The Determination of Tungsten in Biological Material by Activation Analysis

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(Received 21 March 1960)

Tungsten exists at such low concentrations in normal biological material that it has been detected only in tissues from animals previously fed with unusually large quantities of tungsten (Kinard & Van de Erve, 1941), or plants growing in tungsten-rich soils (J. P. R. Polkinhorne, personal communication). In view of the discovery that the related element molybdenum is essential for plants and animals and is a constituent of at least two enzyme systems, it would seem that further measurements of tungsten might yield equally interesting results. The present note describes a technique capable of detecting as little as 1 μ mg. of tungsten.

The method used is that of activation analysis, which is now established as an extremely sensitive technique for determining trace elements in tissue (Loveridge & Smales, 1957; Bowen & Cawse, 1959). A number of workers have studied the distribution of tungsten in samples of geochemical and metallurgical interest by this technique (e.g. Atkins & Smales, 1959; Leliart, Hoste & Eeckhaut, 1959), and a recent paper describes the determination of the element in various marine organisms (Fukai & Meinke, 1959). Tungsten has a relatively high cross-section for thermal neutrons (much higher than that of molybdenum) and, among the radioactive isotopes produced, 24-hr. ^{187}W is much the most abundant. In this work chemical methods were used to isolate the ^{187}W activity from the other radioelements occurring in the activated material. Contamination before activation was minimized as described in the next section, and subsequent contamination would not affect the analysis.

EXPERIMENTAL

Tomato plants were grown in sand culture and seeds collected as described by Bowen & Cawse (1959). Flax plants were grown in soil in a glasshouse. The top parts of these plants were air-dried and ground in a specially made Perspex rotary mill. This mill consisted of a Perspex propeller rotating at 3000 rev./min. in a hollow cylindrical chamber 12.5 cm. in diameter. Nuts were commercial samples of unknown history. About 1 g. of dry tissue, which was handled with plastic apparatus only, was used in each analysis. Blood samples were collected from healthy donors at a collection centre; 1–2 ml. was allowed to run

into weighed polythene ampoules, which had been cleaned with aqua regia. This blood had been in contact with a stainless-steel hypodermic needle and a short length of rubber tubing, so cannot be considered completely free from contamination. The blood samples were weighed, dried under cover at 80° and sealed in their containers. Tissue samples were sealed in small polythene bags and activated together with a standard consisting of 100 μ g. of tungsten absorbed on a single dry tomato seed ('ashless' filter paper was later found to be equally suitable as an absorbent). The standard was delivered from a micrometer syringe attached to a polythene pipette. Standard solutions were made by careful dilution of a stock solution of spectroscopically pure tungsten trioxide in distilled ammonia. Activation was carried out in the Harwell reactor BEPO in a flux of about 10^{12} thermal neutrons/cm.²/sec. for a 24 hr. period.

Chemical separation of tungsten. The separation scheme evolved is original but owes much to the work of Prestwood (1958). Two of the major activities generated in the tissues, ^{24}Na and ^{42}K , were easily removed by simple chemical processes, but a third, ^{32}P , generally co-precipitated with tungsten and proved much more difficult to eliminate. The largest activities generated in trace elements were calculated to be 12 hr. ^{64}Cu and 2.6 hr. ^{56}Mn (Bowen, 1956).

The activated sample or standard was first partially wet-ashed by boiling with 5–10 ml. of 16N-nitric acid containing 20 mg. of tungsten as sodium tungstate in a 50 ml. beaker. No hold-back carriers were employed. After wet-ashing for a few minutes, the contents of the beaker were washed into a 50 ml. centrifuge tube and yellow tungsten trioxide was spun down. The supernatant liquid was poured off, the tungsten trioxide was dissolved in 2 ml. of 15N-ammonia and reprecipitated by boiling with 10 ml. of 16N-nitric acid. This cycle was repeated and scavenging precipitations of ferric hydroxide and magnesium ammonium phosphate were carried out at pH 8 while the tungsten was in solution. Sufficient 50% ammonium acetate was added to buffer the solution, and 1 drop of 10% ferric nitrate, 2 drops of N-ammonium dihydrogen phosphate and 4 drops of N-magnesium chloride were added successively before centrifuging. The supernatant liquid was poured into a 50 ml. separating funnel containing 10 ml. of chloroform, 4 ml. of 5% cupferron and 1 drop of 10% (w/v) copper sulphate pentahydrate. After acidifying with 50% tartaric acid, the funnel was shaken and the chloroform layer was run off. A second shaking with fresh chloroform was used to remove elements such as copper, which form cupferrates soluble in this solvent. The remaining aqueous layer was poured into a fresh 50 ml. centrifuge tube and about 10 ml. of 24N-nitric acid added carefully till the tartaric acid was oxidized

