Spectrographic Determination of Lead in Blood

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Although application of spectrographic methods of analysis to the study of the physiology of the 'trace elements' has been made in this country by Sheldon & Ramage [1931] and by Kent & McCance [1941], little study has been devoted to trace elements from the aspect of industrial metallic poisoning. In America, Cholak [1935] described a spectrographic method for the analysis of lead in blood, using bismuth as the internal reference element. This paper describes a spectrographic method adopted in the investigation of suspected industrial lead poisoning, and some of the results obtained.

METHODS

General. All analytical material was collected and prepared for spectrographing with strict precautions to prevent contamination, by ensuring that, where possible, the vessels and reagents used were Pb-free. Loss of analytical material was eliminated by reducing the handling and chemical treatment of the samples to a minimum.

Spectrograph. The large Hilger quartz spectrograph, which has a range of 1910–8000 Å., was used with the

reference line for measurement against the Pb line at 2833 Å., with satisfactory accuracy when checked by the addition of known quantities of Pb to duplicate samples of blood and synthetic ash. Investigation of subjects exposed to Bi of therapeutic origin confirmed our suspicions that Bi could be encountered normally in blood, and invalidated its use as a standard (Pl. 1, fig. 1).

After investigation of several elements, it was decided to use platinum as the internal reference element, since it fulfilled all the conditions required, and its line at 2830 Å. is in a position of such proximity to that of Pb at 2833 Å. as is ideal for measurement (Fig. 1). After experiment it was found that the most satisfactory Pt solution to add to the ash specimens, following solution in aqua regia, was one containing 0.01 g./100 ml. H_2SO_4 . We consider it worth while to draw attention to the use of Pt in the analysis of several other elements implicated in poisoning by heavy metals. By varying the amount of Pt used, and if necessary the exposure time and type of plate in accordance with the spectrographic sensitivity of the element under investigation, Pt could be used as the internal reference element for the following metals:

As 2860.5 Å.	Ni 3002 Å.	Cr 2986.4 Å.	Au 2676 Å.
Pt 2830 Å.	Pt 2998 Å.	3015 Å.	Pt 2702 Å.
		Pt 2988 Å.	
Th 2837 Å.	V 3063.7 Å.	Mn 2798.3 Å.	Cd 2980 Å.
Pt 2830 Å.	Pt 3064.7 Å.	Pt 2830 Å.	Pt 2998 Å.

With few exceptions all the above pairs of lines are those of *raies ultimes*. All the Pt lines are of high spectrographic intensity and are not overshadowed, which facilitates accurate measurement of line densities. Where the internal reference line is overshadowed by close juxtaposition of another element line, it may be difficult to be certain that the overshadowing line is not in part responsible for the deflexion on the galvanometer scale which is attributed to the internal reference line. Kent & McCance must have experienced this difficulty in their analysis of Au (2676.6 Å.), with Mg (2795.5 Å.) as the internal reference line. Consultation of Harrison's wave-length tables indicates a line of Fe at 2795.54 Å., i.e. 0.01 Å. difference.

Standard ash. The standard material used for preparation of the graph was a synthetic ash which gave a main base spectrum similar to that of blood. Persistent traces of Pb were found in all the supposedly pure chemical reagents used, even after prolonged refining by chemical and electrolytic methods. Spectrographic examination showed that the ash finally contained 1–2 µg. Pb/100 g.

To a number of samples of synthetic ash (0.05 g. the approximate weight of the ash of the blood samples used) different known amounts of Pb and a constant amount of Pt were added. The samples (duplicate) were then spectrographed. The curve from the mean of two sets of results was taken as the standard graph (Fig. 2). It is advisable to use the logarithmic ratios of the line densities, as smooth

Table 1. *Standard conditions for spectrograph*

Wave band	2700–4300 Å.
Slit width	0.015 mm. (fixed slit)
Arc gap	2 mm.
Voltage	120 d.c.
Amperage	5
Upper electrode	H.S. graphite pointed to 80° angle
Lower electrode	H.S. graphite 10 mm. diam. cut to form a crater to receive sample
Exposure	1 min.
Plate	Ilford H. and D. 70
Development:	(a) 90 sec. at 19.5°
	(b) Fixed, acid hypo 15 min.
	(c) Washed running water 15 min.
	(d) Dried in current of warm air

standard conditions outlined in Table 1. For the Pb investigation the region 2700–4300 Å. was employed. Identification of the lines was achieved by use of the Judd Lewis Comparator, an instrument which permits of simultaneous microscopic examination of the test plate and of a standard plate showing the position of all the elements. The line densities of emission spectra are in direct ratio to molecular concentration in a sample of their elements of origin. By use of the Hilger non-recording microphotometer to measure the ratio of the line densities of different known quantities of Pb, and a constant amount of an internal reference element, a graph can be prepared which provides a means of measurement of the unknown amount of Pb in a sample.

