H and *G* are removed and their contents are run off at T_1 and T_2 into a covered flask. *H* and *G* are washed out twice with CO_2 -free water and the washings added to the rest of the alkali. The residual $\text{Ba}(\text{OH})_2$ is titrated with 0.1 N-HCl, using thymolphthalein as indicator. Each determination takes about 30 min.

More dilute alkali and HCl are used for smaller quantities of sample.

Accuracy of method

Five 0.5 ml. portions of sucrose solution, each containing 10.8 mg. of carbon, were dried at 90° before analysis. The results were 11.0, 10.9, 10.8, 11.3 and 10.6 mg. (mean 10.92 mg., s.d. 0.23 mg.). Five determinations of carbon content were carried out upon potassium hydrogen phthalate (A.R.) using approx. 10 mg. of dry solid, giving a mean of 47.0 % (s.d. 0.8 %, calculated 47.1 %). Five determinations

were carried out without prior drying down upon 0.2 ml. of a solution containing approx. 10 mg./ml. of potassium hydrogen phthalate; the results gave a mean of 47.6 % (s.d. 1.3 %; calculated by direct titration 47.2 %). Determinations carried out on mould metabolism solutions, after drying down, and upon dried mycelial felts have given the same order of reproducibility at approx. 5 mg. carbon/sample (s.d. 2.1 and 1.9 % respectively).

SUMMARY

A new apparatus for the determination of carbon in biological materials by wet combination is described.

My thanks are due to Mr K. Bartholemew, glass-blower, for making the apparatus.

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Polarographic Determination of Lead in Urine

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Most of the methods that have been proposed in recent years for the determination of small amounts of lead are based on colorimetry and the use of dithizone (Tompsett & Anderson, 1935; Kerch, 1940). For microdeterminations of metals the methods of polarographic analysis are attractive since they are simultaneously qualitative and quantitative, and the diffusion currents from which values are obtained can be measured accurately and reproducibly. Since extraneous lead is the only substance which can contribute to the reagent blank, the latter is generally smaller than would be found in a colorimetric method, which may also be less specific.

Procedures for the polarographic determination of lead in urine have been described by Bombach & Cholak (1941), Reed & Gant (1942) and Weber (1947). Bombach & Cholak (1941) introduced a stage of electrolytic separation, and each of the three methods involved dry ashing at high temperatures; with the methods described below it has been possible to reduce the time taken for each determination, to avoid dry ashing, and to use less specialized equipment than is demanded by the above authors, while considerably improved accuracy and sensitivity are attained in a special modification.

Lead may be determined polarographically in a variety of conditions (Kolthoff & Lingane, 1941);

when dissolved in acid citrate or tartrate solutions (Suchy, 1931; Shakhov, 1939) the conditions are especially suitable since the half-wave potential is smaller in acid than in neutral or alkaline solution. Since the lead 'wave' is found at a lower applied potential in acid media, there is a greater chance of any other electro-reducible substances giving their waves after lead, in which case their presence will not interfere. The author has found that the diffusion current of lead may be readily and accurately determined in 0.2 M-dipotassium hydrogen citrate (pH 5.2), and that in this medium the waves are not in any way distorted by the formation of maxima; the half-wave potential was found to be -0.49 V. (measured against the Standard Calomel Electrode).

In the present work, the lowest concentration of lead in the polarograph cell which gives a readily measurable diffusion current has been found to be of the order of $500 \mu\text{g./l.}$ Most values reported in the literature for the urinary excretion of lead by normal subjects are below $100 \mu\text{g./l.}$ (Kerch, 1940; Tompsett & Anderson, 1935); a process for concentration of the metal is therefore necessary. Also, since it is possible that urine may contain polarographically active substances which might interfere with the determination of lead, a partial isolation of the lead has been incorporated in the final method. This

twofold requirement is met by a double entrainment and a destructive oxidation of organic material; metals other than lead, and also inorganic anions, are not necessarily eliminated, but are found not to cause any inconvenience. The lead is obtained finally as a citrate complex dissolved in citrate buffer.

It has been shown that lead is entrained quantitatively in the precipitation of calcium either as oxalate at pH 4.5 (Ross & Lucas, 1935) or as phosphate in strongly ammoniacal solution (Ross & Lucas, 1933).

