CCXVI. THE LEAD CONTENT OF HUMAN TISSUES AND EXCRETA.

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THAT lead is normally excreted in human urine and faeces is now well established. The literature has been reviewed by Kehoe *et al.* [1933, 1, 2], who have carried out an exhaustive study of the excretion of lead by normal American adults and children.

The occurrence of appreciable amounts of lead in "normal" human bones is also generally agreed upon, although there is considerable variation in the figures published, *e.g.* Barth [1931] finds 0·01–0·06 mg. Pb per g. ash or approximately 5–30 mg. Pb per kg. fresh bone, whilst Lynch *et al.* [1934] find 14–146 mg. Pb per kg. fresh bone. The position as regards the soft tissues is very unsatisfactory; few analyses are available and the evidence for the occurrence of "normal" lead in tissues is conflicting. Meillère [1903] states that small amounts of lead were present in the organs of nearly all the subjects examined by him (1–2 mg. per kg. on the average in the liver and spleen). Aub *et al.* [1926] state that the lead retained by an apparently normal individual is held almost exclusively by the skeleton. Weyrauch and Muller [1933] found no appreciable amount of lead in the liver, kidney, spleen or brain. Sheldon and Ramage [1931], using a spectrographic method, found lead occurring spasmodically in normal organs, whilst Boyd and De [1933], also using a spectrographic method, found lead well marked in the liver and present in all the other organs examined except the brain. Lynch *et al.* [1934] in an analysis of a few organs found 1·5 mg. per kg. in some livers and kidneys and none in others. Kehoe *et al.* [1933, 1] found appreciable amounts of lead in most of the tissues from two cases apparently normal shortly before death.

Whilst the estimation of lead in bones is comparatively easy, the soft tissues, having a high iron content and yielding only a small amount of ash, present considerable difficulties. It is probable that the differences in the published results may be attributed to the methods of analysis used, which are open to several criticisms. A number of methods have been used. Fairhall [1924] described a method in which the lead was precipitated as sulphide and then as the chromate. The lead chromate was determined either (1) colorimetrically with diphenylcarbazide or (2) by titration with thiosulphate after the addition of potassium iodide. Kehoe *et al.* [1926; 1933, 1, 2] used modifications of this method. Cooksey and Walton [1929], in an examination of urine, made a preliminary separation of lead by an electrolytic method. The lead was subsequently estimated nephelometrically as the sulphite. Francis *et al.* [1929] described a process involving the precipitation of lead as sulphide followed by electrolysis and precipitation as the sulphate. Finally the lead was estimated colorimetrically as the sulphide. Weyrauch and Muller [1933] and Litzner and Weyrauch [1932; 1933], investigating the distribution of lead in man, separated lead as the sulphide and then as the peroxide by electrolysis. They estimated the lead colorimetrically by

the blue colour formed by the interaction of the peroxide and tetramethyldiaminodiphenylmethane.

One of the major problems in the determination of lead is to separate it from substances that would interfere in the final stage of the estimation. The chief of these is iron. None of the above methods can claim to perform this process satisfactorily. Electrolytic methods usually fail in the presence of large amounts of iron [Francis *et al.*, 1929], while precipitation as lead sulphate or chromate is unsuitable as these substances have solubilities which are appreciable when fractions of a mg. of lead are being dealt with. In a recent paper [1935] Kehoe *et al.* acknowledge a loss of 0·07 mg. Pb per sample in the earlier method they employed.

A method suggested by Allport and Skrimshire [1932, 1] for separating lead from solutions of the ash of dyestuffs appeared to solve such difficulties. An alkaline solution of the ash was shaken up with a chloroform solution of diphenylthiocarbazone (dithizone). Lead was extracted by the chloroform as a lead-diphenylthiocarbazone complex. Iron was not extracted and other metals, with the exception of bismuth, were not extracted if cyanide were present. Under the latter conditions then, only two metals, lead and bismuth, were extracted. With this method it is recognised that certain difficulties are encountered. The aqueous solutions must be perfectly clear, the slightest turbidity due to phosphates, iron *etc.*, preventing a complete extraction of lead. As the extractions must be carried out on alkaline solutions, this is difficult, even when citrates have been added, for a solution may appear perfectly clear and yet iron, phosphates *etc.* may be precipitated in colloidal form and so prevent a complete extraction. The p_H of the solutions needs careful adjustment which is not always easy when certain classes of materials are being examined. If the organic matter has been destroyed by a wet oxidation method, the nature of the oxidant appears to exert a marked influence. Allport and Skrimshire [1932, 2] found that if nitric acid had been used as the oxidant, extraction of the lead was generally incomplete. This appears to be due to traces of oxidant remaining in the digest. We have found that in practice, when used to separate lead from solutions of the ash of urine, liver *etc.*, the method gave erratic results. In every case the solutions appeared perfectly clear.

In the final stage of the estimation of lead, the sulphide reaction appears to have been most commonly used. Unfortunately this is not specific for lead, bismuth giving a similar reaction. The sulphide reaction also lacks sensitivity. A more sensitive reaction is required for the determination of lead in blood, as the amount of blood that can be taken from a patient under routine conditions is limited. The objection to the tetramethyldiaminodiphenylmethane reaction is that although it is very sensitive, it is not specific for lead peroxide, substances such as manganese dioxide reacting similarly. Apart from the objections to electrolytic methods in general already referred to, manganese tends to be deposited as the dioxide along with lead. This is an especial failure of the method as manganese occurs in human tissues and excreta in appreciable amounts.