and the tungsten precipitated as the trioxide. This precipitate was spun down, dissolved in 2 ml. of 15N-ammonia and a further scavenging precipitation made with magnesium ammonium phosphate in the presence of ammonium acetate. The clear supernatant from this step was treated with 2 ml. of 2% α -benzoin-oxime and made 6N with respect to nitric acid. It was necessary to carry out this cycle of scavenging and α -benzoin-oxime precipitation two or three times to achieve satisfactory decontamination from ^{32}P . The final precipitate was washed three times with water, slurried on to an aluminium counting tray with acetone, dried and heated in a muffle furnace for 30 min. at 540° to convert the oxime into tungsten trioxide, which was weighed to determine the chemical yield. The average chemical yield was about 50%: the count relative to the standard gave the amount of tungsten in the sample. ^{187}W emits a β ray of 1.31 Mev energy, and was counted with an end-window Geiger counter surrounded by an anticoincidence shield to reduce the background to 1.5 counts/min. No self-absorption correction was found to be necessary. The whole chemical procedure took about 3 hr. for eight samples.

Counting was carried out at 24 hr. intervals to establish the radiochemical purity of the separated ^{187}W . In all cases where residual activity was found after 10 days it was shown to be caused by 14-day ^{32}P .

Losses in the chemical steps were measured radiochemically for four replicates, in order to improve the efficiency of separation and the overall chemical yield. In addition to the steps described above, the following were tested: (a) scavenging ammonium tungstate at pH 8 by precipitating copper sulphide, (b) extracting tungsten α -benzoinoxime into chloroform and (c) re-extracting the latter complex into N-sodium hydroxide; these were not used in the actual analysis, but are included in Table 1 for

comparison. The extraction of tungsten α -benzoinoxime into chloroform would have been a useful decontamination step, had it not been so inefficient with the quantities of tungsten carrier used. The sulphide-precipitation step might be necessary for samples containing large amounts of copper.

RESULTS

The recovery of tungsten added to seeds or filter paper in amounts less than 1 μg . was reasonably good. In Fig. 1 the weights of tungsten taken are plotted against the counts/min. obtained in the final precipitate, corrected for chemical yield, and it can be seen that the curve is linear. Since 0.1 μg . of tungsten yielded about 3000 counts/min. and the background of the counter was 1.5 counts/min.,

Fig. 1. Recovery of added tungsten from tomato seeds.

Table 1. Losses of tungsten during chemical separation after activation

The steps are described in detail in the text.

Chemical step	Loss (%)	No. of times used per analysis
WO_3 ppt. from 12N- HNO_3	1.5	4
Tungsten α -benzoinoxime ppt. from 6N- HNO_3	5.6	2
CHCl_3 extraction of tungsten cupferrate	3.0	1
CHCl_3 extraction of tungsten α -benzoinoxime	16.4	0
Re-extraction of tungsten α -benzoinoxime into N-NaOH	3.4	0
$\text{Fe}(\text{OH})_3$ scavenging ppt. at pH 8	3.5	1
MgNH_4PO_4 scavenging ppt. at pH 8	3.4	3
CuS scavenging ppt. at pH 8	3.3	0