Standard of internal reference. In our earlier investigations the bismuth line at 2898 Å. was chosen as the internal

working curves are obtained (Fig. 2). Some of the results from a series of identical samples used to check the accuracy of the graph are shown in Table 2. Errors of more than $\pm 8 \mu\text{g.}/100 \text{ ml.}$ of blood are exceptional, provided that the standard spectrographic conditions are strictly adhered to.

Fig. 2. Lead curve. Relation of spectrographic line intensity ratios to quantity of Pb. Ordinates, log intensity Pb 2833 A./Pt 2830 A.; abscissae, $\mu\text{g. Pb}$.

Table 2. Recovery of added lead

Material analyzed	Ash weight (g.)	Pb added ($\mu\text{g.}/100 \text{ ml.}$)	Pb found ($\mu\text{g.}/100 \text{ ml.}$)	Error ($\mu\text{g.}/100 \text{ ml.}$)
A. 5 ml. whole blood	0.0506	—	18	—
5 ml. whole blood and Pb	0.0522	40	60	+2
B. Whole blood	0.0516	—	17	—
Whole blood and Pb	0.0526	80	100	+3
Standard ash 1 + Pb	0.0500	50	48	-2
Standard ash 2 + Pb	0.0500	100	108	+8
Standard ash 3 + Pb	0.0500	120	116	-4
Standard ash 4 + Pb	0.0500	140	138	-2

Treatment of samples. 5 ml. of blood, urine, etc., were collected, transferred to a Pb-free silica crucible and sulphated by the addition of 3 ml. Pb-free H_2SO_4 . The samples were then ashed in a dust-proof electric furnace by treatment at 110° for 12 hr., followed by 500° for 8 hr.

The low Fe content of serum, urine and cerebrospinal fluid gives spectrographically what is known as a weak base spectrum, which would be a source of error in applying the blood Pb curve to their analysis, as was pointed out by Foster, Langstroth & McCrae [1935]. We therefore added 0.02 g. of synthetic ash to samples of serum and urine; in this way their main base spectrum was intensified to resemble that of the standard samples. Experiment showed that the results obtained with serum and urine were accurate to $\pm 10 \mu\text{g.}/100 \text{ ml.}$ As yet we have not obtained sufficient quantities of cerebro-spinal fluid to check the application of the Pb curve; we assume that since its main base spectrum is similar to that of serum and urine, the error, when the synthetic ash additions are made, will be of the same magnitude. By this method it is possible to report results on 20 samples within 24 hr. after collection, with minimal use of chemical reagents and vessels.

RESULTS

In a series of 80 normal unexposed subjects we have found the mean concentration of Pb in the blood to be $40 \mu\text{g.}/100 \text{ ml.}$; the results ranged from 5 to $120 \mu\text{g.}/100 \text{ ml.}$ of blood, and the majority fell between 20 and $60 \mu\text{g.}$

As was expected, those individuals who were exposed to the swallowing of metallic dusts, or inhalation of metal fumes, showed higher concentrations than office workers and the like. In four office workers, for example, the mean concentration was $16 \mu\text{g.}$, compared with $88 \mu\text{g.}$ in four metal workers. These latter findings are similar to those of Kehoe, Thamman & Cholak [1933a] and of Cholak & Bambach [1943].

The maximum normal concentration is the only thing that matters, since the concentration in an individual varies widely within normal limits from week to week. We have found, in sampling the same three individuals at intervals of four weeks, variations from 21–73 $\mu\text{g.}$, 150–53 $\mu\text{g.}$ and 43–11 $\mu\text{g.}$ respectively. One of us (A.T.) has found a similar variation in blood Pb concentration throughout the day. Although occupational exposure is mainly responsible for this variation in individual concentrations, the Pb content of the diet also contributes to it. From an analysis of foodstuffs we have found bread to contain 168 $\mu\text{g.}$ of Pb/100 g., tea biscuit 216 $\mu\text{g.}/100 \text{ g.}$, sausage and cheese 85 and 43 $\mu\text{g.}/100 \text{ g.}$ Some of the Pb content of these foodstuffs originates no doubt in contamination from vessels. That the Pb content of the diet contributes materially to blood Pb concentrations, conflicts with the view that there is little or no alimentary absorption of Pb by reason of the insoluble nature of the Pb compounds formed during the process of digestion. The total Pb in the circulation is, however, small in amount, and absorption of very small quantities may suffice to cause what is physiologically a substantial increase in its concentration in the blood. The following results (Table 3) show the effects on blood and urine concentrations of 12.5 grains lead

Table 3. Effect of ingesting lead on the content of blood and urine

		$\mu\text{g. Pb}/100 \text{ ml.}$ before ingestion	$\mu\text{g. Pb}/100 \text{ ml.}$ after ingestion
Blood	E.	11	250
	T.	14	155
	McE.	21	49
	B.	23	58
Urine	E.	5	107
	T.	3	72
	McE.	5	45
	B.	5	26

(5 ml. samples of blood or urine were analyzed.)