The present paper is divided into two parts. In the first a method for the estimation of lead, in which the difficulties outlined above have been overcome, is described. The second part deals with the lead content of human tissues obtained *post mortem*, and also the lead content of blood and excreta from hospital patients and normal individuals.

I. THE METHOD FOR THE ESTIMATION OF LEAD.

By S. L. TOMPSETT.

The separation of lead. When an aqueous solution of sodium diethyldithiocarbamate is added to a solution of a copper salt a yellow organic copper complex is formed which may be extracted with ether. The extraction is complete in acid, neutral or alkaline solution, *i.e.* is independent of p_H, but is preferably carried out in alkaline solution in the presence of pyrophosphate to prevent the extraction of iron. The exact adjustment of p_H is unimportant [Tompsett, 1935].

Lead also was found to form an organic complex with sodium diethyldithiocarbamate, which could be extracted with ether. The lead complex is white and therefore ethereal extracts are colourless. Amounts of lead varying from 0·01 to 0·2 mg. could be extracted quantitatively by such a technique. The complex of lead and sodium diethyldithiocarbamate is very insoluble in water, turbidities appearing when the reagent is added to 0·05 mg. Pb or more in 100 ml. water. Sodium diethyldithiocarbamate itself is insoluble in ether, so that the amount of organic material extracted is minimum.

The estimation of lead. Fischer and Leopoldi [1934] have published a colorimetric method using diphenylthiocarbazone for the estimation of small amounts of lead. When an alkaline solution of a lead salt was shaken with a carbon tetrachloride solution of diphenylthiocarbazone, a pink complex with lead was formed, which was extracted by the organic solvent. After the shaking process, the carbon tetrachloride layer contained pink lead complex and also unchanged green diphenylthiocarbazone. Unchanged diphenylthiocarbazone was removed by repeatedly shaking the carbon tetrachloride with 1 % KCN solution. Finally the pink extract was shaken with dilute acid, which changed the colour to green and then compared in a colorimeter with a standard. They stated that the method was quantitative and that amounts of lead of the order 6 to 120γ could be estimated, also that the reaction was specific for lead.

The writer has found that the pink colour is just as sensitive to colorimetric comparison as the green colour developed after shaking with acid. Using 10 ml. CCl_4 to extract the complex it was found that the depth of colour was proportional to the Pb concentration within the range 5 to 70γ. The best depth of colour for colorimetric comparison appears to be in the region of 0·01 and 0·02 mg. Pb. With amounts of lead above 0·03 mg. the colour was too strong for colorimetric comparison.

Reagents. *The determination of lead in urine and faeces.*

(1) Concentrated hydrochloric acid—analar reagent.
(2) Concentrated nitric acid—analar reagent.
(3) Perchloric acid—analar reagent.
(4) Glacial acetic acid—analar reagent.
(5) Ammonia (sp. gr. 0·88)—analar reagent.
(6) Ether—analar reagent.
(7) 10 % potassium cyanide—PbT (B.D.H.). This was diluted 1 in 10 as required.
(8) Carbon tetrachloride—analar reagent.
(9) 5 % sulphurous acid—lead-free.
(10) 20 % sodium citrate—lead-free.

A lead-free solution was prepared as follows. To 1 litre of a 20 % solution in water, 100 ml. of 0·1 % diphenylthiocarbazone in chloroform were added and

the mixture was shaken vigorously. As required, a small portion was passed through a filter-paper to remove suspended particles of chloroform.

(11) 0·1 % diphenylthiocarbazone in carbon tetrachloride.

Commercial diphenylthiocarbazone contains a yellow oxidation product which is soluble in carbon tetrachloride but is not extracted by alkali cyanide solutions. The commercial product was purified as follows. 100 ml. of 0·1 % diphenylthiocarbazone in carbon tetrachloride were extracted with several 100 ml. portions of 0·5 % ammonia. Diphenylthiocarbazone passes into the aqueous phase, leaving the oxidation product in the carbon tetrachloride. The ammoniacal extracts were passed through filter-paper to remove suspended particles of chloroform and then acidified with sulphurous acid. The green precipitated diphenylthiocarbazone was then extracted with 100 ml. of carbon tetrachloride. This solution if preserved under a layer of sulphurous acid (5 %) will keep indefinitely.

(12) 2 % sodium diethyldithiocarbamate.

Before use a small volume was shaken up with ether to remove traces of lead.

(13) Standard solution of lead acetate.

0·1831 g. of lead acetate $Pb(C_2H_3O_2)_2$, $3H_2O$ was dissolved in distilled water containing 5 ml. of glacial acetic acid. The volume was then made up to 1 litre with distilled water. 1 ml. of this solution is equivalent to 0·1 mg. Pb. This solution was diluted as required so that 1 ml. was equivalent to 0·01 mg. Pb.

The water used was glass-distilled. Filter-papers were washed with dilute acid, followed by distilled water. Pyrex glassware was used. Silica dishes were always cleaned out with hot dilute acid prior to use.