Although calcium forms a soluble complex citrate, calcium oxalate is not dissolved by solutions of either citric acid or citrates. Calcium phosphate precipitates, on the other hand, dissolve rapidly even in neutral citrate solutions. Since, however, substances other than calcium (e.g. magnesium and iron) are precipitated from urine by addition of ammonia, the phosphate precipitate obtained in ammoniacal solution is not always completely soluble in citrate buffer, so that with some samples of urine the maximum current for lead was found only after several hours; in one case the final value was 10 % greater than that found initially.

A rapid but rough method (method *A*) was therefore available. A precipitate of calcium phosphate was formed in urine, separated, washed, dissolved as completely as possible in citrate solution and the lead determined polarographically.

In a more accurate procedure (method *B*), calcium was precipitated as oxalate at pH 4.5, the solid was 'ashed' with perchloric acid and the calcium precipitated from alkaline solution as phosphate, which dissolved completely with the lead in citrate buffer. The second precipitation as phosphate was employed since it was difficult to buffer the ashed perchlorate solution reproducibly and with sufficient accuracy without increasing the volume to an undesirable extent.

Determination of, or allowance for, the reagent blank is as important here as in most micromethods. Therefore a compensation was introduced by treating two volumes (25 and 5 ml.) of urine with the same quantities of reagents, the lead content of the difference (20 ml.) being deduced by comparison of polarograms. Lead also forms a soluble complex in tartrate solutions, and it has been shown by Forche (1938) that adsorbed lead salts can be leached from insoluble material by tartrate solutions, although prolonged extraction (2 hr.) is required. Since, however, solutions of tartrates do not dissolve calcium oxalate or phosphate, a citrate medium was chosen, since, with this, complete solution of the solid material can be readily achieved, thus ensuring the presence of the adsorbed or co-precipitated lead in the liquid phase.

EXPERIMENTAL

Urine is collected as 24 hr. specimens in Winchester quart bottles which have been washed with warm HNO_3 (A.R., about 8M) and which contain 10 ml. of conc. HNO_3 (A.R. about 16M).

Reagents

These are all of A.R. quality.

Ammonia, sp.gr. 0.880.

Calcium chloride, 0.225M. The solution is freed from Pb by adding 0.005 vol. of the NH_4 oxalate solution (below), stirring from time to time for 1 hr. and then filtering through Whatman no. 541 paper.

Ammonium oxalate, 0.35M. The solution is adjusted to pH 4.5 with acetic acid and NH_3 ; it is freed from Pb by addition of 0.01 vol. CaCl_2 solution, standing, stirring and filtering as above.

Dilute oxalate solution, for washing precipitates, is prepared by diluting some of the above solution tenfold.

Perchloric acid, 60 % (as supplied by British Drug Houses Ltd.).

Phosphoric acid, 10 % (v/v) syrupy acid (1.9M).

Phosphate solution, for washing precipitates. 1 vol. of 0.067 M-Na phosphate buffer of pH 7.3 is diluted to 5 vol.

Citrate buffer, 0.2M-dipotassium hydrogen citrate, 21.61 g. $\text{K}_2\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$ and 7 g. $\text{H}_3\text{C}_6\text{O}_7 \cdot \text{H}_2\text{O}$ are dissolved together in water and made up to a total volume of 500 ml. This buffer is conveniently used in a burette; other reagents, excepting wash-liquids, are handled with Pasteur pipettes calibrated for the purpose.

Quartz chips are boiled in 8M- HNO_3 and rinsed with water.

The small (12 ml.) centrifuge tubes referred to below are of plain test tube form and are graduated at 2.0, 5.0 and 10.0 ml. Glassware is of Pyrex or other Pb-free glass and is rinsed with 8M- HNO_3 and with water.

Procedure

The urine, collected as described, has a pH between 1.2 and 2.2. A bulk sample is brought within the pH range 4.4–4.6 by the addition of NH_3 . For 500 ml., about 3 ml. ammonia (sp.gr. 0.880) is required. The adjustment was usually made with the glass electrode, but bromocresol green was used externally in a few cases.

Method A. To 25 and 5 ml. of urine at pH 4.5 in 30 ml. centrifuge tubes are added 1 ml. 10 % H_3PO_4 , 0.3 ml. CaCl_2 solution and 1 ml. NH_3 with intermediate mixing. After stirring at intervals for 20 min. the solids are centrifuged off, drained, washed into smaller tubes with the phosphate solution, respun, drained and dissolved in citrate buffer for polarographic determination as described for method *B*.

