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Determination of arsenic in fish by neutron activation analysis

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DETERMINATION OF ARSENIC IN FISH BY NEUTRON ACTIVATION ANALYSIS

BY

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By William Walter Ullmann

Approved by Robert W Schaefer

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Introduction

The use of arsenicals to control aquatic vegetation has become widespread. A study was conducted to determine possible uptake of arsenic by fish in treated waters. Present methods for the analysis of arsenic (1,2) are reliable when applied to water and bottom samples which contain relatively small concentrations of organic matter. In the presence of larger concentrations, arsenic is lost during digestion. Recoveries of from 40-60 per cent were obtained when the method for the analysis of arsenic in food (2) was applied to fish flesh.

Activation analysis has been successfully applied to the determination of arsenic. The method consists of measuring the B- activity of As^{76} after isolation of the arsenic by either a Gutzeit separation (3), a pentabromate distillation (4), or by distillation from hydrochloric acid containing free chlorine (5). The method described utilizes a measurement of the activity of As^{76} which is separated by precipitation as the sulfide and centrifugation. This minimizes sample manipulation and shortens the time required for analysis. Three people were able to complete the analysis of 96 samples in 15 hours.

History

Since the discovery of artificial radioactivity by Curie and Joliet in 1934, nuclear science has experienced phenomenal growth. Analytical chemistry, with special emphasis upon inorganic analysis, has been especially favored by this development. Probably the most important contribution of the nuclear sciences to the field of analytical chemistry has been the development of the technique for the detection of elements by the formation of their artificially radioactive isotopes. This procedure, which has been termed "activation analysis" is at present one of the most sensitive analytical techniques, capable of detecting as few as 10^9 atoms under the most favorable conditions. With a neutron flux of 10^{12} cm.$^{-2}$ sec.$^{-1}$, sensitivities for most elements are in the range of 10^{-16} to 10^{-11} grams. The procedure is unusually free from interferences by other elements by virtue of the unique decay periods of their artificial radioactivities and of the radiations they emit. The ever present danger of contamination encountered in conventional trace determinations is almost completely absent. Finally, the application of the method is extremely broad with 59 elements having thermal neutron activation sensitivities of $10^{-1}\mu$ g or less with a flux of 5×10^{11} neutrons per cm^2 sec.$^{-1}$(6).

Many applications of activation analysis have appeared in the literature since its use was first reported by Hevesy and Levi (7) in 1936. Using neutrons from a 300 mc radium emanation

beryllium source, they were able to estimate the extent to which dysprosium and europium were present in rare earth mixtures. They were able to estimate as little as 0.10 per cent dysprosium contamination in yttrium by comparing the intensity of the distinctive 2.4 hour half-life of dysprosium formed by the irradiation of impure yttrium samples with dysprosium-yttrium mixtures of known composition. They therefore avoided the difficult chemical separation required when conventional analysis is applied to the rare earths. A second application of the technique was reported in 1938 when Seaborg and Livingood (8), using the 36 inch Berkeley cyclotron, were able to detect trace amounts of gallium in iron samples which had been bombarded with 6.4 MEV deutrons. An excellent summarization of many of the more recent applications has been prepared by Taylor and Havens (9).

Following the advent of the chain-reacting pile in 1943, fluxes of slow neutrons millions of times larger than those used by Hevesy became available. As a result of this, in 1943, at the Oak Ridge National Laboratory, analysis by means of radioactivation became an everyday procedure for the determination of impurities in a wide variety of pure chemicals, in metals and alloys and even in organic substances (10). The

The completion of new reactors with increasingly high fluxes has brought a steady improvement in high sensitivities. Until the last decade, most analysts had to forego the reportedly high sensitivities and use the more conventional methods because there were few reactor facilities available and access to them was limited. In 1952, Oak Ridge National Laboratory made an activation analysis service available to the public (11). Since then, many non A. E. C. Laboratories have built research type reactors (12). Small package reactors (13) are also available at a reasonable cost. This increase in accessibility and availability of sources of high neutron fluxes has proportionally increased the ease of activation and has increased research into methods for the application of the technique.

Theory

Activation analysis consists of subjecting a sample to a homogeneous flux of fast or slow neutrons. After sufficient time has elapsed for the production of a sufficient quantity of the element to be determined, the element is identified and assayed by measurement of the characteristic radionuclide formed. Frequently, it is necessary to follow the irradiation by chemical isolation of the nuclide, carried out in the usual manner after the addition of an appropriate carrier.

The net rate of formation of the radioactive atoms is the difference between the rate of formation and the rate of decay. This may be expressed as

$$\frac{dN^*}{dt} = f\sigma_{ac}N - \lambda N^* \quad (1)$$

where: N^* = number of radioactive nuclei

f = flux of bombarding particles in units of particles per square centimeter per second

σ_{ac} = isotopic cross section for the nuclear reaction in units of square centimeter per target atom

λ = radioactive decay constant which is related to the half life by the relation $= \frac{0.693}{t_{\frac{1}{2}}}$

N = number of target atoms at time t

The number of radioactive nuclei N^* present after time t, is found by integrating equation (1) to obtain the following

expression

$$N^* = \frac{f\sigma_{ac}N}{\lambda}(1-e^{-\lambda t}) \qquad (2)$$

The amount of activity A, in umits of disintegrations per second, exhibited by the atoms N* produced up to time t is given by the expression

$$A_t = \lambda N^* = f\sigma_{ac}N(1-e^{-\lambda t}) = A_\infty(1-e^{-\frac{0.693t}{t_{\frac{1}{2}}}}) \qquad (3)$$

The product $f\sigma_{ac}N$ is termed the "saturation activity" A_∞, for it is the activity produced by an infinitely long irradiation. The factor $1-e^{-\frac{0.693t}{t_{\frac{1}{2}}}}$ is termed the "saturation factor" S which will vary between zero and unity. For the case covered by equation (1), the activity A_t produced up to any time t, is given by the product of the saturation activity and the saturation factor.

The factors which govern the sensitivity of the technique become more apparent when equation (3) is written as

$$g = \frac{AM}{(6.02\times10^{23})\times f\sigma_{ac}(1-e^{-\frac{0.693t}{t_{\frac{1}{2}}}})\theta} \qquad (4)$$

where g is the grams of the element to be determined, M is the atomic weight of the element sought and θ is the fractional isotopic abundance of the target isotope in the naturally occuring element. To detect a very small mass of a desired element, the flux must be large, the cross section must be large and preferably, the element must have a relatively low atomic weight and contain the target isotope in high relative abundance.

The validity of the above mentioned equations will depend upon the validity of the assumptions made in their derivation. In these derivations it was assumed that the rate of formation, $f\sigma_{ac}N$, was constant which implies that the flux is constant. The average energy of the incident particles must also be constant since the value of σ_{ac} is dependent upon this energy. Finally, the assumption is made that the number of target nuclei will not decrease perceptibly with time owing to their consumption by the nuclear reaction.

The type of radiation and the conditions under which the measurements are made also play an important role in determining the sensitivity of the procedure. In general

$$A = RFG \qquad (5)$$

where A is the number of counts, G is the geometry factor, F the overall detection efficiency and R the absolute amount of radiation. The value of F will equal the product of correction factors for the branching ratio, window and air absorption factor, backscattering factor, self scattering factor, housing scattering factor, air scattering factor and the detection efficiency of the counting equipment. These must all be considered when estimating absolute disintegration rates for activities. For γ-ray counting most of these factors become negligible with the exception of the geometry factor and the

detection efficiency of the counting equipment.

Fortunately for the application of the activation analysis, it is not necessary to overcome all of the above mentioned difficulities. Comparative measurements with samples of known composition will yield good accuracy. The standards, which should have the same general composition as the unknowns, are irradiated in an identical arrangement and at the same time as the unknowns. If a separation technique is to be applied to the unknowns for the isolation of the activity of interest, it must also be applied to the standards. When counting, the geometry of samples and standards must be identical and the counts, if decay is important, should be corrected to a common time. Then, by application of the following formula, the amount of unknown can be calculated.

$$\frac{\text{Total activity from element x in unknown}}{\text{Total activity from element x in standard}} = \frac{\text{mass of x in unknown}}{\text{mass of x in standard}} \qquad (6)$$

A calibration curve may be constructed if a number of samples containing varying amounts of unknown are to be analyzed. Usually however, one properly chosen standard will suffice.

β -particles, emitted from radioisotopes, have energies ranging from a maximum value E_{max} to zero with an average energy of about 1/3 E_{max} (14). This wide variation in energy, rather

than monoenergetic β-particles, arises from the random sharing of the transition energy by the β-particle and a neutrino when a neutron changes to a proton.

Although β-spectra are extremely complex, information with respect to identification and analysis can be obtained from absorption measurements, or by scintillation, lens or magnetic spectrometers (15). However, the use of measurements of β-particles, generally requires a rather complete isolation of the isotope of interest.

Gamma-rays, however, are monoenergetic and energy characteristics can be characterized more precisely. This can be accomplished most simply by scintillation spectrometry.

A discussion of all phases of the instrumentation is beyond the scope of this paper. However, a brief discussion of scintillation counting and pulse height discrimination should be included. Fig. 1 shows a block diagram of a typical γ-scintillation spectrometer.

Several organic as well as inorganic crystals, or their solutions have the ability to detect radiation. When an