Method. 500 ml. of urine were evaporated to dryness in a silica dish on a steam-bath and then ignited over a Bunsen burner in a fume cupboard. Final traces of carbon were removed by adding 5 ml. of concentrated nitric acid to the cooled ash and heating further.

The ash was dissolved in 100 ml. of water containing 5 ml. of concentrated HCl, and the solution transferred to a 750 ml. separating funnel. 100 ml. of 20 % sodium citrate solution were added and the mixture was made slightly alkaline to litmus by the addition of ammonia (sp. gr. 0·88). The volume of the solution at this stage should be about 400 to 500 ml. 10 ml. of sodium diethyldithiocarbamate solution were added followed by 25 ml. of ether. The mixture was shaken vigorously. The aqueous layer was run off and the ether extract washed twice with 25 ml. of water. The ether extract was run into a 300 ml. Kjeldahl flask, the separating funnel being rinsed with 10 ml. of ether. The aqueous solution and washings were extracted a second time with ether. The combined extracts were evaporated to dryness on a steam-bath and the organic matter was destroyed by heating with 1 ml. concentrated sulphuric acid and 1 ml. perchloric acid. After digestion fumes were removed by a water pump. The following were added to the digest in order: 10 ml. water, 1 ml. glacial acetic acid, and 5 ml. ammonia (sp. gr. 0·88) and the volume was made up to 25 ml. with water.

The final stage of the estimation was carried out as follows. Three glass-stoppered 50 ml. volumetric flasks were taken. 5–10 ml. of the diluted digest were measured into one of the flasks. Similar amounts of blank solution were measured into the other two flasks. The blank solution was prepared in exactly the same way as the unknown. For every 5 ml. solution in the flasks 6 drops of sulphurous acid were added. Into one of the blank flasks 1 or 2 ml. of standard lead acetate solution (equivalent to 0·01 or 0·02 mg. Pb), depending on the lead

content of the unknown, were measured. To each flask were now added 5 ml. 1 % KCN solution, 10 ml. carbon tetrachloride and 0·5 ml. 0·1 % diphenylthiocarbazone. After vigorous shaking the contents of the flasks were poured into test tubes and the aqueous layers removed with a teat pipette. After being returned to the flasks, excess diphenylthiocarbazone was removed from the carbon tetrachloride layers by repeated extraction with 10 ml. lots of 1 % KCN solution. Usually 4–6 extractions were necessary. The pink extracts were then washed with water and compared with a standard in a colorimeter.

After washing with distilled water the extracts were quite clear and did not require filtration. Filtration should be avoided as yellow tints tend to develop after the extracts have passed through filter-paper. After the diluted digest has been shaken with diphenylthiocarbazone, the aqueous layer should be coloured brown, indicating that excess of the reagent is present, otherwise more must be added. In the event of 5 ml. of diluted digest containing more than 0·03 mg. Pb, a smaller volume should be used which should be diluted to 5 ml. with blank solution. A blank was always done to control contaminations from outside sources. The blanks showed perceptible pink tinges but were too small to be measured. That the blanks showed a reaction at all is due to the extreme sensitivity of the test.

In the case of faeces, 5–10 g. of dried material were ignited in a silica dish and the estimation proceeded with as above.

From the results shown in Table I it will be seen that lead added to urine or faeces may be estimated quantitatively by the above method. The urine and faeces used in these recovery experiments were collected with no special precautions to exclude contamination. The figures therefore cannot justifiably be taken as representative of the lead content of urine and faeces in the normal subject.

Table I. *The recovery of lead added to urine and faeces.*

	Urine. Volume used—500 ml.				Faeces. Weight of dried faeces used—10 g.		
	Initial lead content mg.	Lead added mg.	Lead recovered mg.		Initial lead content mg.	Lead added mg.	Lead recovered mg.
1	0·063	0·100	0·106	8	0·143	0·500	0·482
2	0·053	0·050	0·053	9	0·104	0·500	0·512
3	0·025	0·100	0·096	10	0·100	0·500	0·525
4	0·063	0·200	0·210	11	0·048	0·400	0·423
5	0·062	0·100	0·099	12	0·167	0·250	0·254
6	0·031	0·100	0·095				
7	0·077	0·100	0·105				

The specimens of urine and faeces were collected with no special precautions to exclude contamination from outside sources. The results must not be taken as representative of the normal.

By the use of an ignition method for destroying organic matter, blanks have been reduced to a minimum. There were no losses observed when this method was applied in the estimation of lead in urine and faeces. This is probably due to the high ash content of these materials, much of which is in the form of phosphates. It was not necessary to add pyrophosphates to prevent extraction of iron in the preliminary separation of lead as they are formed in sufficient quantity during the ignition process.

In preliminary experiments the carbon tetrachloride extracts containing the lead diphenylthiocarbazone complex often had yellow tints which made colorimetry difficult. It was considered that this was due to traces of perchloric acid in the digests. The addition of 6 drops of sulphurous acid to every 5 ml. of

diluted digest used prevented the formation of this yellow colour and perfect matching colours could be obtained.

