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A Simplified Rapid Determination of Manganese in Biological Specimens by Neutron Activation Analysis

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The quantitative study of *trace* elements requires a method capable of high sensitivity and good accuracy. Neutron activation analysis (1) fulfills such criteria for a wide list of elements.

Neutron activation analysis procedures for the determination of manganese, involving lengthy, time consuming chemistry have been described (2, 3). However, the study of trace metals in biological research generally necessitates the analysis of many samples. It is clear that a rapid, simplified procedure for the determination of trace manganese in biological materials would be extremely useful.

The procedure to be described involves four basic steps:

1. Irradiation in a thermal neutron flux producing the reaction,

(1) $$^{55}Mn\ (n, \gamma)\ ^{56}Mn$$

2. Rapid simultaneous dissolution of a number of samples.
3. One step precipitation and separation of MnO_2
4. Quantitation of the ^{56}Mn gamma spectrum by means of two NaI (Tl) crystals and a pulse height analyzer (PHA).

MATERIALS AND APPARATUS

The following analytical grade reagents were used: concentrated nitric acid, 16 N, dilute nitric acid, 2 N, manganese carrier containing 50 mg Mn/ml H_2O, as chloride, 10% w/v H_2O solutions of Y $(NO_3)_3$, $Fe(NO_3)_3$, and $NH_4H_2PO_4$ and a 50% w/v H_2O solution of $NaClO_3$. The counting equipment is shown in Figure 1.

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PROCEDURE

Sample Preparation and Irradiation. The tissue samples were placed in preweighed, numbered polyethylene microcentrifuge tubes, dried overnight at 90° C and reweighed. For small samples, a milligram or less, the weight loss of the microcentrifuge tube itself which was from zero to 0.1 mg and occasionally 0.2 mg, required that overnight drying be done at the lower temperature of 80-85° C to minimize such weight losses. Use of thin walled quartz tubes eliminated this temperature restriction and was done in some actual analyses at a later time. Teeth were dried overnight at 100° C, stored in individual polyethylene containers in a desiccator until required and then prepared for analysis and weighed into microcentrifuge tubes. A set of capped tubes containing tissue samples were arranged for irradiation by placing them in a larger polyethylene container along with a set of comparable standards. The standards contained a comparable volume and concentration of manganese in water so as to present a physical arrangement similar to the unknown tissue. While perfect physical similarity is of course impossible, reasonable imitation eliminates uncertainties which could arise when physically

