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HUMAN TISSUES

TO: Dr. K. Z. Morgan

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Methods of Collection, Preparation, and Spectrographic Analysis of
Human Tissues

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Introduction

To determine the maximum permissible concentration (MPC) of a radionuclide which may be ingested or inhaled¹ without exceeding accepted² levels of exposure to the human body, it is necessary to know the average normal concentration of the natural element in the various tissues of the body.

Any element may be ingested or inhaled as the element or as a compound. Although nearly all elements have been observed in living tissue, few quantitative observations had been made before 1950.^{3,4,5,6,7,8}

Because so little information on the average normal concentrations and the distribution of elements in the human body has been available, the Health Physics Division established a subcontract with the Department of Physics at the University of Tennessee to analyze human tissue for the trace elements. Spectrochemical methods were chosen because of their convenience, specificity, and sensitivity and because, by these methods, analyses of small samples could be carried out for a large number of elements simultaneously.

Preliminary studies^{9, 10} showed that individual variations in concentration of all elements were very wide and that geographical variations for certain elements occurred. This indicated that a large number of samples should be analyzed to reduce the standard deviation of the average and that these samples should be collected from different locations to take into

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consideration the geographical variations. Since the quantity desired for the calculation of MPC is the average 'normal' concentration of an element in a tissue, the sample should be from 'normal' tissue--that is, tissues from autopsies of instantaneous death by accident.

With these factors in mind, i.e., number, geographical distribution, and condition of samples, a program for the collection of at least 25 autopsies of adult victims of instantaneous accidental death from each of 15 cities in different regions of the United States was undertaken by the Health Physics Division. The foundations of the program were laid when a prominent pathologist wrote a number of letters to his colleagues in the cities that had been selected, seeking their cooperation and interest. The favorable response to his letters facilitated the collecting of tissues from 9 cities as of February 1957. Results of analyses for three cities have been reported.^{11,12,13}

Procedures. I. Tissue Collection:

After introductions are made and invitations are received from a pathologist in a city, a representative is sent to the city and remains there approximately two weeks. During this time all autopsies suitable for this study are attended and the prosectors are given information as to type and amount of tissue desired. For the larger organs, such as liver, a minimum of 100 grams is taken and for the smaller organs, such as adrenal, the entire organ is taken. The tissues are placed immediately in polyethylene bags, labeled with autopsy number and name of tissue, and frozen.

Figure 1 is a reproduction of the information sheet which accompanies every autopsy. For shipping, the samples are packed in dry ice and sent via air to Oak Ridge where they are stored frozen until processed. (No shipment has required more than 17 hours in transit.)

Procedures. II. Ashing:

Because the amounts of most elements in human tissue are extremely small, some method of concentration must be employed. In this program, dry ashing in a muffle furnace lined with fused silica is the method which has proved most satisfactory, although wet ashing and precipitation methods have been tried.^{14,15}

Contamination is a major problem in all trace element work. Precautions are taken in every phase of the program to keep it to a minimum. In the ashing laboratory the air is brought in through high efficiency filters (CWS-6) composed of plant fibers and asbestos. Except for the stainless steel scalpels which are used in collecting and trimming the samples, nothing but fused silica, polyethylene and platinum touches the sample from collection to loading for arcing. All water used in the program is distilled, passed slowly through a mixed-base ion-exchange column and stored in polyethylene bottles. All silica dishes are washed, soaked for 24 hours in a cleaning solution (5 parts HNO_3 to one part H_2SO_4), rinsed in ion-exchanged water, soaked for 24 hours in a solution of versene, rinsed in ion-exchanged water and dried in an oven at 110°C for 24 hours.

The tissues are thawed, trimmed of fat and fascia, rinsed briefly in ion-exchanged water, weighed in fused silica dishes, and placed in a cold drying oven. The temperature is raised gradually to 110°C ; the tissues remain at this temperature until they reach a constant weight, usually 24 hours. The tissues are cooled, reweighed, and placed in a cold muffle furnace. The temperature is brought up gradually to 450°C and left until ashing is complete, usually 24 hours. The ash is cooled, weighed, and transferred to 1/2 ounce polyethylene bottles. A few platinum beads are added and the beads and ash are shaken on a pipette shaker for 30 minutes. The platinum beads are removed and the ashed samples are stored in these bottles.