Although sodium diethyldithiocarbamate is not a specific reagent for the separation of lead, those metals that are extracted, *e.g.* copper, zinc, bismuth, do not interfere, as the complexes that they form with diphenylthiocarbazone are unstable in the presence of cyanide. The bismuth complex is orange-coloured and if present will be seen in the carbon tetrachloride layer after the first shaking. It is removed however during the subsequent extractions with cyanide. In actual experiment it was found that 0·01 mg. Pb could be estimated in the presence of 0·1 mg. Bi, the bismuth complex being completely removed at the fourth extraction. The complexes of the other metals are so unstable in the presence of cyanide that they are not formed at all.

The separation of lead as the complex with sodium diethyldithiocarbamate serves the very useful purpose of removing those substances such as iron and the phosphates of the alkaline earths which interfere with its reaction with diphenylthiocarbazone.

After extraction of the diethyldithiocarbamate complexes with ether, a few ml. of dilute copper sulphate solution should be added to the residual aqueous solution. The formation of a golden brown colour will indicate that excess of the diethyldithiocarbamate reagent has been added. In the majority of cases the amount of reagent indicated in this paper is in marked excess but in a few cases more may be necessary.

II. THE LEAD CONTENT OF HUMAN TISSUES AND EXCRETA.

By S. L. TOMPSETT AND A. B. ANDERSON.

EXPERIMENTAL.

Urine and faeces. The urine was collected in paraffined bottles and 500 ml. were taken for each estimation. The faeces were collected directly into large pyrex glass dishes in which they were dried. For each estimation 5–10 g. of dried and powdered material were used.

Soft tissues. The organs were first weighed and, except in the case of lung, 100 g. of tissue were chopped into small slices and placed in 100 ml. of lead-free 10 % sodium phosphate solution in a silica dish. The whole right lung was first dried at 110° in a pyrex dish, ground to a powder and an aliquot portion of powder added to the phosphate solution in a silica dish. The mixture of tissue and phosphate solution was then evaporated to dryness on a steam-bath, and the subsequent procedure was the same as that described for urine and faeces. In the final colorimetric estimation 5–10 ml. of diluted digest were taken and compared with standards containing 0·01–0·02 mg. of lead.

Bone. About 5 g. of bone in small pieces were put into a silica dish without phosphate solution and ashed directly. The ash was treated in the same manner as that from other tissues.

Blood. Some modification of the method was necessary when dealing with blood owing to the smaller quantity of material used. The method is therefore given here in some detail. Approximately 20 ml. of blood were drawn from a vein with an all-glass syringe and stainless steel needle. Syringe and needles were sterilised by boiling in distilled water. The blood was immediately poured into a pyrex tube and rapidly pipetted into 100 ml. of lead-free 10 % sodium phosphate solution in a silica dish. The exact volume of blood obtained was

noted. The contents of the dish were then evaporated to dryness on a steam bath and ashed in the same manner as the tissues, using only 1 ml. of nitric acid. The ash was dissolved in 50 ml. of water containing 2 ml. of concentrated HCl and the solution transferred to a separating funnel. The solution, which with the washings amounted to 100–150 ml., was made faintly alkaline to litmus by the addition of ammonia (sp. gr. 0·880), and cooled. After the addition of 2 ml. of 2 % sodium diethyldithiocarbamate and 25 ml. of ether the mixture was shaken vigorously and allowed to separate and the aqueous layer run off. The ether extract was washed with 25 ml. of water and then run into a 150 ml. round-bottomed pyrex flask, a further 10 ml. of ether being used to wash the funnel. The aqueous solution was re-extracted with 10 ml. ether, which were added to the first extract, together with a second washing of 10 ml. of ether. The ether was then evaporated off on a steam-bath and the organic material in the residue destroyed by digestion with 0·2 ml. of sulphuric acid and 0·2 ml. of perchloric acid. The fumes were then removed from the flask by suction with a water pump, and 3·5 ml. water, 0·2 ml. glacial acetic acid and 1·0 ml. ammonia (sp. gr. 0·88) added in that order. After the addition of 6 drops of 5 % sulphurous acid the contents of the digestion flask were transferred to a 50 ml. glass-stoppered pyrex volumetric flask and the digestion flask was washed out with 5 ml. of 1 % KCN into the volumetric flask. To the mixture 10 ml. of carbon tetrachloride and 0·2 ml. diphenylthiocarbazone solution were added. The colour was then developed in the usual way and compared with a standard prepared similarly. For normal blood a standard containing 0·01 mg. Pb was found to be suitable when 20 ml. of blood were used. Sodium citrate was not added in the analysis of blood as any slight haze of calcium or magnesium phosphates did not interfere with the extraction.

Sodium phosphate was added to the blood and soft tissues before ashing for two reasons; firstly to increase the amount of ash and to prevent the formation of insoluble ferric oxide; secondly to form an excess of pyrophosphate which prevents the extraction of iron in the separation process. The sodium phosphate which was kept as a 10 % stock solution was always de-leaded just before use in the following manner. To every 100 ml. of solution in a separating funnel 5 ml. of 2 % sodium diethyldithiocarbamate solution were added and the whole was shaken vigorously with ether. The lead-free aqueous solution was then run off. The phosphate solution was not added to urine, faeces or bone, as these substances contain enough phosphate.