Method B. Duplicate samples of 25 ml. and also of 5 ml. are measured into 30 ml. centrifuge tubes, to each of which are then added 0.3 ml. of the CaCl_2 solution and 2 ml. of the NH_4 oxalate solution with agitation after each treatment. After 20 min., during which period the suspensions are shaken or stirred every 5 min., they are centrifuged (5 min. at 3000 rev./min.), and drained by inversion for 2–3 min. on filter paper. The precipitates are transferred to smaller tubes (12 ml.) by careful washing with a total volume of 10 ml. of the dilute NH_4 oxalate solution in successive small quantities with the aid of a glass rod. Solid material is spun off and drained as before. To each tube is now added 0.4 ml. HClO_4 and a quartz chip; oxidation is carried out for 15 min. on a hot

plate of temperature such that the solutions are clear and refluxing 3 cm. from the base of the tube after 5 min., with no further frothing due to decomposition of oxalate. When cool, the digests are each diluted with 5 ml. water, added so as to wash down the sides of the tubes; 1 ml. 10% H_3PO_4 and 1 ml. NH_3 are run in with mixing, and water is added to a final volume of 10 ml. Ten minutes are allowed with two or three agitations before the deposit of Ca salts is spun down, drained, washed with 10 ml. of the phosphate solution, respun and drained. The solid is then dissolved in citrate buffer and made up to a definite volume (2 ml.) with the buffer. The precipitates dissolve readily on shaking. Generally, addition of 1.65 ml. buffer is required, but extraordinary concentrations of urinary Ca will vitiate this working rule.

Tubes containing the final solutions are placed in the 25° thermostat of the polarograph and O_2 is removed by passing a gentle stream of N_2 for 4–5 min. An assembly comprising the dropping electrode and a flexible bridge filled with saturated aqueous KCl is lowered into the solution, the N_2 tube is drawn above the surface and the polarogram is recorded over the range of applied potential -0.3 to -0.6 V. with suitable counter current; all potentials are measured against the Standard Calomel Electrode (s.c.e.). Sensitivity should be of the order of 10 cm. galvanometer deflexion for $1.0 \mu\text{A}$. in the cell. The half-wave potential of Pb in these conditions has been found by the author to be -0.49 V. The differences between the heights of the lead 'waves' in the solutions derived from 25 and 5 ml. samples of urine represent values, corrected for the reagent blank, of the Pb originally present in 20 ml. urine. From calibration experiments this derived current can be interpreted in terms of μg . Pb/l. and hence per 24 hr. excretion.

A modification of method B was evolved for use with urine containing less than $50 \mu\text{g}$. Pb/l. This is described after the account of the results obtained with larger amounts of Pb using methods A or B.

Calibration

For clinical purposes it is preferable to analyse samples of urine at pH 4.5 to which Pb has been added to give known

Fig. 1. Diffusion current of lead as a function of concentration. Curve A, Pb in 0.2M-dipotassium hydrogen citrate; Curve B, as (A) in presence of Ca (approx. 0.03M).

concentrations in the range 100–1800 μg ./l. Thus a curve can be constructed from which concentrations of Pb in μg ./l. can be read off directly against the difference in currents derived

from 25 and 5 ml. samples. In the present work, however, curves were built up (Fig. 1) showing current as a function of concentration in the final solution with Pb (as nitrate) both in pure 0.2M-citrate buffer and in a citrate solution in which Ca phosphate had been dissolved. This latter solution was made up by applying method A on a tenfold scale to 50 ml. water in place of urine.

Polarographic data

The instrument used is of the non-recording type, designed and constructed by the author. An enclosed galvanometer (Cambridge Spot, 450 Ω) is incorporated; damping is by condenser only with the sensitivity ($\frac{1}{2}$) employed in the present work; $1 \mu\text{A}$. in the cell gives a deflexion of 10.9 cm. A saturated calomel electrode is used for reference, connected to the cell by means of a flexible bridge, with sintered-glass ends, which is filled with saturated KCl solution.