For drying an alternate procedure was used prior to October 1, 1956. The tissues were placed in a cold muffle furnace and the temperature gradually raised to 300°C. The tissues were left at this temperature over night, after which the temperature was raised gradually to 450°C and left for 24 hours or longer as necessary. To obtain the dry weight a small piece of the original tissue was weighed, placed in a cold drying oven, and the temperature raised to 110°C. The tissue was kept at this temperature for 24 hours, then cooled, reweighed, and destroyed.

Procedures. III. Spectrographic Analysis:

The direct current arc is the most sensitive method of excitation of a sample for spectrographic analysis for a large number of elements.¹⁶ This method has the disadvantage of being the least precise. In this study, a large number of elements in widely varying concentrations, most of them in extremely low concentrations, were to be sought and so the d-c arc method was chosen since maximum sensitivity was felt to be the most important characteristic to be considered. It has developed subsequently, that the variation in concentration from sample to sample is so great that for purposes of establishing average values for a large number of samples the precision of the d-c arc method is adequate.

The spectrographic methods employed are summarized in Table II. For soft tissue two methods of d-c arc excitation are employed.

1. For the elements Ag, As, Au, Bi, B, Cd, Ga, Pb, Sb, Sn, Tl and Zn, the cathode layer method with In as an internal standard is used. Four mg of tissue ash and 4 mg of graphite containing the internal standard are mixed and placed in a cavity 5mm deep and 1/16 inch in diameter in a 1/8 inch

spectroscopically pure graphite electrode. This electrode is made the cathode in a 10 amp d-c arc, the counter electrode is 1/8 inch graphite rod, the arc spacing is 10 mm. The cathode spot is focused on the slit of a Bausch and Lomb Quartz Littrow spectrograph. The region from 2400 A - 3400 A. is photographed on Eastman SA - 2 plates, standards being included on each plate.

2. For the elements Al, Au, Be, Ba, Ca, Cs, Co, Cr, Cu, Fe, La, Mg, Mn, Mo, Nb, Ni, P, Sr, Ti, V, and Zr, a method with Pd as an internal standard is employed. Five mg of tissue ash are mixed with 10mg of graphite containing the internal standard and the mixture is placed in a cavity 0.0787 inch deep and 0.1470 inch in diameter in a 3/16 inch pure graphite electrode. This electrode is made the anode in a 5 amp d-c arc with a spacing of 8 mm and the central portion of the arc is focused on the grating of a Bausch and Lomb 1.5 meter grating spectrograph. The spectrum is photographed on SA - 2 film. A portion of the slit is covered with a neutral filter. Low concentrations are determined from the unfiltered portion of the line and high concentrations from the filtered portion. An unfiltered line and filtered line of Pd are used as internal standard lines for unfiltered and for filtered lines of analysis elements.

All samples are run in duplicate.

Plates and film are calibrated using an iron arc source and rotating sector.

The densities of the analytical lines are read on an NSL Spec-Reader which has been altered to read directly in intensities.

Working curves are made using standards of known quantities of the analysis elements in a matrix which simulates tissue ash. The composition of the matrix for soft tissue is:

Na (as NaCl)	15%
K (as KPO_3)	18.6
Mg (as MgO)	1.0
Ca (as $CaCO_3$)	1.0
Fe (as Fe_2O_3)	1.0

The methods for bone ash are essentially the same as those for the anode excitation of soft tissue ash. Because the ash percent for bone is about 20 times that for soft tissues, the absolute amount of an element in a 5 mg sample of bone ash is about 1/20 the amount in an equivalent sample of tissue. The method for bone is therefore less sensitive than that for soft tissue. To detect Zn in the bone, the most sensitive line of Zn must be used. This line occurs in the visible region of the spectrum, necessitating the use of a film sensitive to this region and a filter to cut out the overlying second order in the spectrum as formed by the Bausch and Lomb 1.5 meter grating spectrograph. In the determination of Zn, K, Li, Mg, and Na, the same method is employed as in the anode excitation method for soft tissue except that Eastman 103-F film is used instead of Eastman SA-2 and a glass filter is placed in front of the slit to remove the second order.

