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TRACE ELEMENTS IN HUMAN TISSUE

PART I. METHODS

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Abstract—The concentrations of trace elements in normal human tissues and the distribution of these elements throughout the body have been determined by emission spectroscopy. The methods of collection and preparation of samples, of spectrographic analysis, and of statistical treatment of data are described.

PROBLEMS which arise in the field of Health Physics may bring up questions which themselves have little to do with radiation but to which answers are needed in the solution of Health Physics problems. As indicated in the Report of Committee II of the International Commission on Radiological Protection,[1] one such question has to do with the concentration and distribution of stable elements in the organs and tissues of "standard man" upon which MPC calculations are based. At the time the concept of "standard man" was adopted in 1949, a number of elements had been detected in the human body but very few attempts had been made to measure concentrations in the various organs. To provide this information for MPC calculations, a cooperative program was set up in the Department of Physics at the University of Tennessee and the Health Physics Division of the Oak Ridge National Laboratory. The main objective was to determine the concentration of as many tissues and organs from as many normal individuals from as many locations in the United States in as short a time as possible and thus to throw light on the elemental composition of "standard man".

At the outset it was recognised that the elemental composition of the human body should vary with age and geographical location and it has become increasingly evident that this is true, and that an MPC set up on the basis of a "standard man" must be modified and adapted if it is to be applied to a population or a segment of a population which "standard man" may not represent. A further objective of the program has been, therefore, to estimate the variation of the elemental composition of human beings with age and geographical location.

A. COLLECTION AND PREPARATION OF SAMPLES

(1) *Collection*

Collection of samples from subjects from the United States was carried out for the most part by Miss COOK. Samples from Africa were collected by Dr. PERRY and from Switzerland and the Near and Far East by Dr. SCHROEDER.

An individual who had died suddenly and who had no apparent diseased condition at the time of death was considered to be a normal subject. Since autopsies are not usually performed on such subjects except in cities with a Medical Examiner, the source of samples in the United States was limited to these cities. Programs for collection of tissues from autopsies of victims of instantaneous death (by traumatic accident, for the most part) were set up in nine major cities in the United States. It was

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Human Studies Project

TO: ..... DATE: .....

FROM: .....

HOSPITAL: ..... CITY OF: .....
RACE: .....
SEX: ..... AGE: ..... YEARS RESIDENCE: ..... OCCUPATION: .....
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	Testis .....
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: .....

FIG. 1. Sample autopsy information sheet.

impossible to impose the same restrictions on the foreign subjects since very few autopsies are performed on victims of accidents in the countries from which the foreign tissues were drawn. Only tissues showing no gross pathological condition were analyzed, however, for both American* and foreign subjects.

The American tissues were taken at autopsy, with special care to prevent contamination, placed in individual polyethylene bags, frozen quickly, and transported by air to Oak Ridge where they were kept frozen until prepared for ashing. For large organs like liver, samples of 100–200 g were taken, for smaller organs, the entire organ. The foreign tissues were trimmed of fat and connective tissue at autopsy, placed in individual polyethylene bottles, and

* The word American is used to denote subjects from the United States.

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preserved in an approximately equal volume of specially prepared metal-free formalin.[2]

Figure 1 shows the information sheet which accompanied each autopsy.

(2) *Sample preparation*

To increase the sensitivity of the analytical method, concentration of elements was necessary, but, because of the danger of contamination, as little manipulation of the sample as possible was desirable. Several methods of concentration were explored: wet ashing and reduction to dryness; wet ashing and precipitation with 8-hydroxyquinoline; dry ashing.

Wet ashing with nitric and perchloric acids was found to be time consuming and to provide opportunity for the introduction of contaminants. The method of wet ashing and precipitation with 8-hydroxyquinoline,[3] in addition to being slow and increasing the danger of contamination, also decreased the number of elements which could be determined. Dry ashing proved to be the most satisfactory method for the largest number of elements.