Table 2. Tungsten in plant tissues

Species	Tissue	Origin	Tungsten found ($\mu\text{mg./g. dry wt.}$)
<i>Arachis hypogaea</i> (pea nut)	Seed	Commercial	2.6, 9.0
<i>Bertholletia excelsa</i> (Brazil nut)	Seed	Commercial	7.3, 16.7
<i>Castanea sativa</i> (chestnut)	Seed	Commercial	1.6, 3.1
<i>Corylus avellana</i> (hazel nut)	Seed	Commercial	0.45, 0.46
<i>Juglans regia</i> (walnut)	Seed	Commercial	7.3, 11.0
<i>Lycopersicon esculentum</i> (tomato)	Seed	Commercial*	150
<i>L. esculentum</i> (tomato)	Seed	Sand culture + Mo	140
<i>L. esculentum</i> (tomato)	Seed	Sand culture - Mo	23
<i>L. esculentum</i> (tomato)	Top parts	Sand culture	52, 58
<i>Linum usitatissimum</i> (flax)	Top parts	Greenhouse culture	71, 84, 92

* Suttons, Reading.

the amount of tungsten needed to double the background was $50\text{ }\mu\text{g}$. This is not the ultimate limit of detection, since neutron fluxes much larger than those used in this work are now available, which would extend the sensitivity still further.

The amounts of tungsten found in the plant tissues studied are shown in Table 2.

Nuts were chosen for analysis because of their relative freedom from surface contamination. The variation found in the commercial samples is not surprising. Only the last two entries show the reproducibility of the method, as here samples were taken from homogeneous powdered tissue: the errors to be expected are of the order of $\pm 10\%$. The molybdenum contents of the tomato seeds grown in sand culture with and without added molybdenum were known to be 1900 and $210\text{ }\mu\text{mg./g.}$ respectively (Bowen, 1959).

Tungsten was also determined in human blood samples from two adult males and seven females: the results were: males, $0.75\text{--}1.20\text{ }\mu\text{mg.}$ of tungsten/g.; females, $0.46\text{--}2.34\text{ }\mu\text{mg.}$ of tungsten/g. The mean value of all the samples was $1.09 \pm 0.51\text{ }\mu\text{mg.}$ of tungsten/g., or $0.103\text{ }\mu\text{g.}$ of tungsten/100 ml. Previous work indicates that the molybdenum content of mammalian blood is about $16\text{ }\mu\text{mg./g.}$ (Bowen, 1959).

DISCUSSION

Atkins & Smales (1959) have discussed the errors involved in activation analysis for tungsten and have concluded that spurious results might be obtained if large amounts of rhenium or osmium were present relative to tungsten. Unfortunately the content of these elements in plant tissue and blood has never been measured, but it is unlikely to have affected the present results. There are no tungsten isotopes among the fission products, so fission of any uranium in the samples during activation would not interfere.

There seems little doubt that the method described here is very much more sensitive and more selective than other techniques which have been used to determine tungsten in biological material. Colorimetric techniques (Aull & Kinard, 1940; Ward, 1951; Allen & Hamilton, 1952; Jeffery, 1953) cannot be used to detect less than $1\text{ }\mu\text{g.}$ of tungsten, and are more or less subject to interference by molybdenum. The first restriction means that the techniques cannot be used to determine the amounts of tungsten occurring in normal biological tissue, and the second is equally serious since it seems likely that molybdenum is 10–100 times more abundant than tungsten in both biological and geological material (see below). Interference from molybdenum has not been troublesome in this type of analysis, as it has a much lower

cross-section for thermal neutrons than tungsten, and it is also effectively removed by the chemical procedure.