Working curves are made from standards of known quantities of analysis elements in a matrix with the following composition:

Ca (as $CaCO_3$)	38.9%
Mg (as MgO)	0.97
Na (as NaCl)	0.51

Earlier standards were made in a matrix of $(Ca)_3(PO_4)_2$ which more nearly simulates bone ash. The working curves from these standards, however, do not differ significantly from the curves made from the $CaCO_3$ standards.

The plates and film are developed for three minutes at 72°C in D-19 developer, placed in a stop bath for 1 minute, fixed for 15 minutes and washed for 20 minutes.

Table III shows the method of excitation, analytical lines used, internal standard line, ranges of concentrations that can be determined, and coefficients of variation for soft tissues. The coefficients of variation were determined from replicate runs of a standard sample. The coefficients of variation for actual samples are higher, especially at the high and low ends of the range. Table IV gives the same information for bone.

Since this program has been in progress others^{17, 18, 19, 20, 21} also have reported spectrographic analyses on normal human tissue and except for Al the agreement has been very good. The values for Al reported by Butt et al are very much higher and those reported by Koch et al are somewhat higher than those reported by this laboratory. As yet there is no satisfactory explanation for the discrepancy in Al.

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Figure I

TO: _____

DATE: _____

FROM: _____

HOSPITAL: _____ CITY OF: _____

SEX: _____ AGE: _____ YEARS RESIDENCE: _____ OCCUPATION: _____

RACE: _____

AUTOPSY NUMBER: _____ HOURS POST MORTEM: _____

CAUSE OF DEATH: _____

OTHER INFORMATION BELIEVED TO BE OF SIGNIFICANCE TO THIS STUDY: _____

Adrenal glands _____	Muscle _____
Aorta _____	Psoas _____
Bone _____	Other (Specify by name) _____
Rib _____	Omentum _____
Vertebra _____	Ovaries _____
Other (specify by name) _____	Pancreas _____
Brain _____	Pituitary _____
Breast _____	Prostate _____
Cartilage _____	Skin _____
Diaphragm _____	Mid ventral _____
Esophagus _____	Spleen _____
Fat _____	Stomach _____
Gall Bladder _____	Testes _____
Heart _____	Thymus _____
Intestine - large _____	Thyroid _____
Cecum _____	Trachea _____
Sigmoid colon _____	Urinary bladder _____
Rectum _____	Uterus _____
Other (specify by name) _____	Vagina _____
Intestine - small _____	Vena cava _____
Duodenum _____	_____
Jejunum _____	_____
Ileum _____	_____
Kidneys _____	_____
Larynx _____	_____
Liver _____	_____
Lungs _____	_____
Lymph nodes _____	_____
REMARKS _____	_____

Table I

Mass and Effective Radius of Organs of the Adult Human Body

	Mass, m, (g)	Per cent of Total Body*	Effective Radius, X (cm)
Total body*	70,000	100	30
Muscle	30,000	43	30
Skin and subcutaneous tissue**	6,100	8.7	0.1
Fat	10,000	14	20
Skeleton: Without bone marrow	7,000	10	5
Red marrow	1,500	2.1	--
Yellow marrow	1,500	2.1	--
Blood	5,400	7.7	--
Gastrointestinal tract*	2,000	2.9	30
Contents of GI tract			
Lower large intestine	150	--	5
Stomach	250	--	--
Small intestine	1,100	--	--
Upper large intestine	135	--	--
Liver	1,700	2.4	10
Brain	1,500	2.1	--
Lungs (2)	1,000	1.4	30
Lymphoid tissue	700	1.0	--
Kidneys (2)	300	0.43	7
Heart	300	0.43	--
Spleen	150	0.21	7
Urinary bladder	150	0.21	--
Pancreas	70	0.10	--
Salivary glands (6)	50	0.071	--
Testes (2)	40	0.057	--
Spinal cord	30	0.043	--
Eyes (2)	30	0.043	--
Thyroid gland	20	0.029	3
Teeth	20	0.029	--
Prostate gland	20	0.029	--
Adrenal glands or Suprarenal (2)	20	0.029	--
Thymus	10	0.014	--
Miscellaneous (blood vessels, cartilage, nerves, etc.)	390	0.56	--