The American samples were thawed, trimmed of excess fat and connective tissue, placed in tared fused silica dishes and weighed. After drying at 110°C for 24 hr they were weighed, placed in a muffle furnace lined with fused silica, brought gradually to 450°C, and ashed at this temperature for 24–48 hr. After cooling and weighing, the ash samples were transferred to polyethylene bottles and mixed and ground by shaking, with a few platinum beads added, on a pipette shaker for 30 min. After the beads were removed, the polyethylene bottles were closed tightly with polyethylene-lined caps and sent to the spectrographic laboratory at the University of Tennessee where they were kept closed until aliquots were taken for analysis. The foreign samples were treated in essentially the same way except that no wet weight could

Table 1. Spectrographic methods for tissue

Sample excitation	Cathode layer (sample in cathode)	Anode (sample in anode)
Elements determined	Ag, Au, B, Bi, Cd, Ga, Pb, Sn, Zn	Al, Ba, Be, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Sr, Ti, V
Internal standard	Indium	Palladium
Spectrograph	Bausch and Lomb Littrow (Quartz optics)	Bausch and Lomb 1.5 m grating
Slit width	20 μ	Same
Slit width	1.8 mm	1.8 mm filtered portion; 1.8 mm clear portion
Power supply	Rectified 220 V a.c. $<$ 2 per cent ripple	Same
Current	10 A d.c.	5 A d.c.
Burning time	10 sec	to completion ($\sim$95 sec)
Photographic record	Eastman SA-2 plates	Eastman SA-2 film
Densitometer	NSL spec reader	Same
Electrodes	Lower, cathode: 1/8 in. graphite, 1/16 in. hole, 0.20 in. deep. Upper, anode: 1/8 in. graphite rod	Lower, anode: 3/16 in. graphite rod, 0.147 in. hole, 0.0787 in. deep. Upper, cathode: 1/8 in. graphite rod.
Sample size	4–5 mg ash + 4–6 mg graphite and internal standard	5 mg ash + 10 mg graphite and internal standard
Arc gap	10 mm	8 mm
Portion of arc used	Layer 1 mm above cathode focused on mask gap which is focused on collimator of spectrograph	Central portion focused on slit
Intermediate aperture	Mask gap width 0.10 mm	Diaphragm, diameter 4.0 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
Distance, source to slit	70 cm	48 cm

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be obtained. The formalin in which the tissues were preserved was dried and ashed with the tissues.

Precautions against contamination were taken at every step of the procedure. Dishes were washed in a cleaning solution of 5 parts HNO_3 and 1 part H_2SO_4, rinsed, soaked for 24 hr in a solution of Na_4EDTA (pH 11) and rinsed with metal-free water that had been prepared by passing distilled water slowly through a mixed bed ion exchange column and had been stored in polyethylene bottles. A careful check was kept on the dishes, and those which lost their glaze or changed appreciably in weight were discarded. Air was brought into the ashing laboratory through CWS-6 high-efficiency filters. Analyses were made in an air-conditioned room with double doors.

