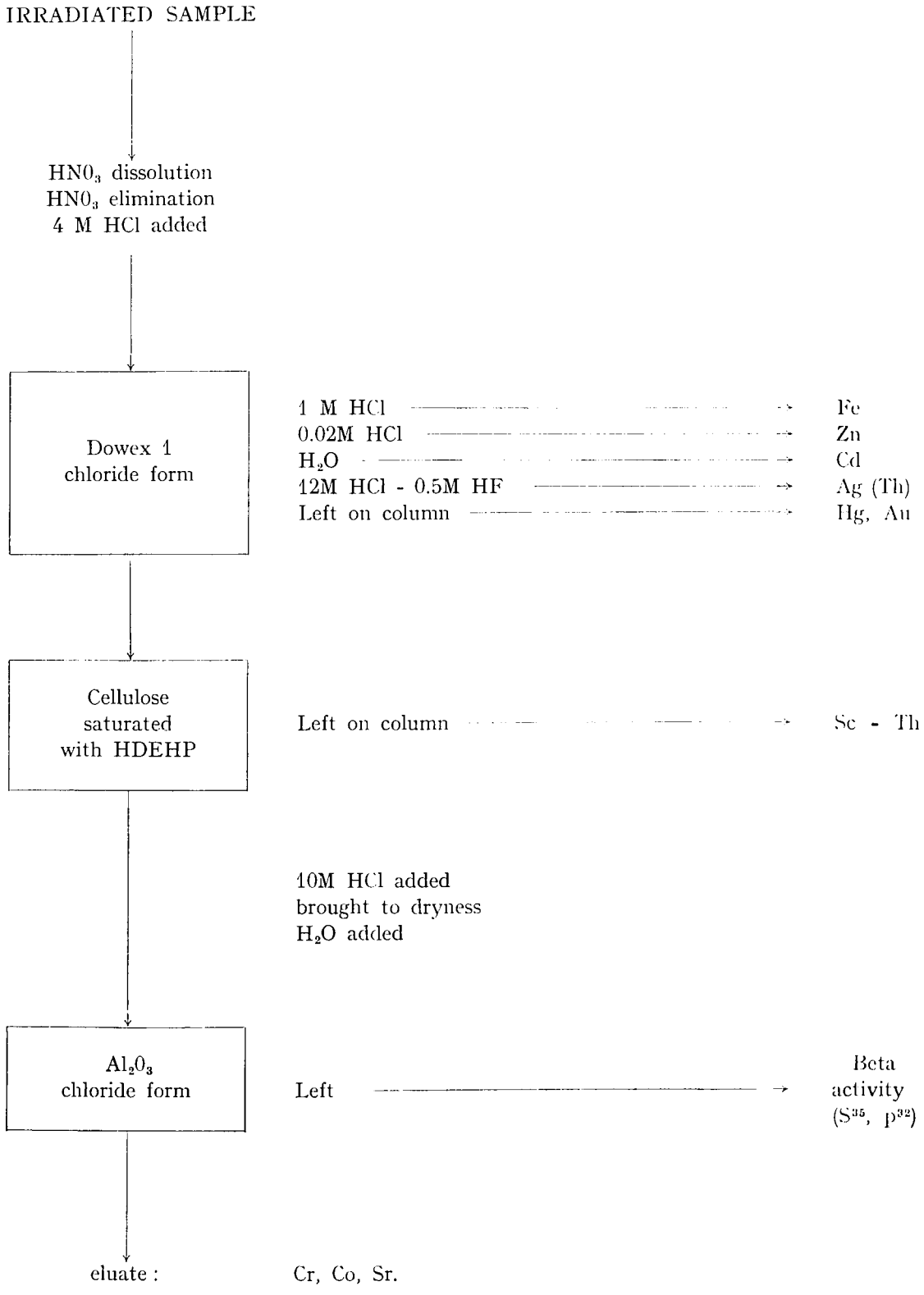

Fig. 3 — Flow sheet of the calculation of weights and ppm from experimental data

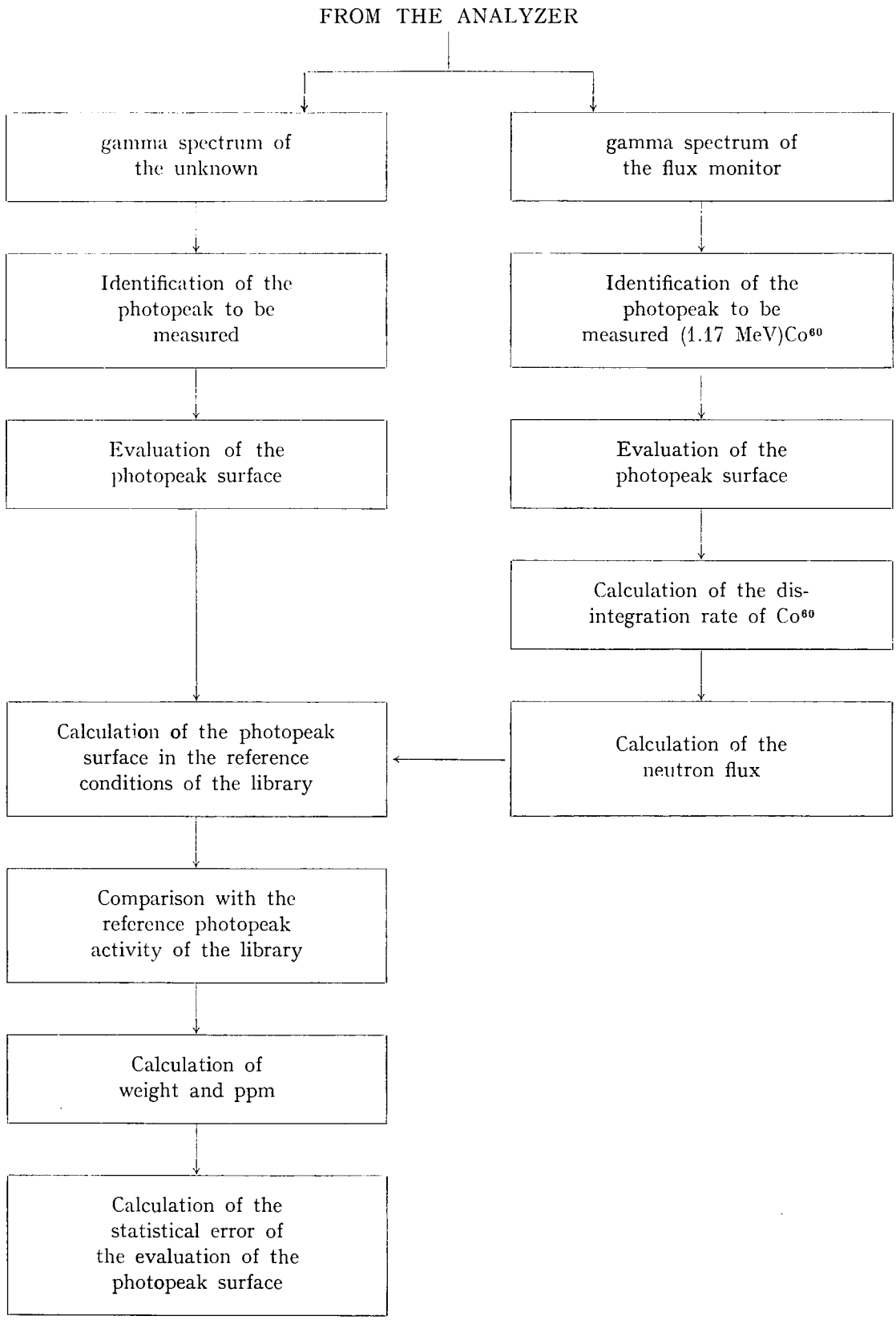

TABLE I

Elements determined in the mollusk *Unio mancus elongatus* given in parts per million (based on dry weight)

ANIMAL No.	SHELL											
	Sc	Au	Zn	Cd	Fe	Hg	Cr	Co	Th	Sr	Ag	Mn
1	0.014	0.0032	10.5	<0.65	215	0.025	5.6	0.05	0.038	860	<0.10	770
2	0.0092	0.0021	7.0	<0.55	69	<0.049	<3.1	<0.035	0.015	400	0.26	720
3	0.020	0.0014	14.0	<0.56	158	0.10	<1.9	0.046	0.016	480	<0.090	755
7	0.00013	0.0021	12.4	0.093	360	0.017	0.10	0.075	<0.026	—	<0.05	715
8	0.0039	0.0023	15.7	<0.071	175	0.086	<0.21	0.02	0.05	370	0.18	730
9	0.0091	0.0004	11.3	0.13	125	0.049	—	—	0.013	230	0.16	545
10	0.012	0.00093	6.3	0.07	490	0.05	<1.8	<0.01	0.062	300	0.66	600
11	0.0091	0.0016	180.0	0.073	500	0.052	<1.9	<0.025	0.027	500	<0.61	450
12	0.0067	0.0026	5.4	<0.036	400	0.046	<2.0	0.049	0.092	265	<0.10	500
13	0.027	0.00035	19.0	0.17	274	0.0092	0.28	0.059	<0.0026	420	0.20	615
14	0.014	0.0012	18.0	<0.10	105	0.052	<0.36	0.022	0.046	355	0.05	500
15	0.0077	0.0023	14.0	<0.10	115	<0.47	<0.21	0.024	<0.0043	630	<0.06	530
16	0.001	—	25.8	—	76	—	—	0.08	<0.05	—	<0.041	594
17	—	—	43.0	—	230	—	0.035	0.082	0.014	505	<0.046	446
18	0.033	0.0025	95.0	0.51	205	0.013	0.11	0.083	0.10	480	<0.056	473