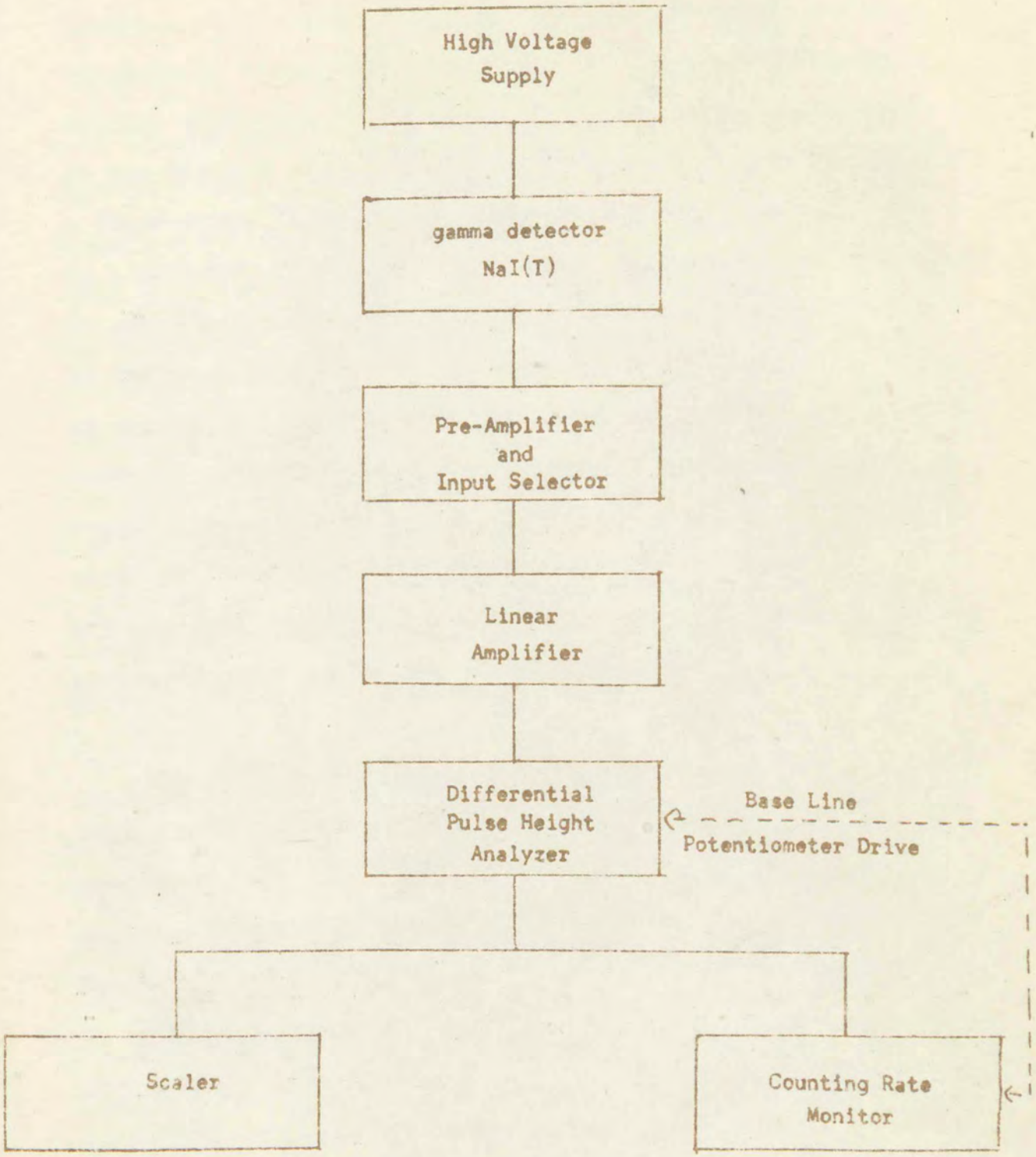

Fig. 1. Block Diagram of gamma scintillation spectrometer.

ionizing particle or photon passes through one of these cyrstals it loses energy resulting in the excitation of orbital electrons of the atoms or molecules in the crystal. As the excited electrons fall back into lower energy levels, a fluorescent light is emitted. In certain crystals there is a delayed emission of light owing to trapping of electrons at crystal imperfections. If the lifetime of such electrons is sufficiently short (10^{-5} sec.), useful flashes of light or scintillations will result. A further requirement of such crystals is that they be transparent to their own radiation. Frequently, a second substance will be added to a crystal to shift the wave length of the emitted light, so that transparency will result. The light flashes produced in a crystal are reflected on to a photomultiplier tube.

The processes that occur when γ-rays pass through a solid, such as NaI, are the photoelectric effect, which produces electrons of an energy equal to the energy of the original γ-ray less the binding energy of the electron; the Compton effect, in which only part of the energy is imparted to an electron; and pair production of a $\beta-$ and $\beta+$ for γ-rays with energys greater than 1.02 MEV. To light flashes from the electrons of the photoelectric effect is added that energy

corresponding to the energy required to overcome the binding of the electrons in the K or L shell. The X-rays emitted when these shells are refilled are absorbed or produce Auger-electron emission. Since X-rays are electromagnetic radiations similar to γ-rays, possessing only slightly lower energies (16), the pulse from the photoelectric effect corresponds to the full energy of the γ-ray.

The scintillation detector is optically coupled to a photomultiplier tube (fig. 1). The pulses from the tube pass through a condenser and resistor to a cathode follower preamplifier which couples the signal into a linear amplifier which feeds the pulse height analyzer. This discussion shall be confined to a single channel pulse height analyzer similar to the one used in this study. In this instrument two discriminators and usually an anticoincidence arrangement are used to pass pulses of such height that they fall between two discriminator settings. The two discriminators may be controlled separately or up and down the voltage scale together with a constant voltage separation between them. The lowest setting is called the base line and the separation the "channel" or "window" width. The usual pulse height

range is from 0-100 volts with a 1-5 volt channel width. Very high stability is required in the discriminators, the amplifier and the high voltage supply. Several multichannel analyzers have been developed. Up to 50 equally spaced discrimination voltages may be used, the pulse from each being fed into an appropriate scaler. Multichannel analyzers are useful in the study of short lived radioactivities since they allow simultaneous measurement of a large number of points on a pulse height spectrum.

Fig. 2 shows a typical γ-ray scan. The recoil electrons from the photoelectric effect produce the major response known as the photopeak. This is the peak of interest in most analytical determinations. The broad Compton distribution with its spectrum of lower pulse height has an energy range extending from that of the γ-ray to 0 MEV. The magnitude of the Compton effect is proportional to the extent that the detector crystal is not large enough to contain the entire sequence of processes that consume the initial γ-ray. The use of larger crystals will therefore lessen the Compton effect. Those γ-rays which lose only a portion of their energy by Compton scattering may lose the remainder by the photoelectric effect thus contributing to the photopeak. The

small peak at 1.02 MEV in fig. 2 results from pair production. This peak is absent for γ-energies below 1.02 MEV. The small peaks at the lower energies arise from 180° scattering and backscattering from the walls surrounding the crystal. At very low pulse heights the noise from the photomultiplier is counted.

Another type of interference not shown in fig. 2 is caused by bremsstrahlung which is the continous X-rays produced when electrons are decelerated in a Coulombic field of an atomic nuclei. It is produced whenever fast electrons pass through matter. The efficiency of the conversion of binding energy into bremsstrahlung increases with increasing electron energy and with increasing atomic number of the absorbing material. The spectrum of bremsstrahlung from monoenergetic electron sources extends from the electron energy down to zero with approximately equal amounts of energy in equal energy intervals.

The basis for the qualitative identification of a particular radioisotope is based on the observation that the pulse height varies linearly with the γ-ray energy from 25 KEV to 3 MEV (13). Therefore the photopeak energy can identify the isotope or at least limit it to a few possibilities. Fig. 3

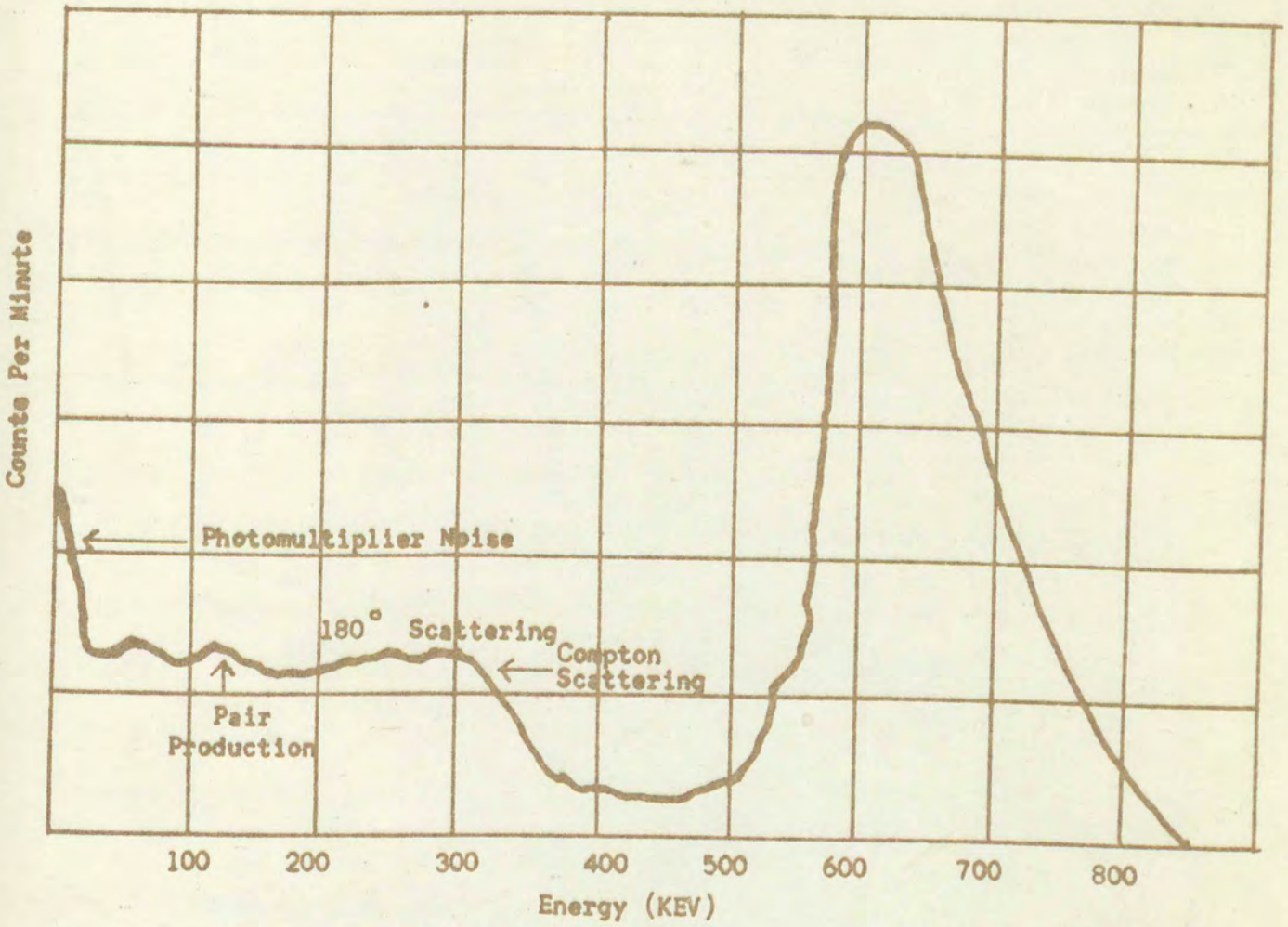

Fig. 2. Schematic representation of a gamma ray spectrum.