Table 2. Analytical lines, range of concentration

Element	Method of excitation	Analytical line	Internal standard line	Limits of method (concentration in ash)
Ag	Cathode	3383	In 3039	0.1–50 μg/g
Al	Anode	3082	Pd 3027	10–200
Al	Anode	3082(F)	Pd 3421	100–4000
Au	Cathode	2676	In 3039	10–
Au	Anode	2676	Pd 3027	10–400
B	Cathode	2497	In 3039	10–1000
Ba	Anode	4554	Pd 3027	0.2–4
Ba	Anode	4554(F)	Pd 3421	1–100
Be	Anode	2349	Pd 3027	4–
Bi	Cathode	3068	In 3039	4–400
Ca	Anode	3179	Pd 3027	0.03–1%
Ca	Anode	3179(F)	Pd 3421	0.20–10
Cd	Cathode	3261	In 3039	50–10000 μg/g
Co	Anode	3454	Pd 3027	2–100
Co	Anode	3454(F)	Pd 3421	100–1000
Cr	Anode	4254	Pd 3027	0.1–6
Cr	Anode	4254(F)	Pd 3421	2–100
Cr	Anode	3593(F)	Pd 3421	10–1000
Cu	Anode	3274	Pd 3027	1–10
Cu	Anode	3274(F)	Pd 3421	10–2000
Fe	Anode	3024	Pd 3027	0.02–2%
Fe	Anode	3024(F)	Pd 3421	0.50–10
K	Anode	3448	Pd 3027	2–40
Mg	Anode	2779(F)	Pd 3421	0.10–6
Mn	Anode	2798	Pd 3027	2–20 μg/g
Mn	Anode	4031(F)	Pd 3421	2–400
Mo	Anode	3170	Pd 3027	4–1000
Ni	Anode	3051	Pd 3027	5–400
Ni	Anode	3051(F)	Pd 3421	100–1000
P	Anode	3246	Pd 3027	1–5%
P	Anode	3270(F) (PO band-heads)	Pd 3421	4–40
Pb	Cathode	2833	In 3039	5–1000 μg/g
Sn	Cathode	2840	In 3039	5–1000
Sr	Anode	4607(F)	Pd 3421	0.1–40
Ti	Anode	3653	Pd 3027	5–400
Ti	Anode	3653(F)	Pd 3421	100–2000
V	Anode	4379	Pd 3027	1–20
V	Anode	4379(F)	Pd 3421	8–400
Zn	Cathode	3345	In 3039	300–20000

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B. SPECTROGRAPHIC METHODS

Emission spectrography was chosen for the analyses because a rapid, sensitive method for a large number of elements was required. A summary of the spectrographic method is given in Tables 1 and 2. Every step of the procedure was worked out to maximize sensitivity and coverage and to minimize time for a determination.

The densitometer was modified to read intensities directly by photographing the values for intensity, as determined from calibration of plate and film, side by side with the transmission scale and replacing the galvanometer scale of the instrument with a positive transparency of this photograph. This cut in half the time for determining intensities of lines, not only because the line intensity could be read directly but also because background correction could be applied immediately. For most determinations Eastman SA-2 films and plates were used. A large quantity of plates and film were purchased at the same time, so that the emulsion on each would be from the same batch and both were calibrated according to the method suggested by Committee E-2 of the American Society for Testing Materials.[4] For each new calibration, a new galvanometer scale was devised. Other types of emulsion were calibrated separately and determinations with these emulsions were made in the usual manner from transmission readings. It was found that the response of any one batch of film remained fairly constant over as long a time as two years if the film was kept at 0°C. Plates, even those from any one box, were more variable. To minimize the error arising from this variation in response for the plates, spectra of standards were put on every plate and the working curve shifted accordingly.

(1) *Standards*

Any method of analysis by comparison such as the spectrographic method depends on the standards used for comparison. In spectrographic analysis it is especially important that the standards have as nearly as possible the same composition as the unknowns because of the influence of certain elements in a sample on the intensity of the spectral lines of other elements in the sample. In this program a matrix having the gross composition of the ash of most soft tissues was devised[5] and several sets of standards were prepared by adding known quantities of elements to this matrix. Table 3 shows the composition of the matrix and Table 4 the composition of a typical set of standards. Other sets contained additional elements. Standards for the determination of the major constituents of ash, potassium, phosphorus, calcium, magnesium and iron, were made by diluting the matrix with graphite and by altering the relative concentrations of calcium, iron, and magnesium.

Table 3. Matrix for analysis of human tissue ash

Element	Compound	Element concentration in matrix (per cent)
K	KPO_3*	18.6
P		14.7
Na	NaCl	15.0
Mg	MgO	1.0
Ca	$CaCO_3$	1.0
Fe	Fe_2O_3	0.98

* KH_2PO_4 in furnace at 400°C for 24–48 hours becomes KPO_3 which is stable if kept dry.

All reagents were "specpure" from Johnson & Matthey; all were heated in the furnace at 300°C for 2–24 hr and ground by hand in agate or mullite mortars before weighing. The matrix was made up in 30 g quantities and mixed in a polyethylene bottle mounted in the chuck of a lathe and turned for 80 hr, part of the time end over end and part of the time around an axis through the median line of the bottle. Individual standards were ground by hand for 2 hr in mullite mortars.