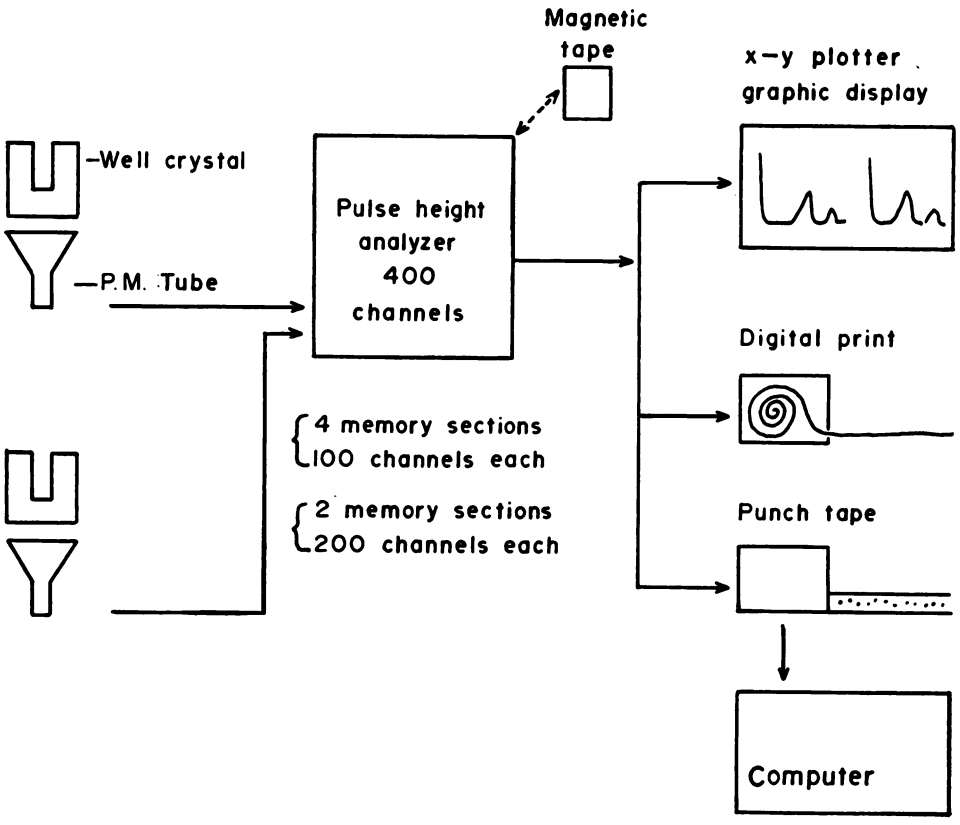

Fig. 1. Dual input, pulse height analysis system with choice of data readout.

differing samples are placed in a reactor with any given neutron flux distribution. Thus, the comparison of a dry powdered standard ($Mn\ Cl_2$ salt) with a tissue sample containing manganese in an organic matrix would not be valid (4).

After sufficient irradiation for a given set of samples, generally one half-life of 156 minutes,[1] in a thermal neutron flux of approximately 5×10^{11} n/cm^2 sec, the individual sample tubes were removed from the outer irradiation vessel.

Chemistry. The contents of the polyethylene microcentrifuge tubes were transferred quantitatively to a 50 ml Erlenmeyer flask. The samples were moistened with a few drops of water and 20 mg manganese carrier, 7.5 ml concentrated HNO_3 and three drops each of the other carrier solutions (Cu^{+3}, Y^{+3}, Fe^{+3}, PO_4^{-3}) mere added. The Erlenmeyer flask was placed on a sand bath and the solution boiled gently until the sample was dissolved. The resulting clear colorless or brown solution was transferred to a centrifuge tube, combined with an equal volume of water used to rinse the Erlenmeyer, and heated to boiling. One ml 50% $NaClO_3$ was added drop wise to each centrifuge tube which was then set aside until all the resulting MnO_2 precipitations were completed. After centrifugation the precipitate was washed with 2 ml dilute nitric acid, then with 2 ml water and the washes were discarded. The sample was transferred to another preweighed polyethylene microcentrifuge tube for counting according to procedures given in C. After counting, the samples were dried overnight and weighed to establish the chemical yield. The chemical yield generally was about 60-75 per cent. Most of the loss occurred in transferring the MnO_2 precipitate to the small polyethylene tubes for counting and eventual drying and weighing. Had intensity or sample size ever been a problem, more painstaking transfer could have given a yield approximating 100 per cent.

Counting. The standards were taken to the counting room as soon as the outer irradiation vessel had been opened and the individual micro tubes were available. The standards and samples were counted in a two crystal input to a 400 Channel PHA with each crystal signal being stored in half of the memory (200 channels), Figure 1. The duplicate standards were counted alternately in each crystal to afford internal checks on the standards and to evaluate differences in overall crystal and amplifier sensitivity and behavior for the conditions of the particular day's experiment. In addition the use of several standards minimized uncertainties from the positioning of standards and unknowns in the reactor.

A typical plot of the resulting count (Fig. 2) shows the counts observed as a function of energy to be relatively free from interferences. The ^{56}Mn peak is at 0.84 MeV for both the standard and the unknown. The data readout from the PHA memory is recorded graphically by an x,y plotter. The same data could be handled by a computer using punched tape or direct transmission readout. In our

[1]Because of the high sensitivity of this procedure it is entirely feasible to use a much shorter irradiation period, longer counting times and smaller sample sizes. All these options, though readily available, do not change the essential procedure. The possible contribution from ^{56}Fe (np) ^{56}Mn was considered and found to be negligible under the conditions; had the Fe content of samples been relatively high compared to the Mn a correction for this would have had to be determined for each case.

work the heights of the peaks were determined manually by use of an ordinary ruler with millimeter scale. The display value, full scale value, time and duration of the counts were noted. Since each unknown was generally counted at the same time as a standard, no decay correction was needed. A crystal correction determined each day by intercomparison of standards in the two crystals was always used.