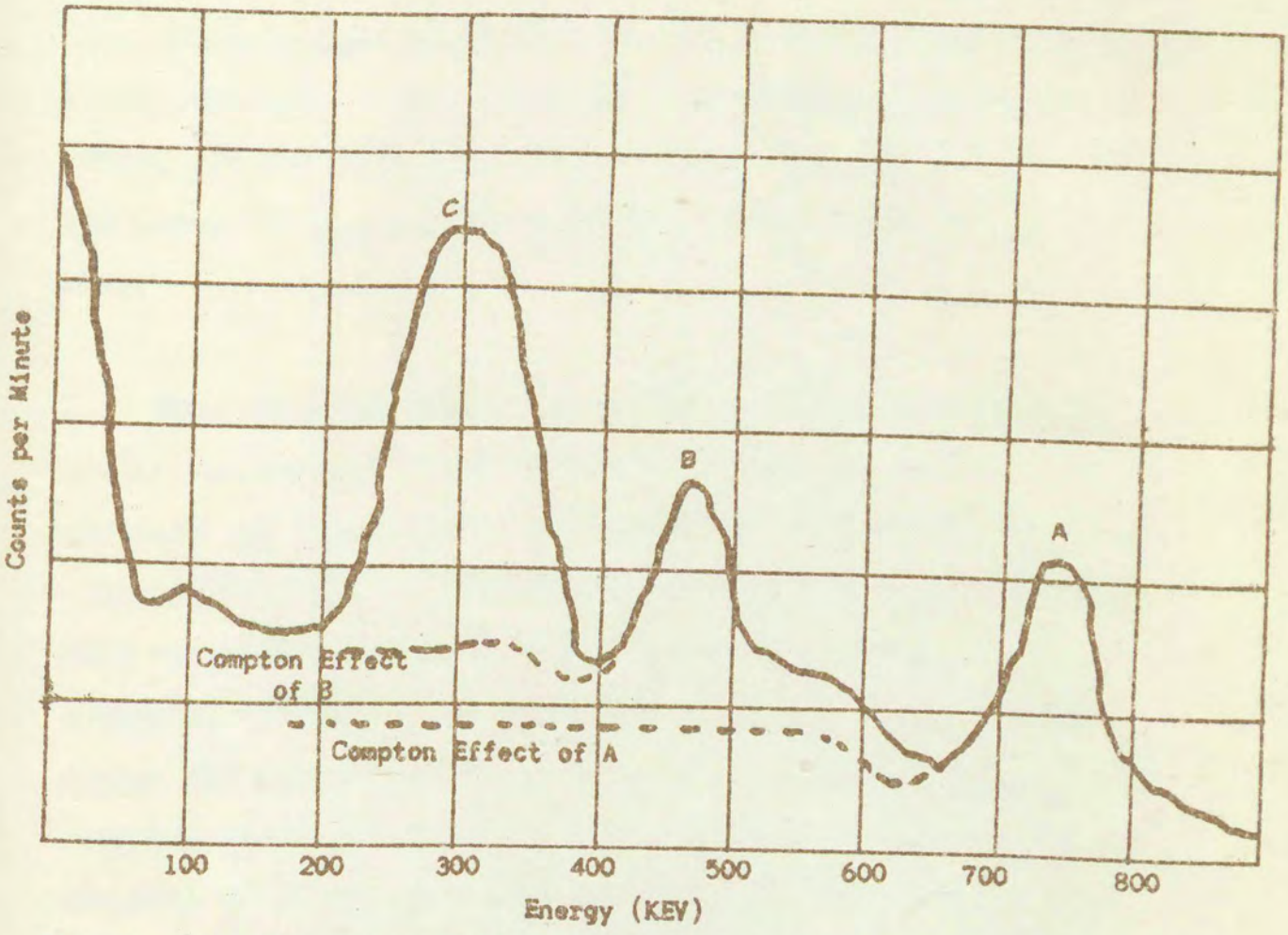

Fig. 3. Schematic representation of gamma ray spectrum of a mixture of three radioisotopes.

shows a typical scan for a mixture of three γ-activities. The contributions of background from the Compton continuum, pair peaks, minor photopeaks and bremsstrahlung must be subtracted to give the correct count for an individual peak. Interferences arise when the principal photopeaks do not differ by more than $\frac{1}{2}$ rho where rho equals the peak width at $\frac{1}{2}$ maximum in units of channel width. If the activity of interest is less than 10 per cent of the total γ-activity, the accuracy of its determination will be low.

When two photopeaks can not be resolved, the analyst generally can do one of two things. If the half-lives of the nuclides are sufficiently different, half-life determination will possibly aid in identification or an interfering photopeak may become insignificant with time leaving the one of interest. Usually however, the analyst must devisesome method for isolating the activity of interest from those activities which interfere with the determination. Generally, the mass of radioactive material produced in a nuclear reaction will be very small. Therefore, if ordinary analytical procedures involving precipitation, filtering, centrifugation, etc. are to be applied, larger concentrations of the material must be present. Usually some inactive material, isotopic with with the radioactive transmutation product is added to act as a

carrier for the active material before chemical analysis is applied. The carrier must be in the same chemical form as the active substance. Usually about 10 - 20 mg. of carrier is most conveniently used. In general, accuracies of 90 per cent and greater are obtainable using γ-spectrometry preceded by some form of chemical separation.

Experimental

Apparatus:

1. Baird Atomic model 312 high voltage power supply
2. Baird Atomic model 510 single channel pulse height analyzer
3. Baird Atomic model 215 non-overloading amplifier
4. Baird Atomic model 810 well type scintillation detector with NaI crystal
5. Baird Atomic model 131A glow tube scaler
6. Texas Instrument Incorporated recti-riter recorder.

Preparation of samples:

Several fish of the type under consideration (Calico Bass) collected from a lake which was not known to have received any application of arsenicals and which had a low natural arsenic content (0.10 PPM), were used in the development procedure. These fish were cut into small pieces and dried in 250 ml porcelain evaporating dishes. After apparent dryness had been reached, desiccation was completed by overnight drying in an oven at 103° C. The dried material was then reduced to a powder in a Waring blender. This material was used for the preparation of samples throughout the study. The same prodedure was used for processing individual specimens when the method was applied.

Preparation of Standards:

Standards were prepared by adding various amounts of standard arsenic solution to portions of dehydrated fish which had been weighed on an analytical balance. The standards were dried in an oven at 103°C. and placed in sample containers. In some cases, the arsenic solution as well as sodium and phosphate solutions were added directly to empty containers which were oven dried. This permitted study of these constituents of the fish individually and in various combinations.

Preparation of Solutions:

A 50 PPM arsenic solution was prepared by making a 1:20 dilution of a solution prepared by dissolving 1.3163 grams of As_2O_3 in 25 ml of 1N NaOH, neutralizing with 6N H_2SO_4 and diluting to one liter with distilled water. A 10 PPM solution was prepared from the 50 PPM arsenic solution by making a 1:5 dilution with distilled water.

Other solutions which were prepared included a 10,000 PPM chloride solution as NaCl and a 100 PPM phosphate solution as K_2HPO_4. All weighings were made to ±0.2 mgs. on an analytical balance. In all cases, reagent grade or C. P chemicals were used.

Sample Containers:

For the first two runs, sample containers were prepared from polypropylene tubing (Nalgene 1249 tubing 3/8 x 0.040) one end of which had been heat sealed. Caps were made from polyethylene tubing (Nalgene 1248 tubing 3/8 x 1/10) which had been heat sealed at one end. The caps were pierced to allow pressure release during activation. Tubes, in groups of ten were tied together for activation.

For the third and fourth runs, containers were prepared by shaping aluminum foil around a glass rod and folding the ends. These tubes were $\frac{1}{4}$ x 3/4 inches. They were identified with a metal punch which imprinted the number on the tube. Several of these tubes, after being filled and identified, were wrapped in aluminum foil for shipment to Brookhaven.

Activation:

Activation was accomplished in a water cooled hole in the Brookhaven National Laboratory reactor on Long Island at a flux of 10^{12} neutrons cm^{-2} sec^{-1}. The flux was monitored with aluminum strips which contained 0.15 per cent cobalt. For the first run, the samples were delivered to Brookhaven and returned to Albany for counting by car. For

the remaining three runs the samples were shipped to Brookhaven and returned to Albany in lead pigs via air-express.

Counting:

All counts were made using the equipment described previously. This equipment is shown in fig. 4. The large "pot counter", shown in the foreground of fig. 4, was not used in this study. This type of detector is a scintillation type, shielded with mercury, and used in counting bulk samples.

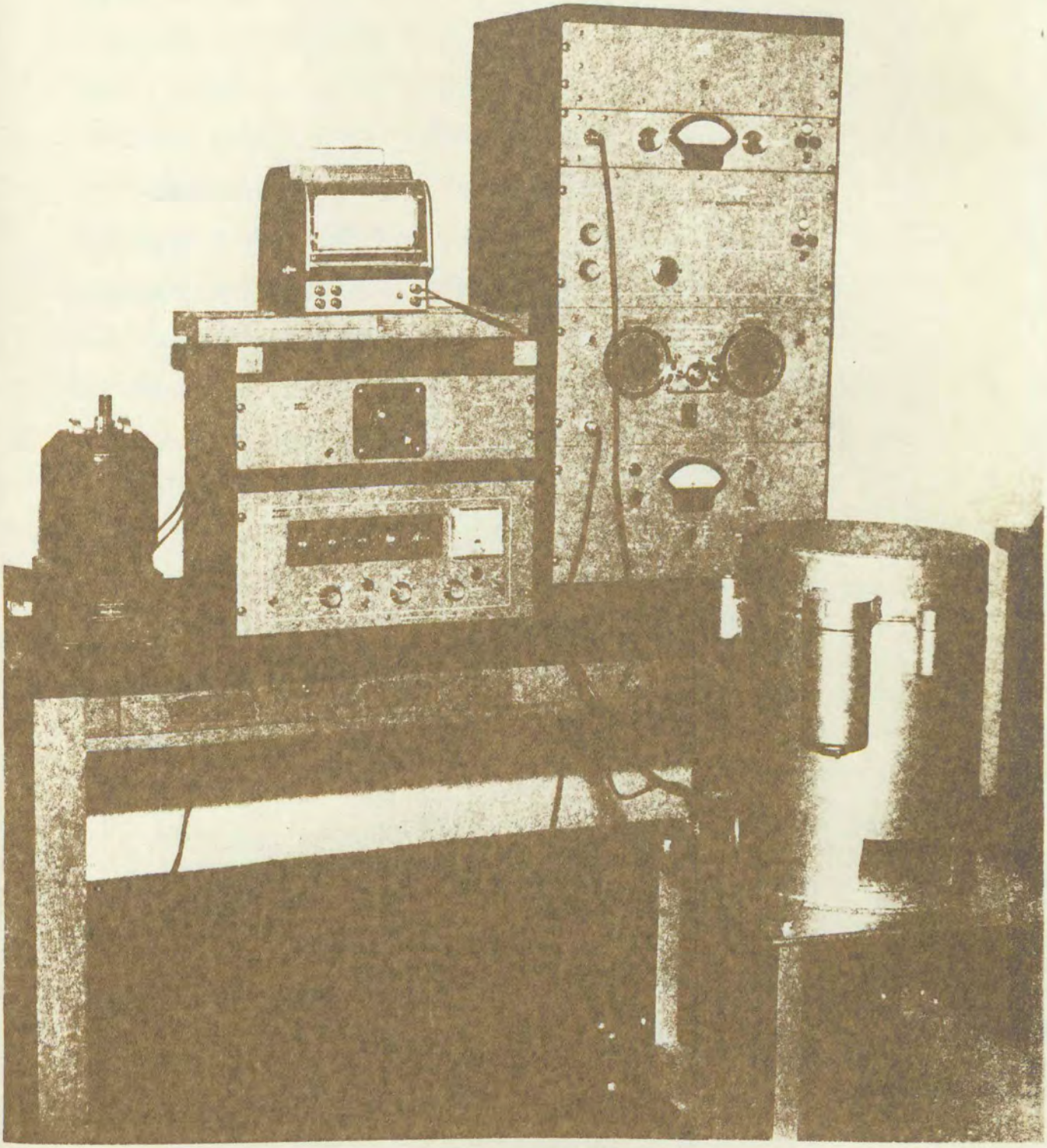

Fig. 4. Gamma-ray spectrometer with scintillation counter

Discussion

The feasability of the application of activation analysis, as was mentioned previously, depends upon the strength of flux, the magnitude of the neutron absorption cross section and the radioactive decay constant. The first of these requirements presented no problems since a flux of 10^{12} neutrons cm^{-2} sec^{-1} is available at the Brookhaven National Laboratory uranium-graphite reactor on Long Island. Arsenic, lends itself quite readily to activation analysis. As^{75} which is 100 per cent abundant in nature has the relatively large neutron absorption cross section of 4.2 barns for a neutron velocity of 2.20×10^5 cm sec^{-1}(15). The half-life of As^{76}, which is the product of the (n,γ) reaction of As^{75}, is 26.5 hours. The decay constant λ, has a value of 7.26×10^{-6} sec^{-1}. The half-life is sufficiently long to permit processing before appreciable decay has occured and short enough to produce sufficient activity to give the method a high degree of sensitivity.