The results of analysis of organs from 22 *post mortems* are given in Table II, which is divided into 20 cases with no known occupational lead exposure and 2 cases with occupational exposure to lead, namely a painter and a printer. The figures are given for concentration in mg. Pb per kg. of fresh tissue and also for the total organ where possible. In the cases of rib and vertebra, concentrations alone are given. In order to obtain a representative sample of lung the whole organ was first dried and powdered as described above. For this reason only the total lead content of the lung is given. The cases in the first division range in age from 69 years to 6 weeks and consist of 11 males and 9 females. Lead in appreciable amounts was found in all the tissues examined, and with the exception of the spleen, the amounts present were remarkably constant considering the widely different pathological states involved. The figures for bone: rib, mean concentration 8·55 mg. per kg., and vertebra, mean concentration 7·09 mg. per kg., are the most constant, the greatest deviation from the mean being in the vertebra of No. 7 with a concentration of 14·7 mg., or just more than twice the mean value. The child of 2 years of age, No. 6, shows the

Table II. *Lead content of human tissues.*

	Age	Sex	Occupation	Diagnosis	Liver		Kidney		Spleen		Brain		Rib	Vertebra	Right lung
					mg. per kg.	Total mg.	mg. per kg.	Total mg.	mg. per kg.	Total mg.	mg. per kg.	Total mg.	mg. per kg.	mg. per kg.	Total mg.
				(*a*) Cases with no known occupational exposure to lead.											
1	61	M	None	Carcinoma colon	1·50	1·65	0·80	0·19	—	—	—	—	10·72	4·47	—
2	35	M	Timekeeper	T.B. meningitis	0·85	1·22	1·26	0·44	—	—	0·36	0·50	9·76	8·93	—
3	59	F	Housewife	Diabetes mellitus	1·10	1·96	2·60	0·83	—	—	—	—	8·43	5·58	—
4	69	M	None	Carcinoma rectum	2·50	2·99	1·50	0·41	—	—	—	—	6·90	5·35	—
5	50	F	Housewife	Chronic nephritis	1·20	1·37	—	—	0·63	0·09	0·64	0·73	7·80	5·45	—
6	2	M	—	Bronchopneumonia	1·53	—	0·74	—	—	—	0·32	0·42	9·53	8·23	—
7	35	M	Cinema proprietor	Cerebral aneurism	1·26	2·39	1·43	0·56	—	—	0·55	0·72	12·9	14·7	—
8	40	F	Housewife	Haemorrhage	0·95	0·93	1·73	0·45	—	—	—	—	5·26	6·18	—
9	57	M	Ex-soldier	Syphilitic aortitis	1·57	2·04	0·72	0·22	0·77	0·17	—	—	8·15	6·61	—
10	41	M	Iron dresser	Lobar pneumonia	2·56	3·69	2·04	0·69	5·90	0·83	—	—	7·47	8·75	—
11	64	M	Machinist	Valvular disease	0·99	1·55	1·21	0·51	1·49	0·28	—	—	11·89	7·17	—
12	40	F	Housewife	Myocarditis	2·10	3·82	0·98	0·31	0·95	0·34	0·72	0·86	10·00	10·95	0·65
13	37	M	Storekeeper	Bact. endocarditis	1·35	1·80	0·91	0·29	0·64	0·15	—	—	7·59	5·96	0·28
14	39	F	Housewife	Acute pancreatitis	0·98	1·18	0·87	0·22	0·74	0·05	—	—	10·23	9·75	0·26
15	19	F	Typist	Obstruction	4·63	4·42	1·13	0·23	0·69	0·08	—	—	5·43	7·51	0·88
16	25	F	—	Carcinoma rectum	2·14	3·42	0·87	0·33	0·72	0·19	—	—	5·00	5·03	0·82
17	59	M	Warehouseman	Carcinoma oesophagus	2·13	1·89	0·74	0·16	3·39	0·23	—	—	5·85	4·21	0·28
18	23	F	Shop assistant	Diabetes mellitus	2·00	3·50	0·83	0·31	3·84	0·42	0·43	0·64	11·00	4·28	0·38
19	54	F	Housewife	Diabetes mellitus	1·49	3·73	0·85	0·31	0·37	0·25	—	—	8·55	5·70	0·42
20	6 weeks	M	—	Subarachnoid haemorrhage	1·95	0·39	3·55	0·14	3·08	0·07	0·24	0·07	1·57	2·60	0·079 (both)
	Mean, excluding No. 20				1·73	2·42	1·35	0·38	1·68	0·26	0·50	0·645	8·55	7·095	0·50
				(*b*) Cases with occupational exposure to lead.											
21	41	M	Painter	Otitis media meningitis	4·50	6·75	1·00	0·38	—	—	1·00	1·40	119·4	18·8	—
22	60	M	Printer	Myocarditis	2·40	2·88	1·00	0·49	—	—	—	—	22·0	8·5	—

same concentrations of lead as the adults in liver, kidney, brain and bones. The spleen was not analysed. The figures for the 6-weeks old infant are remarkably high for some of the organs. The concentrations in the kidney and spleen of 3·55 and 3·08 mg. respectively are approximately twice the mean values for adults. The concentrations in the bones, 1·57 mg. for rib, and 2·6 for vertebra, are considerably less than the adult figures, as would be expected. The *post mortem* on this child showed a normal well-nourished baby and the organs, with the exception of the brain, were normal. Of the two cases with occupational exposure to lead, the painter shows an excessive deposition of lead in the bones; the concentration in the rib of 119 mg. is fourteen times the mean value, and that in the vertebra, 18·8, approximately two and a half times the mean. The printer shows a slight excess in the rib only. There is no suggestion of lead poisoning in the clinical notes on these two cases.