(2) *Internal standards*

The intensity of the light of one characteristic frequency emitted by an element excited in a d.c.-arc discharge depends upon a number of variables, some arising from the varying character of the material in the arc and some from variations in the circuit of which the arc is a part, and these two types of variation are not independent of each other. In spectrochemical analysis one way to minimize the

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Table 4. Typical standards for tissue analysis

Standard number	Concentration of (elements μg/g)	Elements added to previous standards
1	10,000	Zn
2	4,000	Cd, Al, B* (400 μg/g)
3	2,000	Ti, Au, Cu
4	1,000	Mo, Ni, Pb, Sn, Mn, Cr
5	400	V, Sr, Ba, Ag, Bi, Co, Ga, Be
6	200	
7	100	
8	40	
9	20	
10	10	
11	4	
12	2	

* Boron goes down from 400 μg/g in number 2 to 0.2 μg/g in number 12.

effects of these variations is to introduce into the arc with each sample a constant quantity of an element (an internal standard) which will respond to variations in the same way as the analytical element. The ratio of the intensity of a line from the unknown element to a line from the internal standard should remain constant. (If this element is not a constituent of the original sample but is added to it as in this study, the terms should perhaps be more correctly called "external standard", but it is quite commonly called an internal standard as it is in this report.)

Table 5. Requirements for internal standards

(1) Concentration in sample negligible
(2) Volatilization rate similar
(3) Excitation potential similar
(4) Self-absorption negligible
(5) Wavelength close
(6) "Extraneous element" effect low
(7) Ionization potential similar
(8) Atomic weight not too dissimilar.

The requirements for an internal standard are listed in Table 5 after AHRENS.[6] It is obvious that one element cannot satisfy all conditions as an internal standard for all other elements. On the basis of rates of volatilization as determined from moving plate studies (Fig. 2) indium was chosen as an internal standard for the volatile elements in tissue ash and palladium for the refractory elements. These two elements were never observed within the limits of detection of the method in any sample of tissue. The excitation potentials of the indium and palladium internal standard lines and the analytical lines with which they were used are shown in Table 6. The self absorption of these Pd and In lines was negligible. The use of SA-2 plates for which the gamma did not change over a wide range of wave lengths made it possible to use one internal standard line for a number of analytical lines.

An "extraneous element effect" on an analytical line is permissible if the extraneous

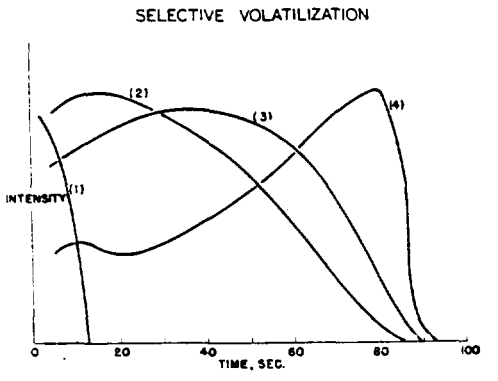

FIG. 2. Variation of intensity of lines with time from start of burn. Curve (1) indium and Ag, Bi, Cd, Ga, Pb, Sn, Zn. Curve (2) potassium. Curve (3) sodium. Curve (4) palladium and Ba, Ca, Co, Cr, Cu, Mn, Mo, Ni, Sr, Ti, V, Zn. (Plate racked 2 mm every 5 sec. Arbitrary scale for intensity.)