Before a satisfactory procedure could be developed, several questions had to be answered. First, the range of arsenic concentrations in the samples had to be determined. From what was known concerning the arsenic concentrations in salt water fish (17), it seemed probable that the concentra-

tions of interest were betwenn 0 and 10 PPM. Secondly, the length of time for activation and the proper sample size had to be determined. As^{76} must be present in amounts which can be conveniently and accurately measured, yet small enough that the samples can be conveniently handled and counted. Finally, the type and concentrations of interfering substances, if any were present, had to be determined.

The activities due to As^{76}, which could be expected following irradiation were calculated using equation (3). A two hour irradiation time was selected. An arsenic concentration of 10γg was used for the calculation.

$$t = 2 \text{ hours} = 7200 \text{ sec.}$$

$$A_t = f\sigma_{ac} N^*\left(1-e^{\frac{-0.693t}{t_{\frac{1}{2}}}}\right) \quad (3)$$

$$f = 10^{12} \text{ neutrons cm.}^{-2} \text{ sec.}^{-1}$$

$$\sigma_{ac} = 4.2 \text{ barns} \quad 4.2 \times 10^{-24} \text{ cm.}^2$$

$$t_{\frac{1}{2}} = 26.5 \text{ hours} = 9.54 \times 10^4 \text{ sec.}$$

$$N^* = \frac{(10^{-5})(6.02 \times 10^{23})}{75} = 8.03 \times 10^{16}$$

$$A_t = \frac{(10^{12})(4.2 \times 10^{-24})(8.03 \times 10^{16})}{\left(1-e^{\frac{(-0.693)(7200)}{9.54 \times 10^4}}\right)}$$

$$A_t = 3.37 \times 10^5 \left(1-e^{-0.0157}\right)$$

$$A_t = 5700 \text{ c/s}$$

The first group of samples were irradiated in an attempt to answer some of the previously mentioned questions. One

gram samples in polypropylene containers were used. Standards containing 0 to 50 μg of arsenic per gram of dried fish were prepared. Samples were irradiated for two hours. Such high activities were obtained as a result of this first study, that the counts were well above the resolving time of the counting equipment. All attempts at decreasing the efficiency of counting by changing the geometry and decreasing the window width failed to decrease the counts within the resolving time of the counter. Therefore, a waiting period was necessary. The samples were eight days old before they could be counted and by this time most of the As^{76} activity had dissapated. A plot of the remaining activity yielded an energy continuum resembling that which would result from bremsstrahlung.

Of all of the elements present in tissue, sodium and phosphorous seemed to be the most likely ones to cause the type of interference encountered in the first run. Phosphorpus is present in tissue in concentrations of about 1 per cent. P^{31} has a thermal neutron absorption cross-section of 2.23 barns. Neutrons activation of the 100 per cent abundant P^{31} would produce P^{32}. This isotope has a half-life of 14.3 days and emits a 1.701 MEV beta-particle.

This B- particle would undoubtedly produce bremsstrahlung with the counting conditions encountered in this experiment.

Sodium is present in tissue in concentrations of about 0.15 per cent. The 100 per cent abundant Na^{23} isotope has a thermal neutron activation cross-section of 0.54 barns. The (n, γ) reaction produces Na^{24} which has a half-life of 15 hours and decays by the emission of a 1.390 MEV β- particle and 1.368 and 2.754 MEV γ-rays. Photons of at least 1.02 MEV are capable of the production of a positron-electron pair. This may be thought of as raising an electron from a negative to a positive energy state. The reverse of this or the falling of an ordinary electron into a hole in the sea of electrons of negative energy with emission of corresponding amount of energy in the form of radiation is the so-called "positron annihilation" of a positron and electron. Energy released either in the form of two 0.51 MEV γ- quanta emitted in nearly opposite directions or if the electron involved in the annihilation is tightly bound, in the form of a single 1.02 MEV γ-ray. The 1.390 MEV β- particle is also capable of producing bremsstrahlung.

The second run was devoted primarily to determine the

effects that Na^{24} and P^{32} would have on the measurement of the As^{76} activities. The following standards were prepared by adding the necessary solutions directly to the polypropylene sample containers and evaporating the water in a drying oven at 103°C.

5 μg of arsenic as As_2O_5

5 μg of arsenic as As_2O_5 and 10 μg phosphorous as K_2HPO_4

5 μg of arsenic as As_2O_5 and 1 mg of sodium as NaCl

5 μg of arsenic as As_2O_5, 1 mg of sodium as NaCl and 10 μg of phosphorous as K_2HPO_4

The size of the fish samples were reduced to 100 mg. to decrease the induced activity and permit immediate counting and easier handling. As in the first run, a two hour activation time was employed in the BNL graphite-uranium reactor. Counting was started 48 hours after irradiation.

Energy-activity plots of the standards described above are shown in figs. 5 - 10. No comparison of counts can be made since changes in geometry were made to bring the counts within the resolving time of the counting equipment. Fig. 5 shows the energy-activity plot for a 100 mg. fish sample containing

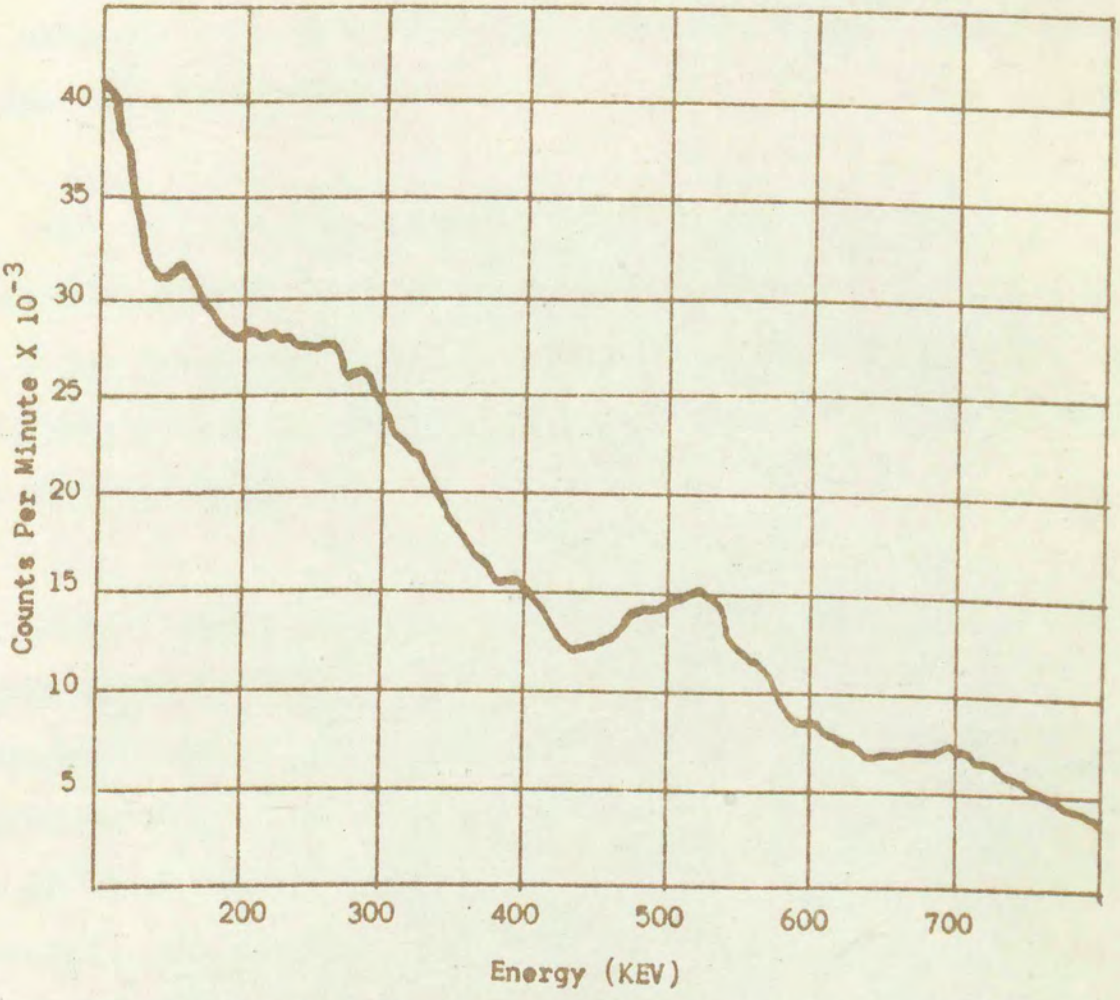

Fig. 5. Sample without arsenic isolation showing effect of interfering activity.

1 μg of added arsenic. This plot illustrates the two major interferences; the energy continuum resulting primarily from bremsstrahlung and the 0.510 MEV γ-ray resulting from positron annihilation. The arsenic activity is completely obscured. All attempts to resolve the 0.550 MEV peak of As^{76} by variation of channel width failed.

Fig. 6 shows the energy activity plot of the standard containing 5 μg of arsenic as As_2O_5 and 1 mg. of sodium as NaCl. The 0.550 MEV γ-ray of As^{76} is completely obscured by the 0.510 MEV γ-ray resulting from positron annihilation associated with the decay of Na^{24}.