Table III. *Lead content of tissues from human foetuses.*

	Months gestation	Sex	Weight g.	Liver		Kidney		Brain		Femur
				mg. per kilo	Total mg.	mg. per kilo	Total mg.	mg. per kilo	Total mg.	mg. per kilo
1	8	M	2400	0·33	0·04	0·66	0·01	0·12	0·03	1·49
2	twin	F	2100	0·83	0·07	0·63	0·01	0·21	0·04	2·66
3	8·5	M	—	0·63	0·06	0·63	0·01	0·18	0·07	1·47
4	7	M	1600	0·95	0·06	0·67	0·01	0·16	0·04	1·30
			Mean	0·68	0·06	0·65	0·01	0·17	0·045	1·73

The tissues of four stillborn foetuses, two of which were twins, were also analysed, using the same method except that, in the case of the kidney, the modifications described for blood were used. For the analysis of bone, both femurs were used. The results are given in Table III. Lead was present in appreciable concentrations in all the tissues examined. The mean values for liver, kidney, and brain are from one-half to one-third of the adult values. The concentration in the femurs approximates to that found in the rib of the infant of 6 weeks. Of the twins, the female shows higher values than the male. For comparison, the copper in these tissues was determined at the same time; the mean values obtained were, in mg. per kg., liver—44·6, brain—1·08, and femur—1·86. These results show the usual selective absorption of copper by the foetal liver. The amount in the livers was at least five times that reported for the adult [Tompsett, 1935].

Urine and faeces were collected from three laboratory workers, as normals, and from ten hospital patients, in periods of two or three consecutive days. The normals were working and eating their ordinary diet. The patients, with the exception of No. 5 who was a case of pernicious anaemia, were in the metabolic wards for determination of basal metabolic rate, which in every case was within the normal limits. The diagnosis in most cases was tachycardia of unknown origin or "neurosis". The figures for the daily excretion of lead in the urine and faeces are given in Table IV. It will be seen that the normals excreted daily 0·16 to 0·03 mg. in the urine and 0·40 mg. Pb in the faeces. The patients' excretion in the urine varied from 0·07 to 0·025 mg., with a mean of 0·05 mg., and in the faeces from 0·26 to 0·20 mg., with a mean of 0·22. A half-day's diet as given in the metabolic ward was dried down in a pyrex dish, and on analysis by the method used for tissues gave 0·22 mg. Pb *per diem*. The hospital water was found to contain 0·03 mg. Pb per litre. Patients on this diet would appear to excrete a fairly constant amount of lead, and this amount is less than that

excreted by the normal laboratory workers. This difference can probably be accounted for by a greater intake of lead in the diet and drinking water of the normals. The occurrence of large amounts of bismuth in the faeces during bismuth medication will interfere with the lead estimation. The only remedy is to discontinue the bismuth, and analyse the faeces in a few days' time, when the small amounts of bismuth that may be present will not interfere, because the bismuth diphenylthiocarbazone complex is unstable when shaken with cyanide.

Table IV. *The excretion of lead in urine and faeces.*

	Sex	Days collection	Occupation	Urine mg. *per diem*	Faeces mg. *per diem*
			(*a*) Normals.		
1	M	3	Laboratory worker	0·16	0·40
2	M	3	Do.	0·03	0·40
3	M	1	Do.	0·085	0·39
			(*b*) Hospital patients.		
4	M	3	Warehouseman	0·04	0·20
5	F	3	Housewife	0·025	0·23
6	M	3	Miner	0·06	0·26
7	M	2	Packer	0·055	0·23
8	F	3	Housewife	0·07	0·24
9	F	3	Do.	0·05	0·20
10	M	2	Miner	0·06	0·22
11	M	2	Engineer	0·05	0·24
12	M	3	Brass moulder	0·04	0·20
13	M	2	Packer	0·06	0·20
			Mean for patients	0·05	0·22

Before estimating the lead in the blood some recoveries of lead added to blood and serum were undertaken to test the method as modified for the smaller quantities. As will be seen from the figures given in Table V, a good recovery of lead added to blood was obtained. It should be emphasised here that the figures for the lead content of blood in Table V have no significance because the blood was mixed and obtained from contaminated sources.

Blood was then collected from the same normals as before and from 18 hospital patients. Twenty-five samples in all were taken. The figures are given in Table VI. The patients were chosen from among those not acutely ill, and where no diagnosis is given it is to be understood that no physical signs of organic disease were present. The blood lead varied from 70 to 40γ per 100 ml., with a mean value of 55γ. The figures are given to the nearest 5γ. In the case of normal No. 3 several figures are given; these represent determinations done on blood collected on different days both before and after meals.