The characteristics of the dropping-Hg electrode were recorded as nearly as possible under working conditions; the electrode and salt-bridge were immersed in air-free 0.2M-citrate buffer maintained at 25° while a potential of -0.5 V. (s.c.e.) was applied. The mean mass in mg. of Hg falling/sec. (m) from the capillary was found by collecting and weighing the Hg falling in a period of 1000 sec. Simultaneously, the time interval between the falling of successive drops (t sec.) was measured. These determinations gave $t = 2.82$ sec; $m = 1.388$ mg./sec., with a hydrostatic pressure at the capillary tip, maintained throughout the present work, of 45 cm. Hg. The 'capillary constant', ($m^{\frac{1}{2}}/t^{\frac{1}{2}}$) therefore has the value 1.479 mg. ^{$\frac{1}{2}$} sec. ^{$-\frac{1}{2}$} . The maximum excursion of the galvanometer in the analytical procedure was ± 0.2 mm. The N_2 used for displacement of air from the solutions to be polarographed was taken from an industrial cylinder and was scrubbed with KOH-pyrogallol and with water.

The diffusion coefficient of Pb complex ion, calculated from the diffusion currents by the Ilkovic equation is 4.5×10^{-6} cm.² sec. ^{$^{-1}$} in pure 0.2M-dipotassium hydrogen citrate. The limiting current was found to have a temperature coefficient of $+2.6\%$ /degree in the range 18–28°. In the more complex medium made up to simulate that found in practice, which contains much Ca in solution, together with small amounts of phosphate, Na and traces of NH_3 , the currents given by Pb are 91% of the corresponding currents in pure citrate, i.e. the diffusion coefficient is now 3.7×10^{-6} cm.² sec. ^{$^{-1}$} .

RESULTS

Recovery is almost quantitative over the range 100–1800 μg . lead/l., as shown in Table 1, where the results of current-difference interpretation made by reference to curve B of Fig. 1 are set out.

The consistency of the methods is illustrated in Table 2, where the results of duplicate estimations are recorded.

Five investigations into four cases of suspected lead intoxication have been made. In two of the cases, abnormally large amounts of urinary lead were found. Urine was collected over three consecutive periods of varying duration (Table 3); in the central period a course of 2:3-dimercaptopropanol (BAL) injections (individual doses of 1.5 mg./kg.

Table 1. *Recoveries of lead added to stocks of urine*

(Urine at pH 4.5. All determinations done in duplicate and on the same stock of urine (*P*) unless stated otherwise.)

Pb added ($\mu\text{g./l.}$)	Pb found ($\mu\text{g./l.}$)	Added Pb recovered	
		$\mu\text{g./l.}$	%
Method A			
0	208	0*	—
0†	11	0	—
180	390	182	101
360	540	332	92
500†	483	472	94
720	839	631	88
1000	1168	960*	96
1624	1776	1568	97
Method B			
0	197	0	—
0†	0	0	—
100†	100	100	100
240	427	230*	96
333	330	330	99
480	685	488	98
1000	1043	846*	85
1400	1496	1299*	93
1800	2080	1883	105
Average recovery		{ Method A 95 %	{ Method B 97 %
		{ Method A 97 %	

* Not done in duplicate. † Second stock of urine (*Q*).

body weight) was given as described by Carleton, Peters & Thompson (1948), and in two cases this appeared to increase the excretion of the metal as found by Ryder, Cholak & Kehoe (1947), Telfer (1947) and Ginsburg & Weatherall (1948). In case

III calcium gluconate was administered in single doses of 2 g./day by injection as indicated. Sample polarograms are shown in Fig. 2, and some excretion figures in Table 3.

Urine containing low concentrations of lead

For measurements of low concentrations of urinary lead, a simple extension of method *B* would appear to lie in the use of larger samples, e.g. 125 and 25 ml. However, the precipitation of 125 ml. of urine with the amount of oxalate hitherto used results in deposition of a quantity of calcium that is often too great to dissolve in the volume of citrate buffer added to give a final volume of 2 ml. Further, the difference between the concentrations of calcium in the final solutions from the two samples of urine taken would be large. The first difficulty may necessitate the use of final volumes of 4 ml. or more, an expedient which would seriously limit the greater volumes of urine that could be treated profitably. Secondly, the disparity between the concentrations of calcium in the two final solutions introduces an error not readily assessed in any given case since the absolute quantities of calcium are not known. Thus, the use of greater volumes of urine is feasible only if the lead can be separated quantitatively in the presence of controlled amounts of calcium that do not vary greatly between the larger and the smaller urine samples.