ANIMAL No.	SOFT TISSUES											
	Sc	Au	Zn	Cd	Fe	Hg	Cr	Co	Th	Sr	Ag	Mn
1	0.078	0.028	3680	20	2420	<1.0	7.8	0.32	0.024	745	<0.91	7470
2	0.041	0.0041	3480	15	2180	0.052	8.3	0.13	0.062	205	0.46	7910
3	0.087	0.0051	3490	37	3550	0.25	17.0	0.19	0.016	920	0.71	10800
7	0.11	0.015	4360	26	2780	0.072	—	0.39	0.075	210	0.56	8000
8	0.044	0.0079	3580	16	2690	0.25	—	0.29	<0.14	750	0.81	13100
9	0.051	0.0046	2500	28	4170	0.036	—	0.10	<0.042	220	0.16	6000
10	0.029	0.015	3800	25	4000	<0.44	<10.6	0.35	<0.076	1300	<0.45	13450
11	0.036	0.0099	5900	29	4300	<2.5	<19.0	0.41	0.0072	1400	<1.3	15500
12	0.022	0.0084	3800	20	2750	<0.51	< 3.0	0.31	<0.13	970	<0.53	9900
13	0.28	0.0096	7876	12	3864	0.14	3.4	0.56	0.033	1040	0.43	20500
14	0.052	0.0067	3705	8.5	2420	0.27	4.2	0.23	<0.092	710	0.61	10300
15	0.098	0.034	4195	12	3165	0.077	2.6	0.26	0.0045	660	0.74	8400
16	0.028	0.039	4800	23.4	3410	0.5	0.45	0.38	0.23	1070	0.84	10000
17	0.04	0.012	4260	23.1	2640	0.33	11.0	0.22	0.056	350	0.33	9000
18	0.09	0.021	4340	23	2750	0.13	2.3	0.38	0.13	1050	0.61	9300

TABLE II

The total amount of Mn, Sr, Fe, and Zn in *Unio*, given as micrograms and based on dry weight (100° C)

Animal No.	Length (cm)	Mn	Sr	Fe	Zn
1	6.50	15,144	9720	4544	3595
2	6.70	19,267	5178	3721	4676
3	6.75	16,941	5603	4586	3173
7	6.30	16,356	—	6898	4805
8	6.75	19,754	4405	4307	3565
9	6.35	11,027	2496	5153	2444
10	6.55	16,987	4469	8691	3556
11	6.75	13,975	5671	7478	5403
12	6.65	13,043	3295	6950	3719
13	7.15	25,905	6234	6883	7202
14	7.75	20,283	6226	4578	4858
15	6.30	10,650	5797	3298	3205
16	6.25	7779	—	3444	3886
17	6.05	11,144	4913	4180	3731
18	5.75	11,309	4179	3787	4422
Standard Deviation		15,304 ± 4751	5245 ± 1744	5232 ± 1691	4149 ± 1145

TABLE III

The distribution of manganese, strontium, iron, and zinc in the shell and soft tissues of *Unio* as per cent of dry weight of each part

ANIMAL No.	SHELL				SOFT TISSUES			
	Mn	Sr	Fe	Zn	Mn	Sr	Fe	Zn
1	0.08	0.09	0.02	0.001	0.75	0.07	0.24	0.37
2	0.07	0.04	0.01	0.001	0.79	0.02	0.22	0.35
3	0.08	0.05	0.01	0.001	1.08	0.09	0.36	0.35
7	0.07	—	0.04	0.001	0.80	0.01	0.28	0.44
8	0.07	0.04	0.02	0.002	1.31	0.07	0.27	0.36
9	0.05	0.02	0.01	0.001	0.60	0.02	0.42	0.25
10	0.06	0.03	0.05	0.006	1.34	0.13	0.40	0.38
11	0.04	0.05	0.05	0.018	1.55	0.14	0.43	0.59
12	0.05	0.03	0.04	0.005	1.00	0.10	0.38	0.38
13	0.06	0.04	0.03	0.002	2.05	0.10	0.39	0.79
14	0.05	0.04	0.01	0.002	1.03	0.07	0.24	0.38
15	0.05	0.06	0.01	0.001	0.84	0.07	0.32	0.42
16	0.06	—	0,01	0.002	1.00	0.11	0.34	0.48
17	0.04	0.05	0.02	0.004	0.90	0.03	0.26	0.43
18	0.05	0.05	0.02	0.009	0.93	0.10	0.27	0.43
Average	0.06	0.045	0.02	0.004	1.011	0.07	0.32	0.43

TABLE IV

The estimated concentration factor for Sr, Fe, and Mn in *Unio*, based on dry weight. Water content : Sr — 0.39 ppm; Fe — 0.1 ppm; Mn — 0.2 ppm.