Of several cases of suspected lead poisoning investigated only one showed definite plumbism and this case is discussed here. The clinical history is briefly as follows. A young man of 30 years of age was admitted to the medical wards

Table V. *Recovery of lead added to serum and blood.*

Initial lead content γ	Lead added γ	Recovered lead γ	Initial lead content γ	Lead added γ	Recovered lead γ
(*a*) Serum. 10 ml. samples.			(*b*) Blood. 10 ml. samples.		
5·5	5	6·0	12·9	5	5·6
5·5	10	9·0	12·9	10	10·5
5·5	5	4·5	12·9	100	102·1
5·5	10	10·5	14·0	5	5·5
			14·0	10	9·0

Table VI. *Lead content of blood.*

	Age	Sex	Occupation	Diagnosis	Blood lead γ per 100 ml.
1	32	M	Laboratory worker	Normal	50
2	19	M	Do.	Do.	40
3	29	M	Do.	Do.	70, 40, 60, 50
4	30	M	Motor driver	Duodenal ulcer	50
5	26	F	Housewife	Hysteria	60
6	—	M	—	—	55
7	53	M	Brass moulder	Debility	50
8	32	F	Housewife	Epilepsy	55
9	—	M	—	—	45
10	62	F	Housewife	Hypertension	60
11	—	M	—	—	45
12	25	M	Engineer	Duodenal ulcer	50
13	65	M	Steelworker	Hemiplegia	65
14	—	M	—	—	55
15	50	M	Packer	Neurasthenia	60, 50
16	45	M	Colliery repairer	Osteoarthritis	50
17	52	M	Park labourer	Gastric ulcer	60
18	26	F	Housewife	Vasovagal attacks	60
19	—	M	Miner	—	50
20	72	F	Teacher	Neurosis	65
21	15	M	Unemployed	Abdominal pain	65
				Mean	55

complaining of severe pains in the stomach with vomiting of a week's duration. The pain had no relation to food and was not affected by food. His occupation was that of a solder-maker. In this trade, which he had followed all his adult life, he mixed molten lead, tin and other metals. The clinical findings were a moderate anaemia, blood count showed 3,000,000 red cells, and 40% haemoglobin, with some punctate basophilia, no wrist drop or other signs in the nervous system. There was a marked blue line round the gums. The stools contained blood. An X-ray of the stomach did not reveal any lesion.

Table VII. *Case J. C. admitted to medical ward 27. i. 35.*

Date	Blood lead γ per 100 ml.	Urine		Faeces	Diet lead
		mg. per litre	mg. *per diem*	mg. *per diem*	mg.
28. i. 35	—	0·27	0·12	—	—
29. i. 35	135	—	—	—	—
19. ii. 35	125	0·073	0·135	0·27	0·13
21. ii. 35	Started medication with potassium iodide 15 gr. *per diem*				
5. iii. 35	135	0·107	0·17	0·19	—
18. iii. 35	Discharged on potassium iodide 15 gr. *per diem*				
15. iv. 35	Readmission to metabolic ward complaining of return of symptoms				
16. iv. 35	380	—	—	—	—
19. iv. 35	—	0·036	0·041	0·44	0·22
24. iv. 35	240	—	—	—	—

The chemical investigation is summarised in Table VII. It will be seen that soon after admission the urinary and faecal excretion of lead was not higher than would be expected in a normal person. In this connection the intake of lead must also be considered. For the first few days he was on a milk diet and later was given a peptic ulcer diet. A sample of the latter diet was analysed and found to contain 0·13 mg. of lead *per diem*. This alkaline milky diet was probably increasing the storage of lead in his bones. The figures for urine show the importance of calculating the excretion per day and not relying on the amount per litre. In contrast to his excretion of lead the blood lead was high,

being at least twice the normal. On 21. ii. 35 medication with potassium iodide, 15 grains *per diem*, was started. Ten days afterwards blood lead and the excretion of lead were the same as before. He was now free from pain and was discharged and given the potassium iodide, which he continued to take until he was readmitted 4 weeks later to the metabolic ward complaining of sharp pains in the stomach. The potassium iodide was discontinued on admission, and blood taken the next day gave the surprisingly high figure of 380γ Pb. He was on the ordinary ward diet, and a 3-day collection of excreta showed a normal urinary excretion but a faecal excretion about twice that of patients on the ward diet. The pain became less and less severe, and 10 days after admission his blood lead had fallen to 240γ. The lead line had almost disappeared when he was readmitted.

This man had been subjected to a prolonged occupational exposure to lead, both as solid and vaporised, and must have had large deposits in his bones. His symptoms were of the gastro-intestinal type probably with intestinal ulceration giving blood in the stools. Though showing clinical signs of lead poisoning and having a high blood lead, he showed little increase in lead excretion. The results of administration of potassium iodide for a period of weeks were a return of the abdominal pain and an increase in the blood lead to nearly treble the original figure.

Discussion.

The salient features of the analysis of "normal" soft tissues are the presence of lead in all the tissues examined, and the comparatively constant concentration for the adult irrespective of age, sex or cause of death. The presence of small quantities of lead in the soft tissues might be deduced from the fact that lead is a constant constituent of blood. It is not suggested, however, that lead is necessarily present in these concentrations in the normal during life. The changes in metabolism preceding death may bring about a mobilisation of lead from the deposits in the bones. Lead was found in the bones in amounts of the same order as those reported by Kehoe *et al.* [1933] and Barth [1931]. The figures show less variation from the mean than those for the soft tissues. Analysis of bones other than the ribs and vertebrae taken in this investigation would probably give different results. The higher figures reported by Lynch *et al.* [1934] were obtained in the analysis of the shaft of the femur principally.