Calculation of the manganese content of the unknown was done by use of a simple equation:

$$(2)\qquad \frac{\text{ug in}}{\text{unknown}} = \frac{\text{ug in}}{\text{standard}} \times \frac{\text{peak height unknown}}{\text{peak height standard}} \times \frac{\text{crystal correction}}{\text{chemical yield fraction}}$$

The chemistry used proved reproducible and selective enough for manganese[1] quantitation on the pulse height analyzer by simple peak height measurement. Each new material analyzed had its 0.84 MeV peak followed for a period of time

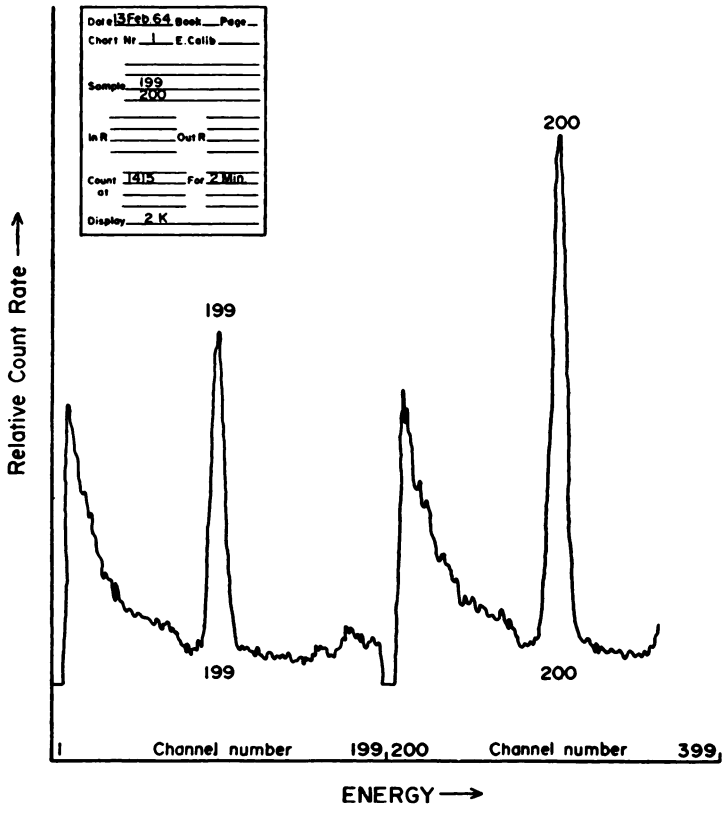

Fig. 2. Spectrum showing *standard* and *unknown* manganese peaks.

[1]More detailed spectrum analysis was unnecessary because of the relatively uncomplicated spectrum obtained.

to establish by its decay period its nature as manganese-56. This means that a chemical separation, an energy determination, and a half-life determination have contributed to the conclusion that manganese is in fact being measured. After this was done for each new material investigated, the height of the 0.84 MeV peak was sufficient for routine quantitation of manganese.

RESULTS

Test analyses were carried out on two kinds of standard materials.

Soft Biological Materials. Analyses were carried out on soft materials consisting of pooled homogenized dried rat liver, pooled homogenized beef livers and certain other samples, as a function of age of the rat. These analyses were paralleled by separate analyses on aliquots of the same materials carried out by means of atomic absorption.[1] A comparison of the values obtained by the different methods is shown in Table I. Agreement is seen to be good. The accuracy is presumed acceptable since two different methods carried out at two different labor-

TABLE I

COMPARISON OF RESULTS ON POOLED RAT LIVER AND OTHER LIVER SAMPLES

Sample	*Activation Analysis*	*Atomic Absorption**
362**	7.68 ± .75(6)	8.94
363**	7.98 ± .60(6)	8.27
373	5.76	7.8
374	5.60	8.4
375	11.1	8.75
3.76	9.1	6.66
377	2.64	3.2
378	2.85	
379	6.20	7.4
380	5.94	6.8
381	9.65	8.5
382	9.30	8.3
383**	8.31 ± .02(2)	7.3
384**	7.48 ± .08(2)	7.4

*Atomic absorption data by Gerry Powell, M.D. and Donald B. Cheeck, M.D., Johns Hopkins Hospital. Samples weighed 500-1000 mg.

**Pooled liver samples; mean values and average deviations from mean in a set (6) of samples. All results are ug/g dry weight; samples were approximately 5-25 mg.

[1]We are indebted to Dr. D. B. Cheek and Dr. Geraldine Powell, of the Johns Hopkins Hospital, Baltimore, Maryland, for supplying the standard materials along with the results of their analyses.