In method *B* the first precipitation of calcium occurs in the presence of an excess of oxalate ions. It is impossible to decide whether the lead is removed from solution in these conditions by co-precipitation of sparingly soluble lead oxalate or by adsorption of

Table 2. *Variations of the diffusion current in duplicate determinations of urinary lead*

(In the first columns of figures the results of duplicate determinations and the means of these are shown. The values for Pb contents of 25 ml. and 5 ml. samples are calculated from Fig. 1, curve *B*. The results for $\mu\text{g. Pb/20 ml.}$ and $\mu\text{g. Pb/l.}$ urine are calculated from the means in the preceding columns.)

Method	Readings of current in galvanometer deflexion, i.e. wave height (mm.)		Pb in urine sample corresponding to currents found ($\mu\text{g.}$)		Pb in urine	
	From 25 ml. urine	From 5 ml. urine	25 ml. urine	5 ml. urine	($\mu\text{g./20 ml.}$)	($\mu\text{g./l.}$)
<i>A</i>	20.5 } 20.35 20.2 }	5.2 } 5.1 5.0 }	22.6 } 22.5 22.3 }	5.4 } 5.5 5.5 }	17.0	850
<i>A</i>	13.8 } 13.75 13.7 }	3.7 } 3.85 4.0 }	15.2 } 15.1 15.0 }	4.2 } 4.3 4.4 }	10.8	540
<i>A</i>	9.2 } 9.1 9.0 }	2.0 } 2.0 2.0 }	10.2 } 10.1 10.0 }	2.2 } 2.2 2.2 }	7.9	395
<i>B</i>	48.6 } 48.2 47.6 }	10.3 } 10.15 10.0 }	53.6 } 53.1 52.6 }	11.4 } 11.2 11.0 }	41.9	2095
<i>B</i>	30.2 } 30.1 30.0 }	10.5 } 10.4 10.3 }	33.4 } 33.3 33.2 }	11.6 } 11.5 11.4 }	21.8	1090
<i>B</i>	18.2 } 18.2 18.2 }	5.8 } 5.7 5.6 }	20.1 } 20.1 20.1 }	6.4 } 6.3 6.2 }	13.8	690
<i>B</i>	16.0 } 15.8 15.6 }	6.5 } 6.75 7.0 }	17.7 } 17.5 17.2 }	7.2 } 7.5 7.7 }	10.0	500
<i>B</i>	2.4 } 2.3 2.2 }	2.2 } 2.15 2.1 }	2.7 } 2.6 2.4 }	2.4 } 2.4 2.3 }	0.2	10

the lead ions on the calcium deposit. If the mechanism of entrainment is that of co-precipitation, the concentration of oxalate ions must be in excess of the sum of lead and calcium ion concentrations for complete removal of the lead from solution. However, if physical adsorption of lead ions on to calcium oxalate will effect the same quantitative separation, then complete removal of lead from a solution containing excess of calcium over oxalate ions should be possible. That this is so was shown in the following trial, where recoveries of lead from urine, in which

oxalate, as used for washing precipitates, was employed.) The two deposits, subjected to the procedure of method *B*, gave results indicating that recovery of lead in (*Y*) was more than 90 % of that found in the comparison experiment (*X*).

Satisfactory recoveries having been obtained with a urine rich in lead by entrainment with calcium oxalate precipitates, formed by interaction of oxalate with excess calcium ions, this modification was then applied to the analysis of a normal urine containing very little lead, estimated at less than 30 $\mu\text{g./l.}$ by method *B*.

Fig. 2. Polarograms of lead from duplicate samples of pathological urine (method *B*). Upper curves 5 ml. urine. Lower curves 25 ml. urine. Applied potential measured against Standard Calomel Electrode; 10.9 mm. deflexion = 0.1 μA . Deduced value for lead content of urine is 410 $\mu\text{g./l.}$

the lead content was raised to 500 $\mu\text{g./l.}$, were compared by two methods: in the first (*X*), method *B* was applied, whereas in the second (*Y*), the precipitate was formed in the presence of excess calcium over oxalate ions. Two 25 ml. samples of urine were treated with calcium chloride solution according to the foregoing methods. To (*X*) was added 2 ml. ammonium oxalate solution in the usual way; the other sample (*Y*) was treated with 2 ml. of a diluted oxalate solution whose strength had been adjusted so that calcium equivalent to 0.3 ml. stock calcium chloride solution would be precipitated. (It was found by trial that 0.3 ml. 0.225 *M*-calcium chloride was equivalent to 1.85 ml. 0.035 *M*-ammonium oxalate (theoretical volume 1.92 ml.). Therefore, as oxalate of controlled calcium equivalence, 0.035 *M*-ammonium

Table 3. *Urinary excretion of lead in cases of suspected lead poisoning. Effects of therapy with BAL and calcium gluconate*

(*B*, BAL (1.5 mg./kg. body wt.) administered; *G*, calcium gluconate (2 g./day) administered; —, not determined.)