There are virtually no figures in the literature with which to compare the present analyses. Recently, Fukai & Meinke (1959) have found $0.13\text{--}0.18\text{ }\mu\text{g./g.}$ of ash in a marine alga by activation, which corresponds to roughly $15\text{ }\mu\text{mg./g.}$ dry wt. Our results are of the same order of magnitude. The figures for blood, though small, vary only by a factor of 5. This constancy does not necessarily mean that tungsten is an essential element, since the results may simply reflect the solubility of stainless-steel hypodermic needles in blood, or indicate the tolerable level of a trace contaminant which the kidneys are not able to remove. Normal steels contain about 0.2% of tungsten (Atkins & Smales, 1959), and the solubility of the element in blood is not known. Hypodermic needles are slightly soluble in water, and it was shown by weighing on a microbalance that a needle lost $174\text{ }\mu\text{g.}$ after 12 l. of water had passed through it. This would correspond to a solubility of $0.03\text{ }\mu\text{mg.}$ of tungsten/ml. The limited data available support the hypothesis that the tungsten/molybdenum ratio in biological material may be largely determined by its environment: in the earth and in terrestrial organisms the ratio is approximately 1:10, whereas in the sea and in marine organisms it is nearer 1:100 (Kuroda & Sandell, 1954; Ishibashi, Shigematsu & Nakagawa, 1954; Atkins & Smales, 1959; Bowen, 1959; Fukai & Meinke, 1959). Molybdenum is known to be an essential element for living creatures, and many studies have shown it to be accumulated by them. The present results indicate that tungsten is accumulated to about the same extent, showing that it, too, may be essential. There are already a number of reports in the literature on the stimulating effects of tungsten on plant growth (Scharrer & Schropp, 1934; Young, 1935; Krisciunas, 1936; Arnon, 1938; Hoagland, 1941; Davies & Stockdill, 1956). Further studies of this problem, with controlled ratios of molybdenum to tungsten in the culture medium, would appear to be warranted. Tungsten does not become toxic until it equals or exceeds molybdenum in abundance (Higgins, Richert & Westerfield, 1956). The method of activation analysis has sufficient sensitivity to measure the element at concentrations well below the toxic level.

SUMMARY

1. A method is described for determining tungsten in biological material by neutron-activation analysis. The theoretical sensitivity is $50\text{ }\mu\text{g.}$ of tungsten, and in practice $1\text{ }\mu\text{mg.}$ can be determined with an accuracy of $\pm 10\%$.

2. Tungsten was determined in various plant

tissues and found to be present in amounts from 0.5 to 150 $\mu\text{mg./g. dry wt.}$

3. The metal was also determined in nine samples of human blood and found to be present in amounts from 0.5 to 2.3 $\mu\text{mg./g. of whole blood,}$ with a mean of 1.1 $\mu\text{mg./g. (0.10 } \mu\text{g./100 ml.)}$

4. The limited data on tungsten/molybdenum ratios indicate that tungsten, though much less common than molybdenum, is accumulated to an approximately equal extent from the environment by living organisms.

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Biochem. J. (1960) **77**, 82

The Phospholipids of Ox Spleen with Special Reference to the Fatty Acid and Fatty Aldehyde Compositions of the Lecithin and Kephalin Fractions

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(Received 2 March 1960)

The fatty acid compositions of lecithin and kephalin fractions from different animal tissues have not yet been studied in a systematic manner, though several excellent papers have been published by Klenk and his co-workers (Klenk & Bohm, 1951; Klenk, Debuch & Daun, 1953; Klenk & Krickau, 1957) on the whole glycerophosphatide fraction and some purified phospholipids from a number of animal tissues. From their results it is apparent that different tissues contain lecithin and kephalin fractions which differ widely in their fatty acid composition.

The plasmalogens differ from the classical ester phosphatides in having only one fatty acid esterified with the glycerol moiety instead of two.

Instead of the other fatty acid there is a carbon chain of similar length attached to the glycerol by an $\alpha\beta$ -unsaturated ether linkage. This linkage is easily ruptured by weak acid to give a long-chain fatty aldehyde and a lysophosphatide. It has been shown that in ox brain (Debuch, 1958) and ox heart (Debuch, 1958; Gray, 1958) the fatty acids of the choline and ethanolamine plasmalogens are mostly unsaturated. Is this a characteristic difference between the plasmalogens and ester phosphatides which is common to all tissues?

Much less is known of the fatty aldehyde composition of plasmalogens from different tissues. Stearaldehyde and palmitaldehyde are the main components of aldehyde mixtures isolated from ox-heart muscle (Klenk, Stoffel & Eggers, 1952; Gray, 1958) and 50% of the aldehydes isolated from

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