* Does not include contents of gastrointestinal tract.

** The mass of the skin alone is taken as 2000 grams.

*** The reports, "The Standard Man" by Herman Lisco, ANL-4253, Nov.1948 - Feb.1949, p. 96 and "A Survey Report of the Characteristics of the Standard Man," Sept.1948, by M. J. Cook were used as the principal sources of reference in the original selection of values given in this table.

Spectrographic Methods

Type of Sample	Soft Tissue		Bone	
Sample Excitation	Cathode Layer	Anode	Anode I	Anode II
Elements determined	Ag, As, Au, Bi, B, Cd, Ga, Pb, Sb, Sn, Ti, Zn.	Al, Au, Be, Ba, Ca, Cs, Co, Cr, Cu, Fe, La, Mg, Mn, Mo, Ni, P, Ru, Sn, Ti, V, Zr.	Ag, Al, Au, B, Be, Ca, Co, Cu, Fe, La, Mg, Mn, Mo, Ni, Pb, Sn, Sr, Ti, V.	Ba, Cr, Fe, K, Li, Mg, Na, Zn.
Internal Standard	Indium	Palladium	Palladium	Palladium
Spectrograph	Bausch and Lomb Quartz Littrow	Bausch and Lomb 1.5 meter grating	Bausch and Lomb 1.5 meter grating	Bausch and Lomb 1.5 meter grating first order
Slit width	20 microns	20 microns	20 microns	20 microns
Slit height	1.8 mm	1.8 filtered portion 1.8 clear portion	1.8 mm filtered 1.8 mm non-filtered	1.8 mm filtered 1.8 mm non-filtered
Power supply	Rectified 220 volt a-c $\leq 2\%$ ripple	Same	Same	Same
Current	10 amp d-c	5 amp d-c	5 amp d-c	10 amp d-c
Burning time	10 seconds	to completion (~ 95 sec)	to completion (~ 70 sec)	5 sec
Photographic record	Eastman SA-2 plates	Eastman SA-2 film	Eastman SA-2 film	Eastman 103-F film
Densitometer	NSL Spec Reader	Same	Same	Same
Electrodes	Lower, cathode: 1/8 inch graphite, 1/16 inch hole, 5 mm deep. Upper anode: 1/8 inch graphite rod.	Lower, anode: 3/16 inch graphite rod; 0.147 inch hole; 0.0787 inch deep. Upper cathode: 1/8 inch graphite rod.	Same	Same
Sample size	4 mg ash + 4 mg graphite and internal standard	5 mg ash + 10 mg graphite and internal standard	Same	Same
Arc Gap	10 mm	8 mm	Same	Same
Portion of arc used	Layer 1 mm above cathode focused on mask gap which is focused on collimator of spectrograph	Central portion, focused on grating	Same	Same
Intermediate aperture	Mask gap width 0.040 inches	Diaphragm, diameter 4 mm	Diaphragm diameter 2 mm	Diaphragm diameter 4 mm
Intermediate optics	83 mm lens to focus on mask gap 150 mm lens to focus on collimator	83 mm lens to focus on grating neutral filter over half of slit	Same	Same lens + filter over half of slit. Filter to cut out second order
Distance, source to slit	70 cm	48 cm	Same	Same