Fig. 7 shows the effect of P^{32} activity upon the detection of the 0.550 MEV γ-ray of As^{76}. The activity of the sample was extremely high. The activity in the energy range of interest, (0.540-0.560 MEV) was many times higher than the amount due to the As^{76}. Therefore, the activity of the As^{76} was completely obscured. The high activity was caused by bremsstrahlung. The degree of bremsstrahlung is directly related to the atomic number of the absorber. Therefore, a sample holder was made which would surround the sample with water and thus minimize the degree of the bremsstrahlung. However, even with this reduction in

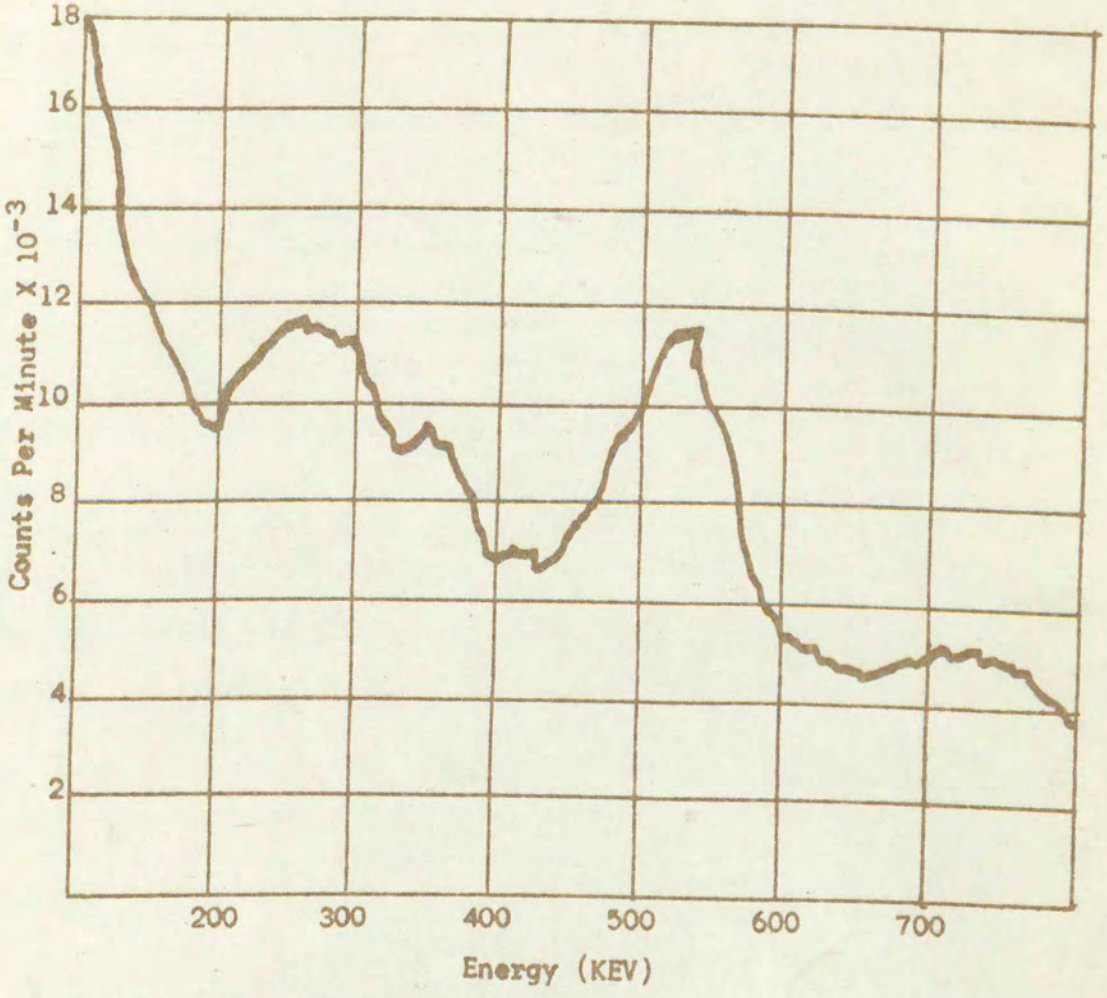

Fig. 6. Standard containing 5 µg of As as As_2O_5 and 1 mg. of Na as NaCl.

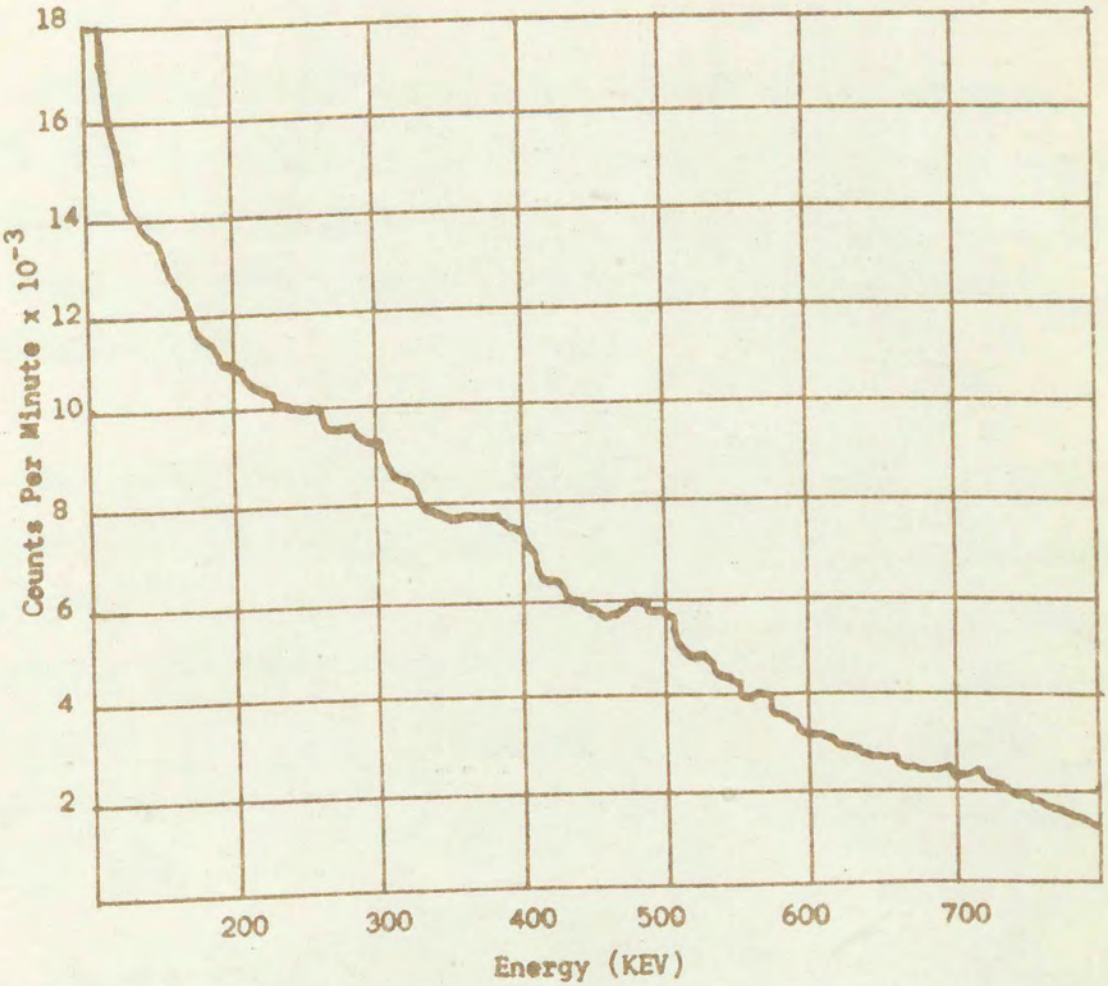

Fig. 7. Standard containing 5 µg of As as As_2O_5 and 10 mg of P as K_2HPO_4.

interference, the As^{76} peak could not be resolved. The B- activity was so intense that extremely high γ-activities resulting from bremsstrahlung, could be detected outside of the lead pig in which counts were made.

Fig. 8 shows the energy-activity plot of a mixture of As^{76}, Na^{24} and P^{32}. The 0.510 MEV γ-ray resulting from positron annihilation is easily seen and completely obscures the 0.550 MEV γ-peak of As^{76}. Bremsstrahlung is evidenced by an increase in total activity.

The energy-activity plot of the standard which contained 5 μg of arsenic as As_2O_5 with no interfering substances is shown in Fig. 9. The 0.550 MEV γ-ray of As^{76} is easily seen. The background activity results in part from Compton scattering, 180° scattering, etc. as illustrated in fig. 2 and in part from bremsstrahlung resulting from B-particle emission associated with the decay of As^{76}.

A better understanding of the nature of the interferences was obtained from the second run. The activity of the 0.550 MEV γ-ray could not be resolved by any available means. A chemical separation therefore was necessary. The techniques

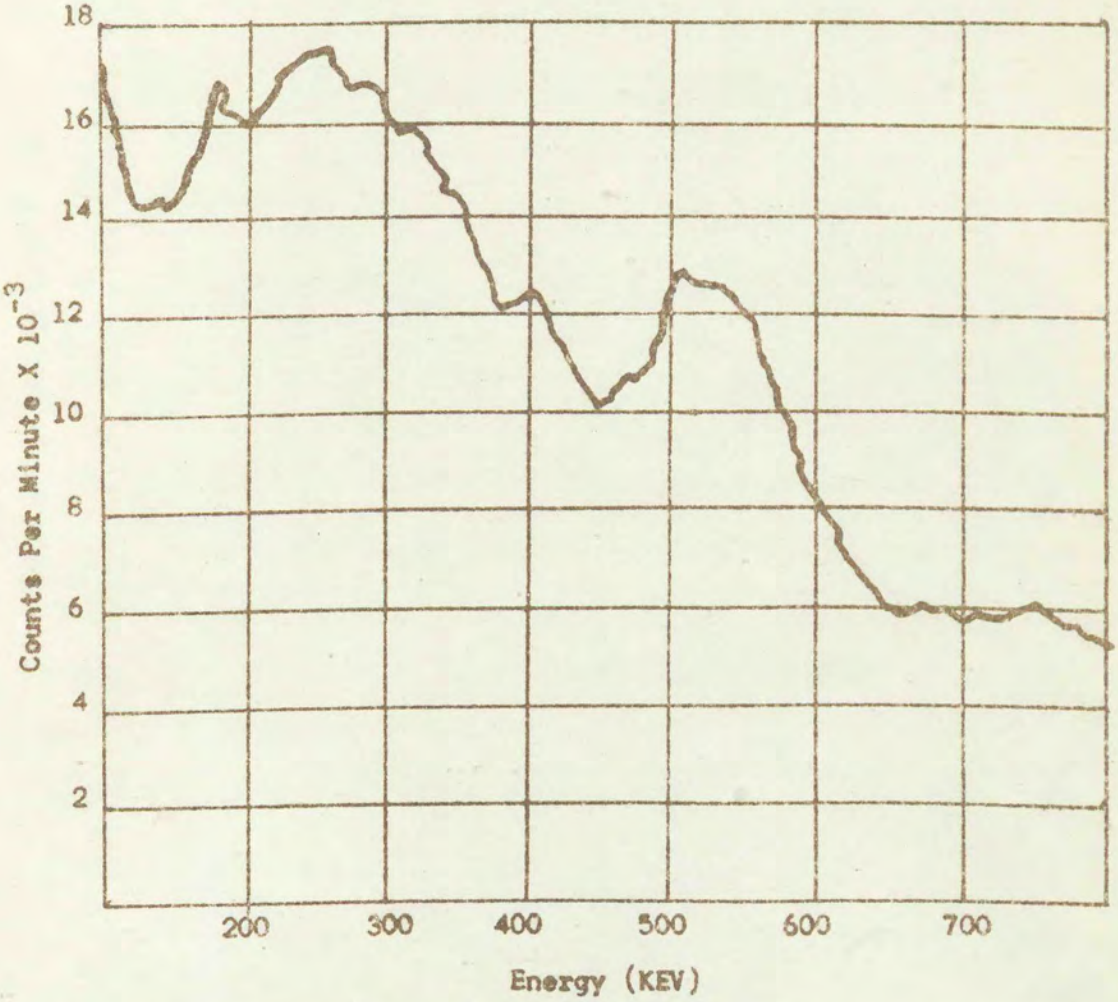

Fig. 8. Standard containing 5µg of As as As_2O_5, 1 mg of Na as NaCl and 10 g of P as K_2HPO_4.

