working with primary amines, the aim was to get a completely substituted amine. In Table I are given the derivatives prepared, with their several characterizations.

TABLE I.—*p*-NITROBENZYL DERIVATIVES OF.

Amine.	Composition, R = $-CH_2C_6H_4NO_2$.	M. p.* ° C.	Color.	Solubilities.
Methylamine[1]	$Me.NR_2$	102	light yellow	v. s. C_6H_6, Me_2CO, Et_2O, less in EtOH
Ethylamine[2]	$Et.NR_2$	67	light yellow	s. EtOH, pet. Et_2O
Ethyl-aminoacetate	$Et.CO_2.CH_2.NR_2$	108	light yellow	v. s. EtOH, Et_2O
Benzylamine	$C_6H_4CH_2.NR_2$	144	light yellow	s. EtOH, C_6H_6, insol. pet. Et_2O
Ethyl-*p*-aminobenzoate	$Et.CO_2C_6H_4NR_2$	117	canary yellow	s. EtOH, Et_2O, C_6H_6
Propyl-*p*-aminobenzoate	$Pr.CO_2C_6H_4NR_2$	114	canary yellow	v. s. EtOH, Et_2O, C_6H_6
p-Aminobenzene-sulphonic acid (sulphanilic acid)	$HSO_3C_6H_4-NR_2$	chars	canary yellow	s. s. organic solvents; sol. in alk.
Aniline[2a]	$C_6H_5.NR_2$	168	deeper yellow	s. s. EtOH, Et_2O; v. s. C_6H_6
Ethylaniline	$C_6H_5.NEt.R$	67	deeper yellow	v. s. EtOH, Et_2O, C_6H_6
Phenylaniline (Diphenylamine)	$(C_6H_5)_2.NR$	96	deeper yellow	s. EtOH, Et_2O, C_6H_6
p-Aminodimethylaniline	$Me_2NC_6H_4.NR_2$	210	brick red	v. s. C_6H_6, Me_2CO
o-Phenylenediamine	$R_2N.C_6H_4.NR_2$	198	brick red	s. EtOH, C_6H_6, AcOH
p-Phenylenediamine	$R_2N.C_6H_4.NR_2$	225	brick red	s. EtOH, less in C_6H_6 and Et_2O
Benzidine	$R_2N.C_6H_4-C_6H_4NR_2$	228	brick red	s. s. in organic solvents

* Uncorrected.

[1] Strakosch, *Ber.*, 6 (1873), 1062. Sealed tube reaction, m. p. 104° C.

[2] Paal and Spranger, *Ber.*, 30 (1897), 64. Sealed tube reaction.

[2a] As by-product in making the mono-*p*-nitrobenzyl derivative.[2]

SUMMARY.

p-Nitrobenzyl halides are useful reagents for the identification of primary and secondary amines. Alkyl amines yield light yellow derivatives, aromatic monoamines give deeper yellow bodies while the diamines yield brick-red derivatives. In general they possess definite melting points and differ in solubility from the mother substance thus adding to the ease of purification.

A TOXICOLOGICAL INVESTIGATION OF MERCURY AND LEAD.*

BY L. W. RISING AND E. V. LYNN.

Because of the numerous deaths attributable to mercury and lead and the subsequent seeking of chemical evidence, it would be well to have available certain experimental data which would indicate: The effect of preservatives or putrefaction on the recoverable amount of these substances, and how long after death an analyst might reasonably expect to find the full quantity of drug which was present in the body at the time of the demise. There is always the possibility of unsuspected organic combinations which might render an unknown quantity of either chemical proof against the present procedures for their isolation and determination.

In order to secure these quantitative data, a series of specimens consisting of lead or mercury in contact with organic material both with and without added preservatives was made up. The series was sufficiently large in number so that the poisons were exposed to nearly all of the conditions which they might encounter if the bodies containing them were embalmed, exposed to the natural action of putrefaction, or the organs removed for analysis and preserved with the usual tissue preservatives.

* Scientific Section, A. PH. A., Miami meeting, 1931.

The carriers for the poisons were stomachs from freshly killed sheep. These were minced and weighed amounts placed in 4-ounce wide-mouthed bottles. To each of these bottles there was added a known quantity of one poison; this was thoroughly incorporated with the tissue. A measured portion of preservative was next added and the bottle stoppered and sealed with paraffin. The samples which were to be permitted to decompose were treated in a like manner except that no preservative was added nor was one set stoppered. The latter were allowed free contact with air and changing weather conditions on the laboratory roof. The preservatives used were alcohol, a 10 per cent solution of formaldehyde, a 1 per cent solution of mercuric chloride, a saturated solution of arsenic trioxide, and embalmer's cavity fluid.