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Table 6. *Excitation potentials* (V)

Internal standards and analytical lines		
Pd 3027 5.0	Pd 3421(F) 4.6	In 3039 4.1
Al 3082 4.0	Al 3082(F) 4.0	Ag 3382 3.7
Ba 4554 2.7	Ba 4554(F) 2.7	B 2497 5.0
Co 3453 4.0	Co 3453(F) 4.0	Bi 3067 4.0
Cr 4254 2.9	Cr 4254(F) 2.9	Cd 3261 3.8
Cu 3274 3.8	Cu 3274(F) 3.8	Ga 2943 4.3
Mn 2798	Mn 4031(F) 3.1	Pb 2833 4.4
Mo 3170 3.9	Ni 3051(F) 4.1	Sn 2840 4.8
Ni 3050 4.1	Sr 4607(F) 2.7	Zn 3345 7.8
Ti 3653 3.4	Ti 3653(F) 3.4	
V 4379 3.1	V 4379(F) 3.1	

element has the same effect on the internal standard line. A study[7] was made of the effect of varying concentration of the major constituents K and Na, on Pd and In and on a number of trace elements. Five matrices containing K and Na in different proportions but constant sum were mixed and the same amount of the trace elements added to samples of each. Aliquots of each matrix with and without trace elements were weighed out, the internal standard in graphite added, and the sample arced in the same manner as tissue samples. The intensity of the internal standard line and the element line were read on the densitometer. The log of the ratio of the intensity of the element line to the internal standard line and the log of the reciprocal of the intensity of the internal standard line were plotted as a function of the ratio of concentrations of K to Na, the sum of the two concentrations being held constant. Figures 3 and 4 are examples of these plots. The standard tissue matrix was diluted with graphite and the effects of varying the sum of concentrations of Na and K, the ratio being held constant, were studied. The intensity ratios were plotted as a function of the sum of the concentration of K and Na. Figure 5 is an example of this plot. In these plots a straight line of slope zero for the internal standard indicates no effect of varying concentrations of K and Na on the internal standard. For the analytical elements, a straight line parallel to the line corresponding to the internal standard alone indicates that the internal standard and the analytical element are affected alike by varying concentrations of K and Na.

The results of this study indicated that Pd and In were satisfactory internal standards for the elements studied and that the use of one set of comparison standards for all tissues would not introduce enough error to warrant spending the time to devise separate standards for every tissue.

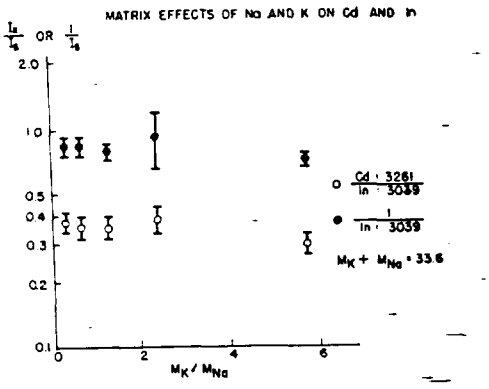

FIG. 3. Effect of changing the ratio of concentrations of K and Na, keeping the sum constant, on the internal standard indium and the element cadmium. I_x/I_s is the ratio of the intensity of the Cd 3261 line to the intensity of In 3039 line. $1/I_s$ is the reciprocal of the intensity of the In 3039 line. The sum of the concentration of K and Na is 33.6%.

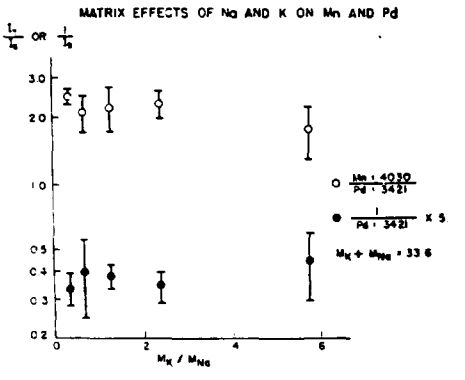

FIG. 4. Effect of changing the ratio of concentrations of K and Na, keeping the sum constant, on the internal standard palladium and the element manganese. I_x/I_s is the ratio of the intensity of the Mn 4030 line to the intensity of the Pd 3421 line. $1/I_s$ is the reciprocal of the intensity of the Pd 3421 line. The sum of the concentrations of K and Na is 33.6%.