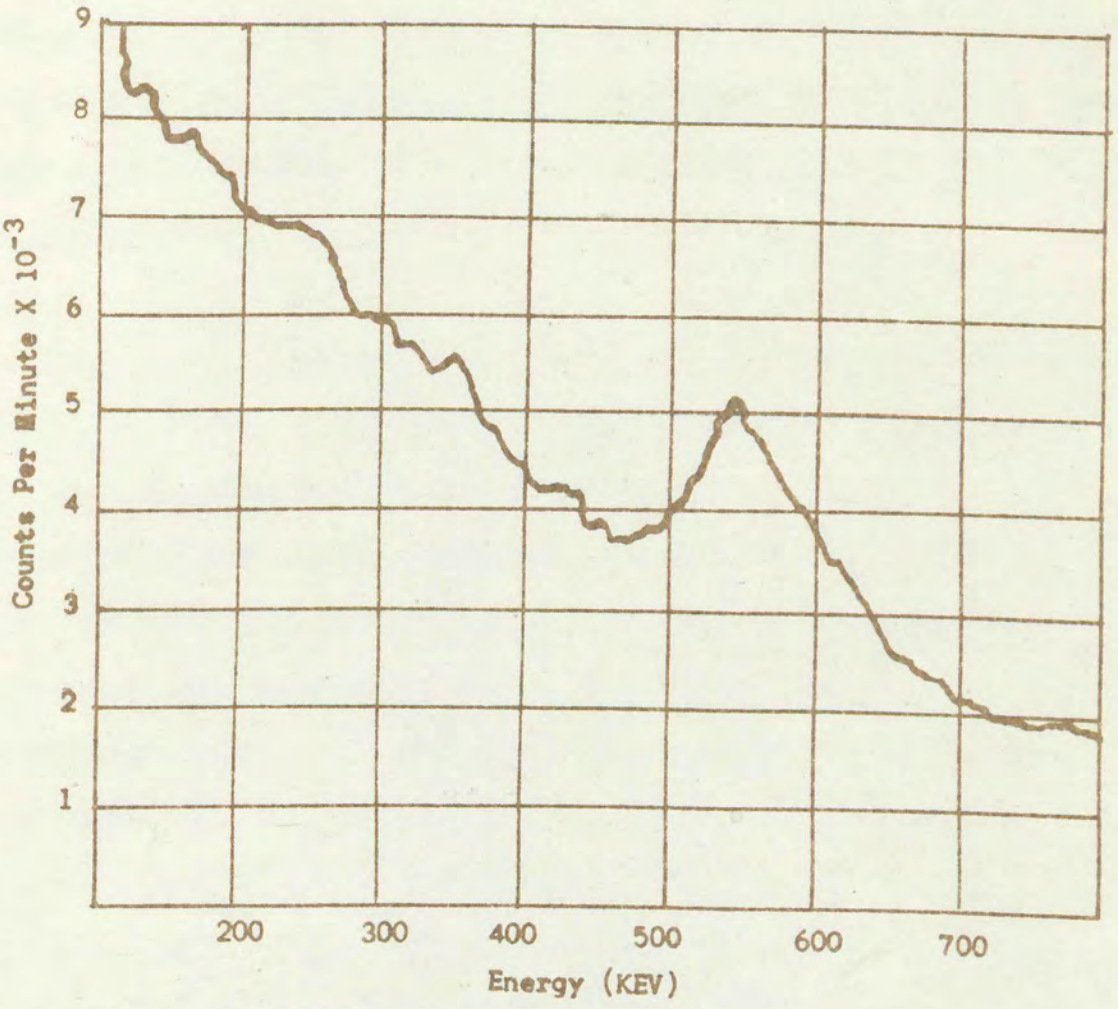

Fig. 9. Standard containing As as As_2O_5

which had been employed for the separation of arsenic activity (3) (4) (5), were time consuming and required considerable handling of the samples. Using 100 mg. samples and the relatively short irradiation time of two hours, the total integrated count of the γ- radiation yielded an activity of about 0.05 μc per sample forty-eight hours after irradiation. Therefore, a rapid method which required a minimum amount of handling was required.

Before any isolation procedure could be applied, a method for the destruction of organic matter had to be developed. The sulfuric acid-nitric acid method commonly used (2) calls for the addition of 25 ml of concentrated nitric acid and 20 ml of concentrated sulfuric acid per 25 grams or more of sample. To prevent the reduction and volatilization of arsenic, oxidizing conditions must be maintained throughout the digestion. An attempt was made to reduce the acid volumes to one ml. each to permit digesting in a test tube. However, the digestion was quite time consuming. A much faster digestion was accomplished by adding the nitric acid dropwise. The procedure finally used consisted of adding the 100 mg. fish sample to a test tube with a few glass beads. Twenty mg. of arsenic carrier

as Na_2AsO_4 are added with one ml. of concentrated sulfuric acid and 0.1 ml of concentrated nitric acid. The mixture is heated to fumes and as the solution darkens, nitric acid is added dropwise until the solution no longer darkens on heating. The procedure requires about 5 minutes per sample.

The next step was to select a method which would isolate the arsenic from the above mentioned interferences. The method selected as the most satisfactory consisted of precipitating the arsenic as the sulfide from an acid solution. Sodium sulfide was selected in preference to hydrogen sulfide because of the ease in handling. The precipitate was isolated by centrifugation. Sodium lauryl sulfate was used to aid settling of the floating precipitate by reducing the surface tension of the liquid.

For the third run, standards containing 0-10 μg of arsenic per 100 mg of fish were prepared by adding the standard solution to the dehydrated fish and drying in an oven at 103^{o}C. It was discovered in the first two runs that the polypropylene containers became quite radioactive when irradiated. This was probably caused by impurities in the plastic. The material also became brittle and cracked. Therefore, the use of this

type of container was discontinued. A new type of container was made by forming aluminum foil around a ¼ inch glass rod and folding one end. The weighed sample was placed in the container and the tube sealed by folding the open end. Identification numbers were imprinted on the aluminum foil with a metal punch set. To open the tubes, one end was cut and the sample poured out.

To determine the precision of the method, ten replicates containing 5 µg of arsenic per 100 mg of dehydrated fish each, were prepared in the manner described. Samples and standards were irradiated for two hours in the BNL reactor at a flux of 10^{12} neutrons cm.$^{-2}$ sec.$^{-1}$. After irradiation, the sulfide precipitation procedure was applied and the samples counted.

Fig. 10 shows the background activity of a sample which contained no measurable arsenic. The energy continuum results primarily from Compton scattering and bremsstrahlung produced by impurities in the arsenic sulfide precipitate.

A typical energy-activity plot of isolated arsenic activity is shown in fig. 11. If a single count were made with a base

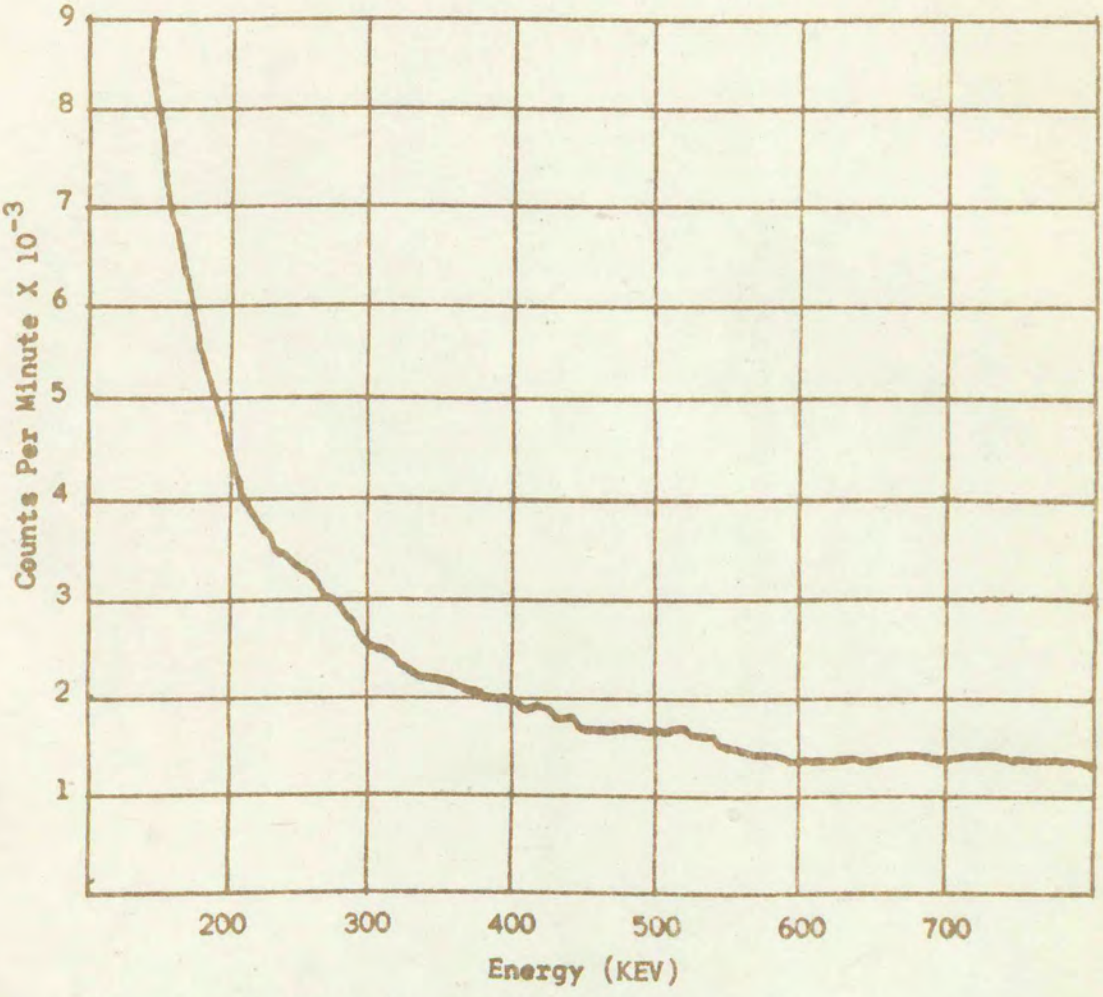

Fig. 10. Background activity of sulfide precipitate obtained from sample which contained no arsenic.