Whilst our results for tissues may be taken as "normal" for cases in the Glasgow Royal Infirmary, it is possible that a similar investigation in another part of the country would reveal different "normals". This "normal" figure is of importance in the evaluation of results undertaken for toxicological purposes. Whilst lead was present in all the foetal tissues examined, the amounts present, which were of the same order as that in normal blood, do not indicate any selective absorption of this element in the foetus. By contrast, copper was concentrated in the foetal livers.

In any investigation of the excretion of lead, the important figures are those for the amount excreted over a given period of time. For this reason we have given all results for urine and faeces as mg. per day. In the case of urine the concentration of lead varies with the volume secreted, and results expressed in mg. per litre, which is the usual practice, may be misleading.

The estimation of blood lead described above has many advantages. It can be completed in 24 hours, and, as only 20 ml. of blood are required, it can be repeated several times on the same subject, if necessary. The lead content of the normal bloods examined showed only comparatively small variations and the

mean value of 55γ/100 ml. agrees with that of 60γ reported by Kehoe *et al.* [1935] for a group of medical students. On the other hand Litzner and Weyrauch [1933] consider that a figure above 40γ indicates an increased lead absorption. The method used by Litzner and Weyrauch is open to several criticisms which have been mentioned already. The estimation of blood lead would appear to be a more satisfactory method of investigation than the determination of the excretion of lead, the significance of which is unavoidably obscured by the presence of unabsorbed lead in the faeces, where the greater excretion is to be expected. This is illustrated by the case of plumbism reported here.

Lead is normally considered to be an accidental body constituent, and the term "normal" has been decried on these grounds. When the general occurrence of lead in foodstuffs and its presence in the tissues generally are taken into account it seems more reasonable to describe lead as a normal constituent of the human body.

With the information at present available the results of analyses of tissues, blood and excreta only justify a statement as to whether normal or abnormal amounts of lead are present. The final diagnosis of lead poisoning is in the province of the clinician, who can correlate the clinical, haematological and chemical findings.

Summary.

1. An accurate method has been described for the estimation of lead in human tissues, blood and excreta. After ashing, the lead was extracted with ether as a complex with sodium diethyldithiocarbamate. The lead in the ether extract, after destruction of the organic material, was determined colorimetrically with diphenylthiocarbazone.

2. Lead was found in all the tissues examined. The mean concentrations in mg. Pb per kg. for adults were: liver—1·73, kidney—1·34, spleen—1·68, brain—0·5, rib—8·55, vertebra—7·09. Tissues from a case of known exposure to lead showed higher figures, more especially the rib, with 119 mg. per kg.

3. Analysis of four foetuses of 7–8 months' gestation gave mean concentrations in mg. per kg. of: liver—0·68, kidney—0·63, brain—0·17, femur—1·73. Lead was found in all these tissues in each case.

4. Figures for the excretion of lead in urine and faeces by normal laboratory workers and hospital patients are given. The mean daily excretion of lead by 10 patients was 0·05 mg. for urine and 0·22 mg. for faeces.

5. The analysis of 25 samples of blood obtained from 3 normals, and 18 patients, none of whom were acutely ill, gave values of 40–70γ per 100 ml., with a mean value of 55γ.

6. A case of plumbism in a solder-maker showing very high concentrations of blood lead is reported.

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REFERENCES.

Allport and Skrimshire (1932, 1). *Analyst*, **57**, 440.
—— —— (1932, 2). *Quart. J. Pharm. Pharmacol.* **5**, 461.
Aub, Fairhall, Minot and Reznikoff (1926). Lead poisoning, p. 56.
Barth (1931). *Virchow's Arch.* **281**, 146.
Boyd and De (1933). *Ind. J. Med. Research*, **20**, 789.
Cooksey and Walton (1929). *Analyst*, **54**, 97.
Fairhall (1924). *J. Biol. Chem.* **60**, 485.
Fischer and Leopoldi (1934). *Z. angew. Chem.* **47**, 90.
Francis, Harvey and Buchan (1929). *Analyst*, **54**, 725.
Kehoe, Edgar, Thamann and Saunders (1926). *J. Amer. Med. Assoc.* **87**, 2081.
—— Thamann and Cholak (1933, 1). *J. Indust. Hyg.* **15**, 273.
—— —— —— (1933, 2). *J. Indust. Hyg.* **15**, 301.
—— —— —— (1935). *J. Amer. Med. Assoc.* **104**, 90.
Litzner and Weyrauch (1932). *Arch. Gewerbepath. Gewerbehyg.* **4**, 74.
—— —— (1933). *Med. Klin.* **29**, 381.
Lynch, Slater and Osler (1934). *Analyst*, **59**, 787.
Meillère (1903). *Compt. Rend. Soc. Biol.* **55**, 517.
Sheldon and Ramage (1931). *Biochem. J.* **25**, 1608.
Tompsett (1935). *Biochem. J.* **29**, 480.
Weyrauch and Muller (1933). *Z. Hyg. Infectionskrank.* **115**, 216.