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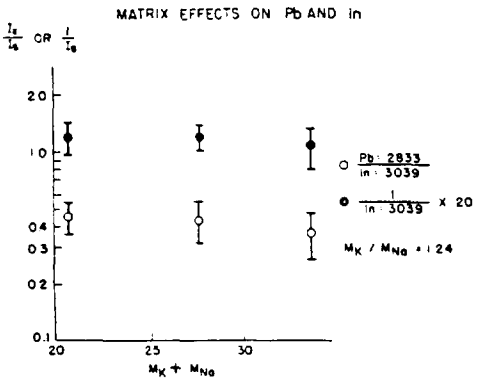

FIG. 5. Effect of changing the sum of the concentrations of K and Na, keeping the ratio constant, on the internal standard indium, and the element lead. I_z/I_s is the ratio of the intensity of the Pb 2833 line to the intensity of the In 3039 line. $1/I_s$ is the reciprocal of the intensity of the In 3039 line. The ratio of the concentration of K to the concentration of Na is 1.24%.

(3) *Precision and accuracy*

For each determination two aliquots from a sample of ash were analyzed. The deviation of either of two concentrations from the mean of these two values, expressed as a percentage of the average of the two values, is given by $(C_1 - C_2)/(C_1 + C_2) \times 100$, where C_1 is larger than C_2. The geometric mean of the values of this fraction for the individual samples is sometimes termed the repeatability of the value. The values of the repeatability of the concentrations of 12 elements in thirteen tissues are shown in Fig. 6. For elements such as chromium for which the analytical line lay in a region of high background and which usually occurred in concentration close to the limit of detection, the difference between duplicates was greater than for elements like copper and iron for which the lines were usually clear and strong. A statistical study of the variation of these differences with time over the years 1954–1961, and with concentration of element, indicated no trends. That is, the agreement between the values for an element in the two aliquots did not depend on the concentration of the element, and did not vary significantly from one time to another.

The ash of several different tissues was mixed as composites to serve as control samples and were rerun from time to time as a check on the variability of the method. Table 7 gives a summary of the random variation of the method over a 16-month period. Since for most elements there was a ten-fold or greater variation in concentration among samples, this precision was felt to be adequate for the study.

The results of a recovery study are shown in Table 8. Known amounts of elements were added to aliquots of a composite sample of ash and thoroughly mixed. Three additions at different levels of concentration were made. The mean of four determinations for the composite sample before addition and for each of the three samples with additions are given in the first two columns of Table 8. The amount recovered, column 3, was obtained by subtracting the values in column 1 from the corresponding values in column 2. Column 4 indicates the amount added in each case.

Samples of ash from this program were analyzed at the Spectrographic Laboratory of Los Angeles County General Hospital and at the Spectrographic Laboratory of the Analytical Chemistry Division at Oak Ridge National Laboratory. Except for Ag, Al, and Ba, the agreement between the Los Angeles[8] and Tennessee values was good, 37 out of 47 values being within 50 per cent and 21 out of 47 within 25 per cent. The agreement with Oak Ridge[9] was better, 114 out of 120 values being within 50 per cent and 60 out of 120 within 20 per cent.

Although they would have been desirable as checks of the accuracy of the method, analyses of the samples by other methods were not feasible.

C. STATISTICAL METHODS

Any set of quantitative data has two important features, central tendency and variation about the central value. If the frequency polygon of the individual values approximates a normal curve the arithmetic mean is taken to indicate central tendency and the standard deviation to indicate variation. Common methods for analyses of variance and of independence are also based on these statistics. If the individual values are not normally distributed, however, common statistical procedures, especially for analysis of independence