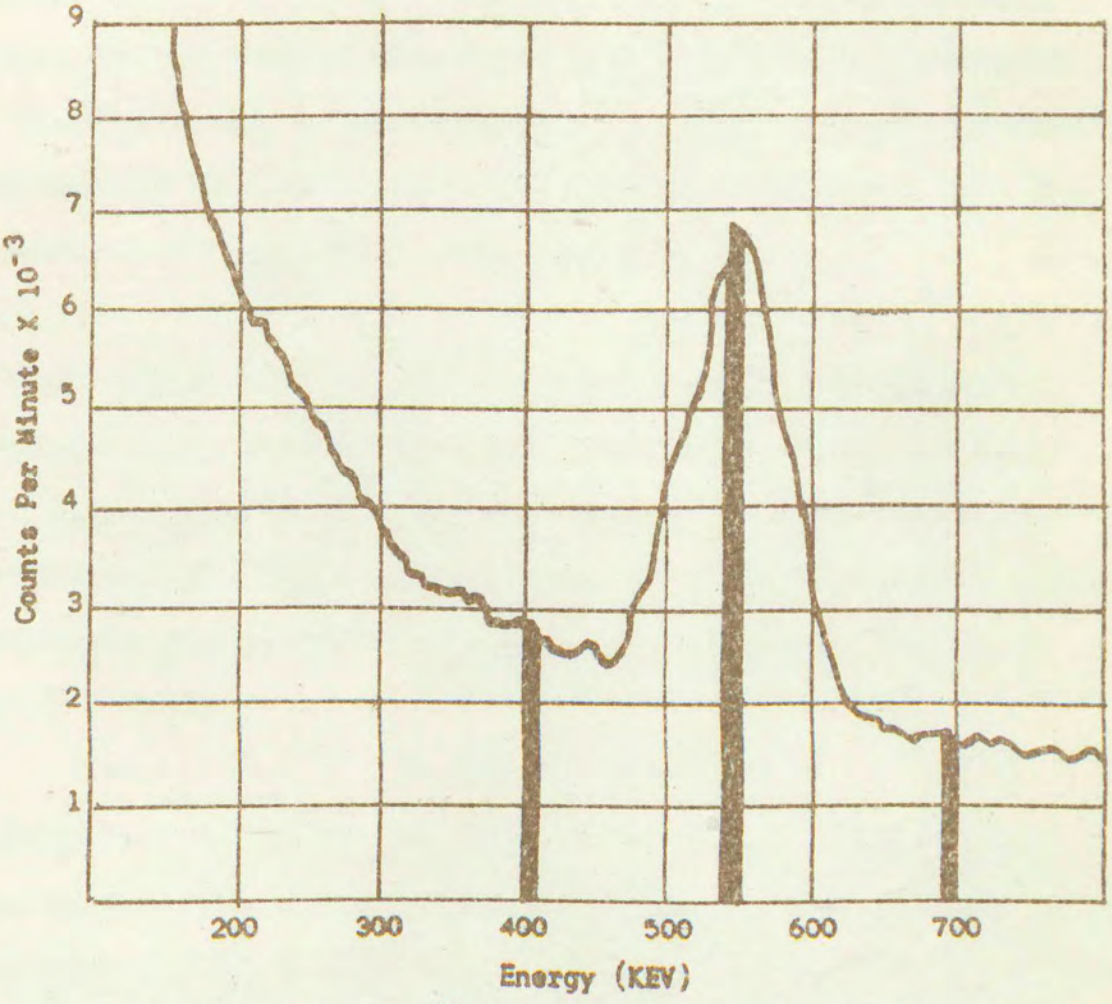

Fig. 11. Isolated As^{76} activity from sample to which 10µg of As as As_2O_5 had been added.

line of 0.540 MEV and a channel width of 0.020 MEV, a portion of the activity would be background. To correct the activities, two additional counts were made on each sample equidstant from the peak, at 0.400 MEV and 0.680 MEV with 0.020 MEV channel widths. The average of these two counts was then subtracted from the count made at 0.540-0.560 MEV. All counts were made at two differenttimes and half-lives were determined to rule out interference from positron annihilation.

The results obtained in the third run showed very poor precision. The precipitates were dried and weighed and the activities corrected to a common weight. This improved the precision and favorable results were obtained. However, weighing the precipitate introduced an additional step which we wished to avoid.

Before a fourth run could be made, a more reliable precipitation technique had to be developed. A more thorough study of the precipitation procedure indicated that the use of sodium sulfide for the precipitation of arsenic did not always give complete precipitation. Therefore, hydrogen sulfide gas was substituted for the sodium sulfide.

The digestion and precipitation techniques were applied to ten, 100 mg. replicates of unirradiated fish to determine the precision and accuracy. Recoveries were based upon the 20 mgs. of carrier arsenic added prior to digestion. The results of this study are shown in Table I. A standard deviation of 0.73 mg. or 2.2 per cent was obtained. To determine the accuracy of the procedure, certain assumptions had to be made. First, it had to be assumed that the precipitate was all As_2S_3. Secondly, it had to be assumed that elemental sulfur was absent and that the precipitate was pure. If these assumptions were correct, an average recovery for the analysis of the ten replicates of 101.5 per cent was obtained. However, if they were not correct, the method would still be valid. Since a comparative technique for determining concentrations is used, good reproducibility is all that is necessary for obtaining accurate results.

In the fourth run, standards containing from 0-10.0 PPM of arsenic were prepared by adding the standard solution to 100 mg. portions of the dried fish and drying in an oven at 103°C.

Table 1

Arsenic Precipitation with H_2S

Arsenic Added mg/100mg	Precipitate(mg) Theoretical	Recovered	Per cent Recovery
20.0	32.6	33.9	103.9
20.0	32.6	33.4	102.4
20.0	32.6	33.8	103.6
20.0	32.6	32.8	100.6
20.0	32.6	34.0	104.2
20.0	32.6	32.9	100.9
20.0	32.6	32.2	98.7
20.0	32.6	32.3	99.0
20.0	32.6	34.0	104.2
20.0	32.6	32.0	98.1

Calculated as As_2S_3

Standard deviation = 0.73 mg. (2.2%)

Range = 1.9 mg. (5.8%)

Average Recovery = 33.1 mg. (101.5%)

Ten replicates containing 1.0 μg of arsenic / 100 mg. of fish were also prepared to determine the precision of the complete method. The results of this study are shown in Tables 2 and 3. The results indicate that the method has an average per cent recovery of 96.5 for concentrations ranging from 0.2 - 8.0 μg of arsenic / 100 mg. of dried sample. The analysis of the ten replicate samples yielded an average per cent recovery of 99.9 and a standard deviation of ±0.039 μg / 100 mg. of dried sample or ±3.9 per cent.

Figs. 12 and 13 show that a straight line relationship exists between induced activity and arsenic concentration. From these two graphs, it is apparent that the precision of the method is slightly poorer for the analysis of concentrations varying from 0.0 - 0.8 μg than those ranging from 1.0 - 8.0 μg of arsenic. However, even in the lower range the precision is sufficiently good for the purpose for which the method was intended.