TABLE II

PRECISION OF ANALYSIS FOR MN IN DENTIN AND ENAMEL OF CERTAIN HUMAN TEETH

Sample	*Enamel ug/g*	*Dentin ug/g*	*Ratio*
559	.289		2.70
560		.107	
563	.581		3.54
564		.164	
565	.284		1.63
566		.174	
567	.723		2.44
568		.296	
569	.336		2.32
570		.145	
571	.622		3.07
572		.202	
573	.785		2.69
574		.292	
675	.076		
676	.051		2.17
677		.037	
678		.021	
679	.145		
680	.110		2.56
681		.042	
682		.058	
683	.454		
684	.533		3.43
685		.172	
686		.117	
687	.445		
688	.458		2.39
689		.189	
690		.188	

Paired and grouped sample numbers, *i.e.*, 559, 560 and 675, 676, 677, and 678 represent enamel and dentin samples from one tooth.

atories gave similar results. It is believed this represents a better procedural test than the *recovery* of added manganese (5).

Hard Biological Materials. Some analyses were carried out on normal caries-free human teeth in order to evaluate the importance of sample preparation for hard biological where separation of the desired sample was more difficult. In Table II, columns 2 and 3, are shown the results of analyses of dentin and enamel from single teeth. The duplicate results seem to be in good agreement. These results also have an internal consistency. For a given tooth, the ratio of enamel manganese content to dentin manganese content is approximately 2.5.

These results are in good agreement with the results of one group (6). But these results are in very substantial disagreement with the higher enamel values reported by other workers on the basis of emission spectroscopy (7). It was felt that this disagreement might be attributed, at least in part, to their method of separating enamel from dentin, grinding and cutting. Therefore, tests were run on the analytical preparatory procedures of grinding and cutting versus our own procedure of chipping and crushing with polyethylene covered instruments. Parallel enamel samples in each case from the same tooth were prepared for analysis by cutting with a tungsten carbide bur or by cutting with a small diamond dust impregnated bur, as opposed to the standard polyethylene protected chipping and crushing procedure. The results of the two kinds of handling procedures when the samples were subjected to the same manganese analysis are shown in Table III, columns 2 and 3. The manganese content of the samples de-

TABLE III

MANGANESE CONTENT OF ENAMEL SEPARATED FROM DENTIN BY GRINDING OR CHIPPING PROCEDURES

Tooth No.	*ug Mn/g Found* *Method of Separation* *Tungsten Carbide Bur*	*Polyethylene Protected Chipping*	*Per Cent Increase of Mn in Ground Enamel Over Chipped Enamel*
10.6	0.98	0.72	27
10.9	1.05	0.43	145
10.11	1.05	0.71	45
10.14	0.65	0.46	40
16.24	1.44	0.90	60
16.25	1.38	0.61	128
	Diamond Stone		
15.19	1.35	1.11	22
15.20	1.11	0.84	32
16.22	1.13	0.72	58
16.23	0.88	0.73	21
16.26	0.94	0.62	51

scribed in the upper sections of columns 2 and 3 of Table III show a difference which is significant at less than the four per cent level[1], as compared to the similarities in columns 2 and 3 of Table II. Manganese is not a major constituent of the tungsten carbide bur used in this test. Tungsten, which is a major constituent of the bur, shows up strongly through the chemical procedure, though not at the same energy as the manganese. The tungsten peaks appear at 0.48 and 0.68 MeV and in the spectrometric measurements used did not interfere with the manganese measurement.

The other prehandling procedural test in which the cutting of the tooth was by means of a diamond dust impregnated bur gave, once again, results higher in manganese than samples of the same tooth which were crushed in a polyethylene sheet with no further handling. The sample dust prepared by grinding was collected by gravity on a clean glass surface within a totally inclosed volume consisting of a small beaker with a diaphragm through which the grinding head was introduced. In the work cited (7) the dust collection was by an air suctioning device which collected in the dust in a stream of air.

CONCLUSIONS

The procedure described for activation analysis of trace manganese in biological material is quite simple and rapid. It is capable of good precision and accuracy as determined by comparison to the atomic absorption procedure done on relatively large samples at another laboratory. Thirty analyses could be run in parallel by one technician and all measurements completed in less than one full working day.

When applied to teeth, the accuracy of the method probably suffers from inability to make a standard of a comparable physical arrangement. However, good agreement with results reported by one group of workers was obtained and a reasonable explanation presented for results substantially lower than some other reported data.

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[1]We are indebted to Major Miriam K. Ginsberg, ANC, for these statistical analyses.

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