Table III

SOFT TISSUES

Element	Method of Excitation	Line Used	Internal Standard Line	Range of concentration (soft tissue ash)	Coefficient of variation (per cent)
Ag	Cathode	3382	In 3039	0.6 - 100 ppm	15
Al	Anode	3082	Pd 3027	5 - 400	15
Al	Anode	3082(F)	Pd 3421	100 - 10,000	
As	Cathode	2780	In 3039	300 -	
Au	Cathode	2675	In 3039	10 - 400	
Au	Anode	2675	Pd 3027	20 - 400	
B	Cathode	2497	In 3039	10 - 400	
Ba	Anode	4554	Pd 3027	0.3 - 10	20
Ba	Anode	4554(F)	Pd 3421	2 - 400	
Be	Anode	2348	Pd 3027	3 - 100	
Bi	Cathode	3067	In 3039	4 - 4,000	
Ca	Anode	3179	Pd 3027	0.1 - 4 per cent	
Ca	Anode	3179(F)	Pd 3421	0.25 - 10 per cent	
Cd	Cathode	3261	In 3039	70 - 10,000 ppm	4
Co	Anode	3453	Pd 3027	4 - 200	
Cr	Anode	3593(F)	Pd 3421	10 - 1,000	
Cr	Anode	4254	Pd 3027	0.1 - 10	
Cr	Anode	4254(F)	Pd 3421	2 - 100	
Cs	Anode	4555	Pd 3027	150 - 2,500	
Cu	Anode	3274	Pd 3027	10 - 100	
Cu	Anode	3274(F)	Pd 3421	20 - 5,000	5
Fe	Anode	3024	Pd 3027	0.1 - 3 per cent	
Fe	Anode	3024(F)	Pd 3421	1.0 - 10 per cent	
Ga	Cathode	2943	In 3039	1 - 40 ppm	
Ge	Anode	2651	Pd 3027	30-	

Table IV

BONE

Element	Method of Excitation	Line Used	Internal Standard Line	Range of Concentration (in bone ash)
Ag	Anode I	3382	Pd 3027	1-25 ppm
Al	Anode I	3082	Pd 3027	5-100 ppm
Au	Anode I	2675	Pd 3027	25- ppm
B	Anode I	2497	Pd 3027	30- ppm
Ba	Anode II	4554	Pd 3027	0.5-10 ppm
Ba	Anode II	4554(F)	Pd 3404	10-300 ppm
Be	Anode I	2348	Pd 3027	4- ppm
Ca	Anode I	3158(F)	Pd 3404	1%-50%
Ca	Anode I	3181(F)	Pd 3404	10%-50%
Co	Anode I	3453	Pd 3027	6-100 ppm
Cr	Anode II	4274	Pd 3027	0.3-10 ppm
Cr	Anode II	4274(F)	Pd 3404	6-100 ppm
Cu	Anode I	3247	Pd 3027	1-40 ppm
Fe	Anode I	3024	Pd 3027	200-5,000 ppm
Fe	Anode I	3047	Pd 3027	100-4,000 ppm
Fe	Anode II	3581	Pd 3027	10-2,000 ppm
K	Anode II	4044(F)	Pd 3404	0.2%-4%
La	Anode I	3337	Pd 3027	100- ppm
Li	Anode II	6707	Pd 3404	0.01-2% ppm
Mg	Anode I	3336	Pd 3027	0.15%-3.5%
Mg	Anode II	5167(F)	Pd 3404	0.1%-4%
Mn	Anode I	2798	Pd 3027	1-10 ppm
Mo	Anode I	3170	Pd 3027	15- ppm
Na	Anode II	3302	Pd 3027	0.5%-4%
Ni	Anode I	3050	Pd 3027	20-400 ppm
Pb	Anode I	2833	Pd 3027	4-1,000 ppm
Sn	Anode I	3840	Pd 3027	10-500 ppm
Sr	Anode I	4077(F)	Pd 3404	20-400 ppm
Sr	Anode I	4607(F)	Pd 3404	10-500 ppm
Ti	Anode I	3653	Pd 3027	10-400 ppm
V	Anode I	4379	Pd 3027	15- ppm
Zn	Anode II	4810	Pd 3027	200-2,000 ppm