Fig. 1. Blood spectrograms. 1, shows Bi absent, while 2 denotes its presence due to absorption. 3, 4 and 5, blood spectrograms compared with a pure Pt spectrum 6 (note proximity of Pb 2833A. to Pt 2830A. and Pt 2834A.). Variation in blood Pb content is evident from densities of Pb 2833A. in 3, 4 and 5. 7 and 8, mother and infant bloods (case E). 9 and 10, infant urine and milk collected 5th day (case E); presence of Pb throughout clearly indicated, as also reduction of Fe lines in milk and urine.

acetate swallowed in the form of pills over three days by four subjects, who experienced no ill effects therefrom.

The bladder was emptied $\frac{1}{2}$ hr. before and the urine for test collected $\frac{1}{2}$ hr. after the blood was drawn. This gave an indication of the rate of excretion for a given blood concentration. From these results it is clear that considerable elevation of the blood Pb is possible by absorption of small quantities of Pb from the intestine.

Investigations on the blood of newly born infants shows that the Pb concentration approximates to that of the maternal blood. The investigation was carried out as follows. In the second stage of labour just before delivery 5 ml. of blood were withdrawn from the mother's arm, and 5 ml. from the umbilical vein prior to severance of the cord. The samples were covered and treated as described for spectrographing (A, B, C, Table 4, Fig. 1). In another

agree with Tompsett & Anderson [1939], that the blood and urine Pb do not run parallel, it is evident that, in health, elevated blood concentrations are balanced by rapid excretion of the Pb absorbed. Our results further indicate that some control over diet and occupational exposure is necessary in the investigation of suspected Pb poisoning, and that errors in diagnosis may arise from single analyses. The Pb content of the diet, and occupational exposure, are, no doubt, accountable for some of the divergencies in results reported by different observers. One other possible factor in the variation of results is the Pb transmitted from mother to foetus. The amount is surprising, as also are the facts that the infant appears to retain it, and that the Pb content of the milk is sufficient to maintain the infant's original concentration. The results on human milk are higher than those reported by Kehoe *et al.* [1933*b*]. This may possibly be attributable to differences in time of sampling and sensitivity of the analytical method used. The unfailing appearance of Pb in blood and tissue fluids, and its transmission to the foetus, raises the question as to whether Pb has a physiological function as yet undiscovered. The opportunities for such investigations are few and collection of the necessary samples considerably more difficult than might be thought. In spite of the use of Pb-free crucibles and test-tubes for direct collection of milk and infant urine, occasional minute contamination is inevitable.

SUMMARY

1. A spectrographic method of lead analysis in blood is described, using platinum as the internal reference element.

2. Blood lead concentrations encountered in 80 healthy subjects ranged from 5 to 120 $\mu\text{g./100 ml.}$ with a mean of 40 $\mu\text{g./100 ml.}$ and greatest frequency of from 40 to 60 $\mu\text{g./100 ml.}$ More than 50% of the results lie between 20 and 60 $\mu\text{g.}$

3. Lead is transmitted to the foetus in amounts approximating to those of the maternal circulation.

4. Lead is present in human milk and is very slowly excreted in infant urine.

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Table 4. Lead content of maternal and of infant blood, urine and milk

Subject	Lead ($\mu\text{g. Pb/100 ml.}$) in			
	Maternal		Infant	
	Blood	Milk	Blood	Urine
A	54	—	57	—
B	43	—	42	—
C	34	—	28	—
D	46	32	71*	2
E	22	8	37	7
F	33	20	18	3
G	35	5	25	—

* Doubtful result: contamination probable.

similar experiment, samples of the maternal milk and urine from the infant on the fifth day (D, E, F, G, Table 4, Fig. 1) were analyzed.