Day	Case I, chronic	Case II, no Pb intoxication	Case III, acute
	Pb excretion ($\mu\text{g./24 hr.}$)		
1	75	50	6000
2	100	30	950
3	—	80	1360
4	85	30	960
5	40	65	—
6	230 <i>B</i>	115	—
7	375 <i>B</i>	40	1530 <i>B</i>
8	430 <i>B</i>	108	500 <i>B</i>
9	225 <i>B</i>	35	800 <i>BG</i>
10	290 <i>B</i>	45	320 <i>G</i>
11	180 <i>B</i>	85	— <i>G</i>
12	110	140 <i>B</i>	— <i>G</i>
13	65	125 <i>B</i>	200 <i>G</i>
14	70	40	200
15	100	65	—
16	130	45	—
17	90	40	—
18	120	—	—

Samples of the urine of 125 and 25 ml. were treated with 0.5 ml. calcium chloride solution and 2 ml. of the diluted oxalate of controlled calcium-equivalence (0.036 *M*). The deposits were collected after 1 hr. by centrifuging, and treated according to method *B*. The second precipitation of the calcium as phosphate gave deposits of equal volumes from the two samples, and the solutions in citrate buffer gave good polarograms from which it was concluded that the urine contained originally $25 \pm 5 \mu\text{g./l.}$ of lead.

Another 24 hr. sample of urine which showed no detectable content of lead when treated by method *B* gave good duplicate results when analysed by the same modified method, as shown below:

Pb current from 125 ml. sample (mm.): 4.0, 3.8, average 3.9.

Pb current from 25 ml. sample (mm.): 2.2, 2.0, average 2.1.

Current difference equivalent to Pb in 100 ml. urine $\equiv 1.8$ mm. Urine contained 20 $\mu\text{g. Pb/l.}$

DISCUSSION

The choice between the two methods given above depends primarily on the nature of the urine under examination. With some samples the final undissolved materials found in the quicker method have been very small, and good results were obtained. In other cases the more exacting procedure had to be followed, since the insoluble residues were larger. Some substance, provisionally identified by the half-wave potential as iron (-0.35 V., s.c.e.) (Lingane & Kerlinger, 1941), giving a wave preceding but clearly distinct from that of lead is usually observed when method *A* is applied; it is not found in method *B* and the latter may have to be used if excessive amounts of the interfering substance are present in the urine. In all cases the shorter method is adequate for ascertaining whether the excretion of lead is abnormally high, although the rare occurrence of erratic results with phosphate precipitates found by Weber (1947) and by Reed & Gant (1942) has been confirmed. Considerations of principle, mentioned below, suggest that method *B* is more reliable in certain cases. However, when both can be employed they appear in practice to be equally accurate.

It could not be shown that any of the prescribed conditions was critical. Times of precipitation, centrifuging and draining, much greater than those prescribed, did not improve results. The times finally adopted seemed reasonable, but by no means constituted a practical minimum. The corresponding intervals used by Weber (1947) and by Reed & Gant (1942) prolong the analysis unduly, e.g. the precipitates stand 2–24 hr. and drain between 0.75 and 2 hr., whereas in the work now reported the precipitates stand for 0.5 hr. at most and drain for less than 5 min. The 15 min. period for oxidation was chosen since a minimum for consistent results was found to be 10 min., and no improvement or deterioration was found when the tubes were heated almost to dryness (40 min.).

Although it is claimed (Weber, 1947) that in 20 % citric acid equally good polarograms for lead are given in the pH range 5–7, the present author has found that in 20 % citric acid adjusted to values in the range pH 5–8 with ammonia the best polarograms were given in the narrow range pH 5.0–5.4. Therefore the value pH 5.2 was chosen for the citric buffer used.

The effect of calcium in reducing slightly the diffusion current of lead has already been noted; this effect, reported qualitatively by Reed & Gant (1942) has been measured (p. 608) and found directly proportional to the concentration of calcium.