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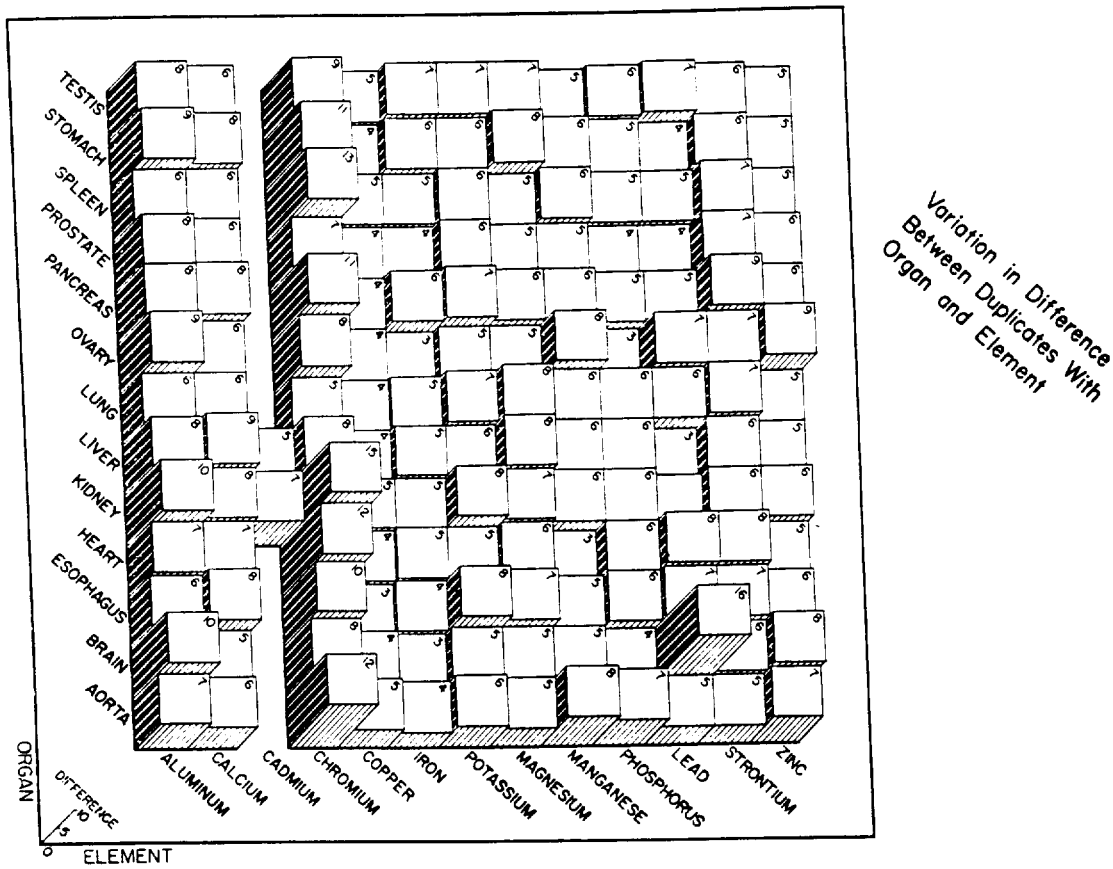

FIG. 6. Variation with organ of the repeatability of the value for concentration of an element. The height of each block indicates the geometric mean over all sample values of the per cent difference between each of the determined concentrations for two aliquots of a sample and the average of the two concentrations, $(C_1 - C_2)/(C_1 + C_2) \times 100$ (where C_1 is the high value and C_2 is the low value).

and of variance, will give misleading results. Distribution-free methods have been developed for the latter type of data.[10] One very successful method of this type involves the ranking of values in order and using the rank rather than the values to measure independence and variance. In this type of analysis the median is used to indicate central tendency, and a measure of variation is based on quartiles or percentiles. Although distribution-free statistical procedures are not usually as efficient as common parametric procedures, according to KENDALL and STUART[11] their use for non-normal data leads to much more dependable results.