DISCUSSION

Some diversity of results has been reported for normal blood Pb concentrations by different workers. Blumberg & Scott [1935], by visual comparison of the line densities, found the normal to vary from 5 to 100 $\mu\text{g./100 ml.}$; a critical concentration between 100 and 150 $\mu\text{g.}$ produced symptoms. Kehoe, Thamman & Cholak [1935], using Bi 2898 A. as the internal reference element, reported 58 $\mu\text{g./100 ml.}$ to be the mean concentration in a series of 71 cases; this to us appears high. We agree with Blumberg & Scott that the maximum normal concentration is 100 $\mu\text{g./100 ml.}$ and consider that, in cases of chronic exposure, the critical concentration is around 130 $\mu\text{g./100 ml.}$ of blood. Although we

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The Vitamin B-complex Requirements of Several Insects

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It has been shown by us [Fraenkel & Blewett, 1943b] that six insects, which normally feed on flour and other dried food, grow satisfactorily on a diet of casein 50, glucose 50, yeast 5, cholesterol 1, McCollum's salt mixture 1, and water 15 parts, but entirely fail to grow when yeast is omitted. With the yeast replaced by yeast extract, the growth rate is considerably slower. No growth takes place if the diet contains a water-insoluble fraction alone in place of whole yeast. Previous work has indicated that insects require several vitamins of the B-group; the position up to 1940 has been reviewed by Craig & Hoskins [1940] and Trager [1941]. The present paper deals with the growth factors required by insects which are contained in the water-soluble and in the water-insoluble fractions of yeast. Short accounts of some of the results have already appeared elsewhere [Fraenkel & Blewett, 1942 a, b; 1943a, d].

EXPERIMENTAL

The following beetles were used: *Tribolium confusum* Duv., Tenebrionidae; *Silvanus (Oryzaephilus) surinamensis* L., Cucujidae; *Sitodrepa panicea* L., Anobiidae; *Lasioderma serricornis* Fab., Anobiidae; *Ptinus tectus* Boield., Ptinidae.

The following preparations and substances were used to compound diets:

Casein A/E, vitamin and fat free (Glaxo Laboratories Ltd.). Dried debittered brewer's yeast (Glaxo Laboratories Ltd.). Yeast extract, prepared from fresh brewer's yeast according to Chick & Roscoe [1930]. 'Insoluble yeast': residue from preparation of yeast extract, after 10 successive extractions with large volumes of boiling water.

Aneurin hydrochloride (B.) (Glaxo Laboratories Ltd.), riboflavin (Glaxo Laboratories Ltd.), nicotinic acid (British Drug Houses Ltd.), pyridoxin (Glaxo Laboratories Ltd.), Capantothenate (Glaxo Laboratories Ltd. and Roche Products Ltd.), choline chloride (British Drug Houses Ltd.), *p*-aminobenzoic acid (British Drug Houses Ltd.), *i*-inositol (British Drug Houses Ltd.), biotin methyl ester (Research Laboratories, S.M.A. Corporation), and biotin (Prof. du Vigneaud).

All the tests were performed in 2 × 1 in. specimen tubes. Each tube contained 2 g. of the dry diet and each diet was

prepared in duplicate. Each test was performed with 20 larvae, 10 in each tube. The tubes were kept in a room of which the temperature was automatically maintained at 25° and the relative humidity at 70%. The criteria for assessing results are rate of growth from the newly hatched larva until pupation (*Tribolium*) or emergence of adult (other insects), and mortality. The methods of experimenting and assessing results are fully described elsewhere [Fraenkel & Blewett, 1943 b, c].

Preparation of a diet deficient in riboflavin. Yeast extract was treated with norit charcoal (Hopkin and Williams, Ltd.) according to the method of El Sadr, Macrae & Work [1940]. The yeast extract was not previously autoclaved, but as a precaution aneurin HCl was added to the diet, which then consisted of casein 50, glucose 50, cholesterol 1, yeast charcoal filtrate 15, insoluble yeast 5, McCollum's salt mixture 1 parts, aneurin HCl 50 µg./g. Riboflavin was added to the diet in graded doses and the results showing a graded response (Table 1)

Table 1. *Pupation of Tribolium on a diet deficient in riboflavin, with the addition of graded doses of riboflavin*

Riboflavin (µg./g. of diet)	No. of pupae	Spread in the pupation period (days)
None	11	60–113
0.625	16	50–74
1.25	18	32–43
2.5	16	29–39
5.0	18	30–39
10.0	16	30–39
50.0	20	28–39
Wholemeal flour (control)	19	27–30

confirmed those reported by Fröbrich [1939], who grew *Tribolium* on a diet containing yeast extract which had been heated in an alkaline medium in order to destroy the riboflavin.

Substitution of yeast extract by pure vitamins. As pure vitamins of the B-group became available, attempts were made to feed mixtures of pure vitamins instead of yeast extract. Except in some earlier tests, wheat-germ oil was added to the diets