The formula for the cavity fluid is approximately as follows:

Absolute alcohol	(Volume)	35.00 per cent
Methyl alcohol	(Volume)	14.70 per cent
Glycerin	(Volume)	2.00 per cent
Ethereal constituents	(Volume)	4.00 per cent
Water and other constituents	(Volume)	44.30 per cent

Enough specimens were made up to permit three quantitative determinations of the drugs under every condition considered. These were made at approximately the ends of the first, third and seventh months of standing.

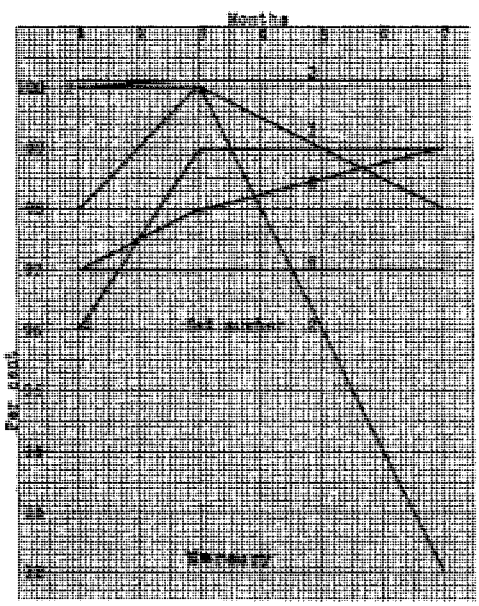

Fig. 1.—Mercury.

A number of processes have been devised for the extraction of mineral poisons in toxicological investigations, nearly all of which begin with the destruction of the contaminating organic material. To consumate this first step Treadwell and Hall (1) suggest the Carius method. Allen (2) makes use of Kjeldahl digestion. Wasterson (3) sulphuric and nitric acids, then potassium permanganate. For this work the destruction was brought about by means of the Fresenius-Babo procedure (4).

The search for simple yet accurate methods for the determination of these drugs led to an investigation of Waldbott's (5) modification of the Reinsch test as a quantitative procedure for mercury. In this method the mercury is precipitated from an acid solution on a copper foil which is then dried and weighed. The mercury is driven off by holding the foil over a flame until the grey film of the element has just disappeared, and the foil is weighed again. The difference in the two weighings represents the mercury originally present in the sample.

It is difficult to drive off the mercury without oxidizing some of the copper at the same time. The most serious objection, however, is the fact that the element

does not always plate out according to theory. A number of solutions were made up containing 0.2 Gm. of mercuric chloride. The mercury was deposited from these on copper according to the procedure of Reinsch. One group of samples stood an hour at room temperature. Another group stood an hour and a half, while two others were heated gently for a half hour and an hour, respectively. In a fifth group, the samples were boiled for 15 minutes. Table I contains the data for this series.

TABLE I.—INCREASE IN WEIGHT OF COPPER FOIL.

	Digested at 20° C.		Heated Gently.		Boiled.
	1 hour.	1½ hours.	½ hour.	1 hour.	15 minutes.
1.	0.0068	0.0098	0.0172	0.0226	0.0410
2.	0.0069	0.0121	0.0116	0.0168	0.0414
3.	0.0200	0.0221	0.0273	0.0290	0.0420
4.	0.0250	0.0170	0.0290	....	0.0425
5.	0.0005	0.0180	0.0368	0.0323	0.0339
6.	0.0200	0.0220	0.0324	0.0289	0.0337

It was thought that the lack of uniformity in the results might be due in part, at least, to variations in the size of the surface of the copper foil available for plating. Therefore, a new series of samples was set up from which the mercury was plated out on foils two and four inches square.

The results are shown in Table II:

TABLE II.—INCREASE IN WEIGHT OF THE COPPER FOIL.

	Two-Inch Foil.		Four-Inch Foil.	
	Boiled 15 min.	Boiled 20 min.	Boiled 15 min.	Boiled 20 min.
1.	0.0366	0.0288	0.0260	0.0310
2.	0.0310	0.0100	0.0300	0.0340
3.	0.0449	0.0040	0.0337	0.0350
4.	0.0338	0.0401	0.0389	0.0169
5.	0.0573	0.0310	0.0378	....
6.	0.0355	0.0171	....	....

As the differences in results still persisted, experiments were carried out using much smaller quantities of mercuric chloride. Table III contains the figures for this work. No better success was obtained with the reduced amount of chemical. It is obvious, therefore, that the modified Reinsch procedure is not a satisfactory quantitative method for mercury.

TABLE III.—DECREASE IN WEIGHT OF COPPER FOIL.