It is not certain in what form excreted lead is present in the urine, either when BAL has been

administered (Spray, Stocken & Thompson, 1947) or in its absence. In the former case, presumably, a chelated sulphur-containing complex of the metal is present. However, identical results were obtained with urine excreted after injection of BAL by application of the methods to the urine both as received and also after boiling it with bromine. The complex of lead and BAL prepared *in vitro* is insoluble in the pH range 1.6–8.2 and in more alkaline solutions will not form a precipitate with phosphates. Further, the compound is insoluble in the citrate buffer used. This gives ground for preference of method *B*, since there is a risk that the alkaline precipitation of the lead may not be quantitative. Further, the oxidation in method *B* will ensure that all the lead will be soluble in citrate and that no polarographically active thiols will reach the final solution to complicate the determination by introducing unwanted waves.

The sensitivity of the polarographic determination is such that 1 μ g. of lead in the final solution (2 ml.) is equivalent to a galvanometer deflexion of approximately 1 mm. The current levels between which the diffusion currents are read are drawn through five or six points lying in a straight line. Each point, after practice, is probably observed within 0.2 mm. Therefore diffusion currents may well be read off with errors of less than 0.2 mm. Since interpretation of the results is based on 20 ml. urine, the corresponding average variation is ± 10 μ g. lead/l. of urine which corresponds roughly to ± 15 μ g. lead/24 hr. When 125 and 25 ml. samples of urine are used, the interpretation is based on 100 ml. urine, so that the same error in the polarographic measurement is now equivalent to ± 2 μ g. lead/l., i.e. ± 3 μ g. lead/24 hr. The error as a percentage of the value for lead excretion will vary inversely with the latter. The overall variation in a series of determinations carried out in duplicate is shown above to be somewhat greater. The polarographic part of the determination is therefore not the limiting factor for accuracy.

The sources of greatest error appear to lie in (1) the adjustment of the volume of the final solution and (2) variations in the final concentration of calcium. When urine contains large amounts of this metal it is practicable to add calcium chloride only to the smaller sample and thus equalize to some extent the final concentrations. The uncompensated error for reagent blank thus introduced will be negligible, since the calcium chloride can be freed from lead very easily with a little oxalate before use.

A few normal urines examined showed values for lead excretion of less than 60 μ g. in 24 hr. With the above methods (*A* and *B*) the determinations on urine containing less than 50 μ g. lead/l. were not satisfactory, since variations between duplicates were of the same order of magnitude as the difference

in diffusion currents corresponding to the lead in 20 ml. urine. Concentrations of lead smaller than 50 $\mu\text{g./l.}$ can, however, be determined by the modified procedure reported at the end of the experimental section.

No attempt has been made to use the present methods for material other than urine.

SUMMARY

1. Two relatively rapid methods for the polarographic determination of pathologically increased lead in urine are described, together with a modification for the estimation of lower concentrations.

2. The quantitative recovery obtained in the

entrainment procedures of Ross & Lucas (1935) is confirmed.

3. Applications to cases of suspected lead intoxication are described; there is evidence that 2:3-dimercaptopropanol (BAL) increases the excretion of lead in the urine.

4. Estimates have been obtained for the diffusion coefficients of the complex lead ion in citrate buffer in presence and absence of calcium.

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Properties of Preparations from the Central Nervous System which Degrade Coenzymes I and II; their Connexion with Carbohydrate Metabolism

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Extraction of the system which degrades cozymase in nervous tissues, and examination of the action of various agents upon it, were considered to be of value as the system is a very potent one which acts also on coenzyme II though not on the dihydrocoenzymes (McIlwain, 1949*a, b*). It could thus affect the main energy-yielding reactions of the brain at several points.

The system has previously been examined in brain slices and in ground brain (for references see McIlwain & Rodnight, 1949*a, b*). In such preparations a large part of the activity remains firmly

attached to the solid particles. There is also evidence that material with cozymase-degrading activity passes into solution in homogenates of brain in water (Utter, Wood & Reiner, 1945; Meyerhof & Wilson, 1947; McIlwain & Rodnight, 1949*a*). We have found that the active material is also soluble in strong salt solutions, and that this property, combined with its solubility in water and relative insolubility in isotonic saline, can be used to separate it from other materials.

Following the separation of the cozymase-degrading system, an examination has been made