ANIMAL No.	SHELL			SOFT TISSUES		
	Sr	Fe	Mn	Sr	Fe	Mn
1	2205	2150	3850	1910	24,200	37,350
2	1206	690	3600	525	21,800	39,550
3	1231	1500	3775	2358	35,500	54,000
7	—	3600	3575	538	27,800	40,000
8	949	1750	2150	1923	26,800	65,500
9	590	1250	2725	564	41,700	30,000
10	769	4900	3000	3333	40,000	67,250
11	1282	5000	2250	3589	43,000	77,500
12	679	4000	2500	2487	27,500	49,500
13	1077	2740	3075	2666	38,640	102,500
14	910	1050	2500	1820	24,200	51,500
15	1615	1150	2650	1692	31,650	42,000
16	—	760	2970	2743	3.400	50,000
17	1295	2300	2230	897	26,400	45,000
18	1231	2060	2365	2692	27,500	46,500
Average	1143	2326	2881	1982	29,346	53,210

LITERATURE

CHAVE, K.E. 1954. — Aspects of the biogeochemistry of magnesium. I. Calcareous marine organisms. *Jour. Geol. 62 :* 266-283.

DAVIS, J.J., PERKINS, R.W., PALMER, R.F., HANSON, W.C., and CLINE, J.F., 1958. — Radioactive materials in aquatic and terrestrial organisms exposed to reactor effluent water. In : *Second U.N. Geneva Conference. Vol. 24 :* 423-428. Pergamon Press, London.

ERMAN, Paul. 1816. — Wahrnehmungen über das Blut einiger Mollusken. *Abh. preuss. Akad. Wiss.*, p. 199.

FRETTER, Vera, 1953. — Experiments with radioactive strontium (Sr^{90}) on certain molluscs and polychaetes. *Jour. Mar. Biol. Assoc. 32 :* 367-384.

FUKAI, R. and MEINKE, W.W. 1962. — Activation analyses of vanadium, arsenic, molybdenum, tungsten, rhenium, and gold in marine organisms. *Limnol. and Oceanogr. 7 :* 186-200.

GIRARDI, F. and PIETRA, R. 1963. — Neutron activation analysis of aluminium. *Analytical Chemistry. 35 :* 173-177.

GOLDBERG, E.D. 1957. — Biogeochemistry of Trace Elements. In : *Geol. Soc. America Memoir 67 :* 1, 345-357.

GOLDBERG, E.D. 1962. — Elemental composition of some pelagic fishes. Supplement to *Limnol. and Oceanogr. VII :* IXXii; IXXV.

GOLDBERG, E.D. and ARRHENIUS, G.O.S. 1958. — Chemistry of Pacific pelagic sediments. *Geochimica et Cosmochimica Acta 13 :* 153-212.

HARLESS, Emil. 1847. — Über das blaue Blut einiger wirbelloser Tiere und dessen Kupfergehalt. *Arch. Anat. Physiol.*, Lpz,. *14 :* 148.

HUTCHINSON, G.E. 1957. — *A Treatise on Limnology.* Vol. I. Geography, Physics, and Chemistry. John Wiley and Sons, Inc. New York.

KORRINGA, P. 1952. — Recent advances in oyster biology. *Quart. Rev. Biol. 27 :* 266-308.

MURRAY, H.D. and LEONARD, A.B. 1962. — *Handbook of Unionid Mussels in Kansas.* Museum of Natural History. University of Kansas, Lawrence, Kansas.

NELSON, D.J. 1962. — Clams as indicators of Sr^{90}. *Science 137 :* 38-39.

NICHOLLS, G.D., CURL. H., Jr., and BOWEN, V.T. 1959. — Spectrographic analyses of marine plankton. *Limnol. and Oceanogr. 4 :* 472-478.

PILKEY, O. and GOODELL, H.I. 1963. — Trace elements in recent mollusk shells. *Limnol. and Oceanogr. 8:* 137-148.

RAO, K.P. and GOLDBERG, E.D. 1954. — Utilization of dissolved calcium by a pelecypod. *Jour. Cellular and Compar. Physiol. 43:* 283-292.

RONA, E., HOOD. D.W., MUSE, L., and BUGLIO, B. 1962. — Activation analysis of manganese and zinc in sea water. *Limnol. and Oceanogr. 7:* 201-207.

UNDERWOOD. E.J. 1956. — *Trace Elements in Human and Animal Nutrition.* Academic Press, Inc., New York.

VINOGRADOV, A.P. 1953. — The Elementary Chemical Composition of Marine Organisms. Sears Found. Marine Res. ,*Memoir 2*:, 647 pp.

CDNA00474ENC