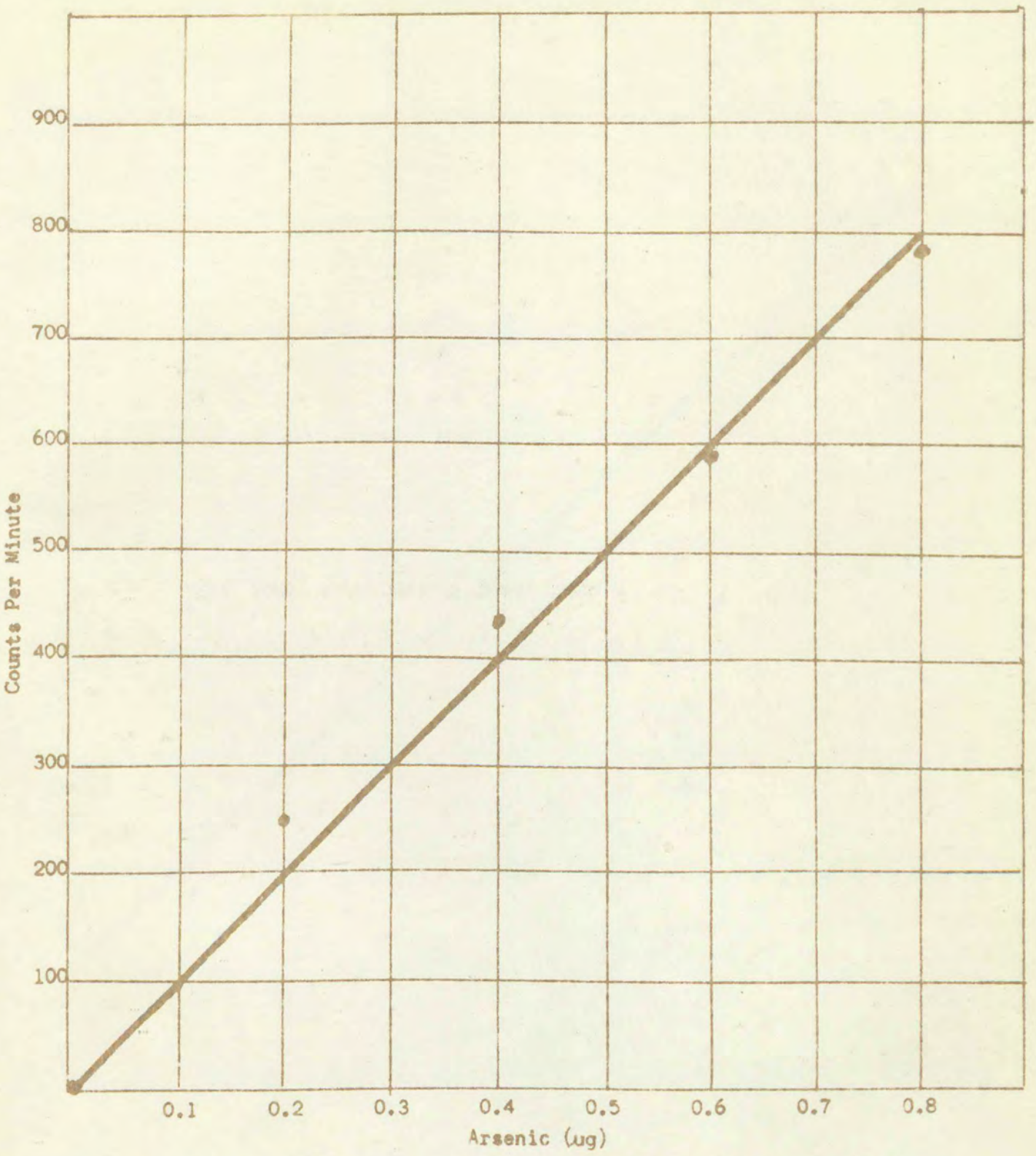

Fig. 12. Relationship between arsenic concentration and induced activity

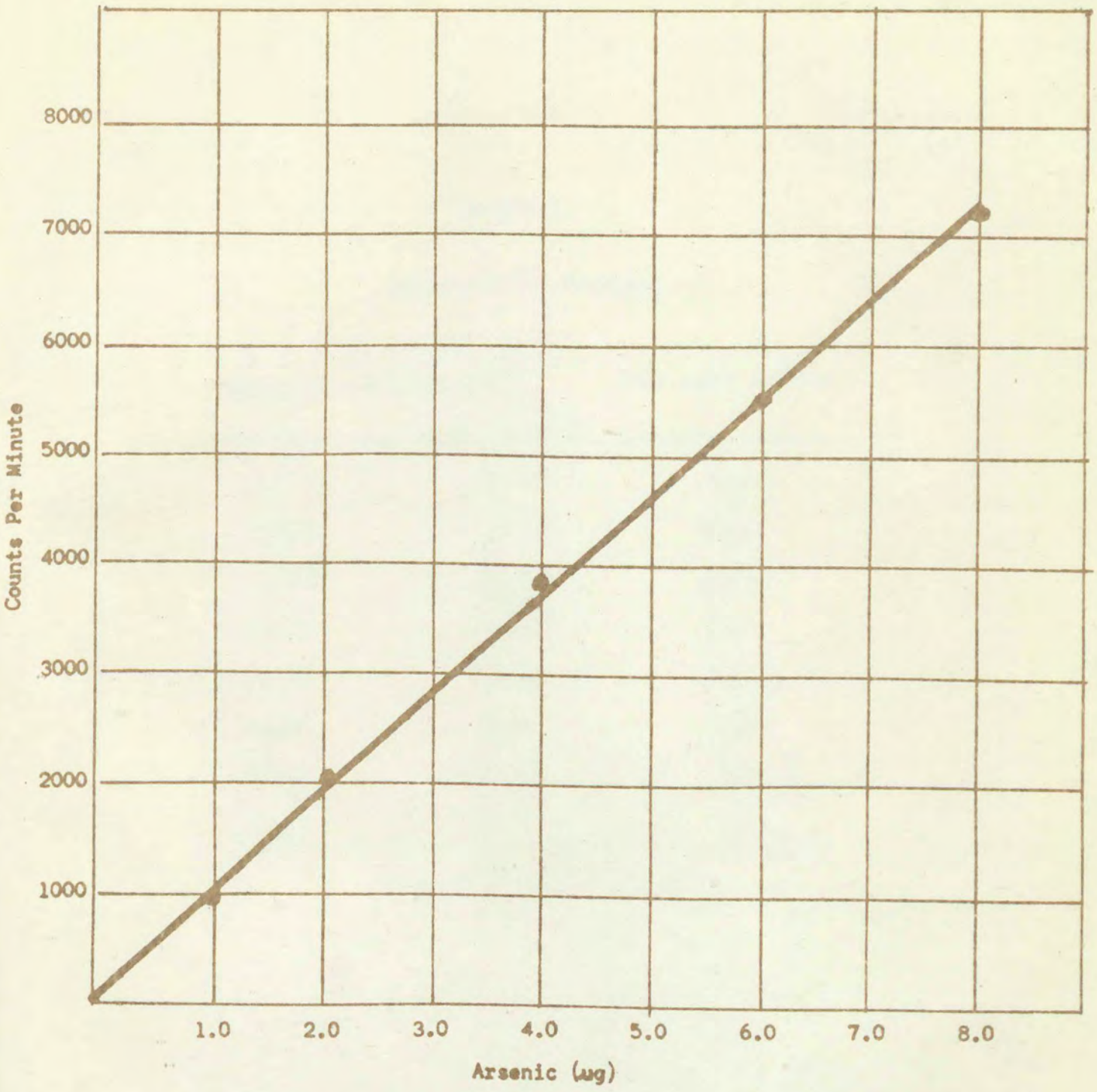

Fig. 13. Relationship between arsenic concentration and induced activity.

Table 2.

Recovery of Arsenic

Arsenic (µg/100 mg.)		Per cent Error
Added	Recovered	
0.200	0.208	4.3
0.400	0.423	5.7
0.600	0.580	3.4
0.800	0.791	1.2
2.00	2.16	8.0
4.00	4.08	2.0
6.00	6.20	3.3
8.00	8.00	0.0

Avg. Recovery = 96.5%

Table 3.

Precision Study

Arsenic (µg/100 mg.)		Per cent Error
Added	Recovered	
1.00	0.948	5.2
1.00	0.983	1.7
1.00	0.04	0.4
1.00	0.982	1.8
1.00	1.00	0.0
1.00	1.04	0.4
1.00	0.970	3.0
1.00	0.941	5.9
1.00	1.08	0.8
1.00	1.01	0.1

Standard Deviation. 0.039 µg (3.9%)

Range..... 0.092 µg (9.2%)

Average Recovery... 0.999 µg (99.9%)

Completed Method (18)

Preparation of samples:

Samples are cut into small pieces (about 6 cm^3) and dried on a steam bath. Desiccation is completed by overnight drying in an oven at 103°C. The dry material is reduced to a powder in a blender.

Irradiation:

One hundred milligram samples are weighed on an analytical balance and placed in $\frac{1}{4}$ x $1\frac{1}{2}$ inch aluminum foil tubes which are sealed at each end. The samples are irradiated in a neutron source for two hours at a flux of about 10^{12} neutrons $cm.^{-2}$ $sec.^{-1}$. Flux strength is determined by foil irradiation.

Digestion:

One end of the aluminum foil tube is cut off. The irradiated sample is placed in a 15 x 125 mm Pyrex test tube with a few glass beads and 20 mg. of carrier arsenic as sodium arsenite. One ml. of concentrated sulfuric acid and 0.1 ml of concentrated nitric acid are added and the mixture heated to boiling. As the solution darkens, nitric acid is added a drop at a time until the solution clarifies.

Precipitation:

Two ml. of concentrated hydrochloric acid are added to the

digested sample and the arsenic precipitated with hydrogen sulfide. The tubes are centrifuged for one minute. A few drops of 5 per cent sodium lauryl sulfate are added to decrease the surface tension and permit settling of the floating precipitate. Addition of the sodium lauryl sulfate before the first centrifugation causes cloudiness. The solution is centrifuged a second time for one minute and the sides of the tube scrubbed with a policeman. The tubes are then centrifuged for two minutes and the supernatant decanted. The precipitate is washed with 5 ml. of 9N hydrochloric acid and with 5 ml. of distilled water. The tubes are centrifuged for two minutes after each washing and the supernatant decanted.

Counting:

Counts are made using a gamma-spectrometer with a well-type scintillation detector. The tubes in which the precipitation is accomplished are placed directly in the well of the detector for counting. Counts are made at 400, 540 and 680 KEV with 20 KEV channel widths. The average of the counts made at 400 and 680 KEV are subtracted from the count made at 540 KEV to eliminate the effect of background.

Calculations:

$$\mu g \text{ of arsenic in unknown} = \frac{(\text{c/m of unknown})(\mu g \text{ arsenic in std})}{(\text{c/m of std.})}$$

Application (19)

Sodium arsenite is one of the most effective herbicides for submerged plants (20). Because of its toxicity, its use in potable water has been restricted. In 1956, weed control by sodium arsenite was started in Chautauqua and Findley Lakes in New York State. Since then, the herbicide has been applied annually to portions of both lakes. The depletion of residual arsenic and the arsenic concentrations in weeds and bottom deposits were studied (21 a, b, c).

An investigation was made to determine the degree of arsenic accumlation in fish from the two lakes. The method described above, utilizing neutron activation analysis was employed. Fish from a third lake, Cassadaga Lake, which had never received an application of the herbicide and which had a low natural arsenic content (0.10 PPM), were used as controls. The study was restricted to Calico Bass, taken from New York State Conservation Department muskellunge traps.

Five fish from Findley and ten each from Chautauqua and Cassadaga Lakes were collected in May 1959 prior to spraying. Twenty-one days after the herbicide was applied to Findley Lake and eleven days after it was applied to Chautauqua Lake, three fish from Findley Lake and ten from Chautauqua Lake

were collected. Although analysis of more fish from Findley Lake would have been desirable, the collectors were unable to obtain them.

Arsenic concentrations in the fillets including skin and in the viscera including bone and scale were determined. Table 4 summarizes the results. Detectable quantities of arsenic were found in the fillets of all fish from Findley Lake, ranging from 0.27 to 0.47 μg/gram of fish. Arsenic was detected in the viscera of seven of these fish. No significant differences in arsenic concentration occured between fish taken before or after spraying. This was also true of fish from Chautauqua Lake.

In none of the fish examined was an amount of arsenic found equal to that present in edible salt water fish (17). The persistance of residual arsenic in Findley Lake is most likely responsible for the arsenic in the fish. Failure to detect differences in arsenic concentrations between fish taken before and after application of the herbicide illustrates that longer contact periods are necessary for a significant accumulation of arsenic in the fish.

Table 4.

Arsenic Concentrations in Calico Bass

From Three New York State Lakes

Lake	No. fish examined	Date caught	Type sample	μg Arsenic per gram wet wt. Minimum	Average	Maximum
Findley	5	5/29/59	Viscera	0.10	0.33	0.78
Findley	5	5/29/59	Fillet	0.27	0.38	0.47
Findley	3	6/19/59	Viscera	0.14	0.56	1.0
Findley	3	6/19/59	Fillet	0.22	0.28	0.31
Chautauqua	10	5/29/59	Viscera	0.10	0.10	0.29
Chautauqua	10	5/29/59	Fillet	0.10	0.10	0.28
Chautauqua	10	6/19/59	Viscera	0.10	0.10	0.14
Chautauqua	10	6/19/59	Fillet	0.10	0.10	0.15
Cassadaga	10	5/29/59	Viscera	0.10	0.10	0.12
Cassadaga	10	5/29/59	Fillet	0.10	0.10	0.14

Summary

A method was needed for the determination of microgram quantities of arsenic in fish. The methods now used are inaccurate when applied to samples containing large quantities of organic matter since arsenic may be volatized during the digestion of the organic matter.

Neutron activation analysis has been used for the estimation of arsenic concentrations. These methods utilize either a Gutzeit separation, a pentabromate distillation or distillation of the arsenic from hydrochloric acid in the presence of free chlorine. Each of these methods require considerable time and handling of the samples. Fish contains high concentrations of sodium and phosphorous. Activation produces high activities due to P^{32} and Na^{24}. Therefore, the chemical separation should be rapid and require a minimum amount of handling

The method finally selected utilizes a precipitation of the arsenic activity from the digested sample after the addition of carrier arsenic. The organic matter in the dried fish samples is destroyed by sulfuric acid-nitric acid digestion. Although it was previously stated that digestion of the organic matter frequently caused loses of arsenic, the use of

small samples, made possible by the high sensitivity of activation analysis, minimizes these loses. The arsenic is precipitated by passing hydrogen sulfide through the solution. The precipitate is separated by centrifugation.

The method yields a per cent recovery of 99.9 with a standard deviation of 3.9 per cent for the analysis of standards containing 1 μg of arsenic/ 100 mg. of dried fish. Three chemists analyzed 96 samples using this method in 15 hours.

The minimum value recorded in this study was 0.1 μg per gram of sample. By increasing the time which the samples remained in the flux, the sensitivity could be increased up to 6 fold. This would permit the use of smaller samples or the measurement of smaller quantities of arsenic. The method could easily be adapted to the analysis of arsenic in other types of samples.

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