In this study, with the exception of potassium and phosphorus, values for concentration of an element in a tissue, and for the per cent ash of wet, per cent dry of wet, and per cent ash of dry were not normally distributed, the frequency polygon being skewed toward high values. The mean value of these quantities was higher than the median, in many instances much higher. A further complication arose from the fact that some elements were not observed above the limit of detection in every sample. It was impossible to calculate an exact value of the mean for these elements. The assumption that the concentration of an unobserved element was zero would have lead to an erroneously low value for the mean, whereas arbitrarily considering the concentration of an unobserved

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Table 7. Precision of method

Element	C Mean concentration (μg/g of ash except as indicated)	n Number of determinations over 16-month period	v Relative standard deviation $v = \frac{1}{\bar{C}}\sqrt{\left[\frac{\Sigma(C - C_i)^2}{n - 1}\right]} \times 100$
Ag	1.8	16	27%
Al	20	5	13
	100	5	25
	420	6	21
	1200	8	8
	2200	8	17
B	180	16	18
Ba	7	5	14
	26	8	35
Ca	0.60%	5	20
	1.9%	5	22
Cd	220	16	19
	3400	16	18
Co	22	8	6
Cr	1.5	5	34
	77	5	14
Cu	180	5	12
	480	8	14
Fe	0.35%	8	13
	0.85%	5	28
	2.8%	5	18
K	7.2%	8	12
	17%	5	25
Mg	1.1%	5	12
Mn	17	7	19
	47	7	20
	90	5	20
Mo	50	5	13
Ni	20	5	26
P	13%	5	11
Pb	56	8	17
	125	16	20
Sn	20	16	28
	70	16	20
Sr	3	5	28
	15	5	24
	27	8	11
Ti	42	7	20
	440	8	20
	2000	8	22
V	10	8	41
Zn	2400	8	19
	7000	16	19

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Table 8. Recovery study

Element	Composite tissue ash (μg/g)	Composite tissue ash + addition (μg/g)	Recovered (μg/g)	Added (μg/g)
Al	275	282	7	4
		300	25	40
		350	75	100
B	17	22	5	4
		36	19	40
		87	70	100
Ba	8	14	6	4
		54	46	40
Bi			51	40
			100	100
Cd	150	248	98	100
		1140	990	1000
Co			4	4
			45	40
			84	100
Cr	9	19	10	4
		78	69	40
Cu	330	360	30	40
		430	100	100
Mn	42	48	6	4
		80	38	40
		142	100	100
Mo	29	33	5	4
		99	70	40
		159	130	100
Ni	6	8	2	4
		50	44	40
		112	106	100
Pb	95	83	−12	4
		161	66	40
		194	99	100
Sn	93	77	−16	4
		130	37	40
		168	75	100
Ti	20	24	4	4
		55	35	40
		109	89	100
V			1	4
			46	40
			89	100
Zn	4800	6900	2100	2000

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element to be equal to the lower limit of detection would have given an erroneously high value for the mean. These two values did set limits on the range of the mean, however, and the arithmetic mean of the two values afforded a single value which might be assumed to approximate the mean.

Although mean and standard deviation were calculated for concentrations and for ash and dry per cents, the median was considered to be a more appropriate measure of central tendency and the 80 per cent range (10th–90th percentiles) a better indication of variation,[12] except for potassium and phosphorus which showed a normal distribution.

In population surveys such as this, correlation, according to FISHER,[13] is extremely useful as a descriptive quantity. The correlation coefficient, which may have values -1 to -1, is a measure of the extent to which two variables vary alike. If in every case a high value of one is accompanied by a high value of the other, the coefficient would be $+1$. If in every case a high value of one was accompanied by a low value of the other, the coefficient would be -1, etc. The correlation between two variates admittedly does not measure the influence of one variate on another, but it does assess, on a conventional scale, the importance of factors which act alike on both variates, as against the remaining factors which affect the variates independently.

The method described by KENDALL[14] was used to compute and test for significance the rank correlation coefficient for pairs of elements in a tissue and to measure the correlation between values for the same element in different tissues. To determine the significance of differences between groups of subjects, the Two-Sample Sign Test as described by WHITE[15] was used.

Most of the statistical treatment of data was carried out at the Computing Center of the University of Tennessee on an IBM 1620 Data Processing Unit, although some of the computations were done at the Union Carbide Nuclear Corporation Computing Center at Oak Ridge on the IBM 7090.

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