	Ten Mg. of Mercuric Chloride.		Twenty Mg. of Mercuric Chloride.	
	Boiled 15 min.	Boiled 30 min.	Boiled 15 min.	Boiled 30 min.
1.	0.007	0.0021	0.0002	0.0068
2.	0.005	0.0017	0.0039*	0.0081
3.	0.0060	0.0070	0.0009	0.0074
4.	0.0008	0.0009	0.0020	0.0020
5.	0.0008	0.0002	0.0009	0.0010
6.	0.0020	0.0039*	0.0017*	0.0090
7.	0.0007	....	....	0.0043

* Increase instead of loss in weight.

The assay method of the United States Pharmacopœia was employed in the quantitative work for mercuric chloride. The assay often gives high results, because of the inability to always completely free the mercuric sulphide precipitate of elementary sulphur. However, its simplicity recommends it.

TABLE IV.—RESULTS OF MERCURIC CHLORIDE ANALYSES.

Set Number.	1.	2.	3.	4.	5.	6.	7.
						Unpreserved.	
Preservative % found in.	Alcohol.	HCHO.	$HgCl_2$.	As_2O_3.	Cav. Fld.	Exposed.	Unexposed.
1.	100	98	101	97	97	96	100
2.	100	100	101	97	98	99	101
3.	98	92	101	97	99	99	..

1. Per cent recovered at the end of one month.
2. Per cent recovered at the end of three months.
3. Per cent recovered at the end of seven months.

Lead was determined as the sulphate. The liquid resulting from the digestion of the organs is treated with an excess of dilute sulphuric acid and concentrated over a free flame until dense white fumes are given off. Care must be exercised to avoid loss by spattering. The mixture with its char is diluted and filtered through a Gooch crucible. It is then dried, ignited and weighed.

TABLE V.—RESULTS OF THE LEAD ACETATE ANALYSES.

Set Number.	1.	2.	3.	4.	5.	6.	7.
						Unpreserved.	
Preservative % found in.	Alcohol.	HCHO.	$HgCl_2$.	As_2O_3.	Cav. Fld.	Exposed.	Unexposed.
1.	95	97	92	95	92	95	95
2.	95	94	90	95	93	94	95
3.	96	94	94	97	93	97	97

1. Per cent recovered at the end of one month.
2. Per cent recovered at the end of three months.
3. Per cent recovered at the end of seven months.

The data clearly indicates that both mercury and lead can be recovered in practically the full amount under the ordinary conditions encountered in a body after death. Putrefaction, or preservation with any of the common agents used for that purpose, in no way interferes seriously with their recovery. The toxicologist, by careful choice of procedure and the exercise of reasonable care in manipulation, should be able to recover 92–98 per cent of either poison. While this would normally be expected there is as yet little confirmatory evidence in the literature, and since the unexpected frequently occurs in medico-legal cases it was deemed advisable to demonstrate the point.

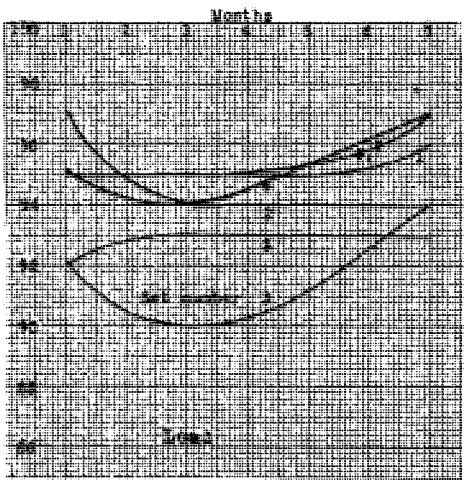

Fig. 2.—Lead.

SUMMARY.

1. The modified Reinsch method was examined with respect to its use as a quantitative procedure. It was found to be not satisfactory.

2. Practically the full quantity of mercury present in a body at the time of death can be recovered whether it has been preserved with alcohol, a 10% solution

of formaldehyde, a 1% solution of mercuric chloride, a saturated solution of arsenic trioxide, embalmed, unpreserved and exposed, or unpreserved and protected from the elements. Time is a negligible factor in the disappearance of the poison.

3. Lead is recovered to about the same extent as mercury when it is exposed to similar conditions.

REFERENCES.

(1) Treadwell and Hall, Analytical Chemistry, 1924.
(2) Allen, Commercial Analysis, 1923.
(3) Wasterson, *Sv. Farm. Tids.*, 21–54 (1917), 9.
(4) Peterson, Haines and Webster, Legal Medicine and Toxicology, 1923.
(5) Waldbott, *Sci.*, 1 (1919), 441; and Peterson, Haines and Webster, *Legal Medicine and Toxicology* (1923), 197.

VIII. THE STANDARDIZATION AND STABILIZATION OF ERGOT PREPARATIONS.*

BY EDWARD E. SWANSON, CLARENCE E. POWELL, ASA N. STEVENS AND E. H. STUART.

Since 1923, when Broom and Clark (1) first introduced the Isolated Rabbit Uterus Method, there has been some discussion as to which of the many methods give the more accurate results for the standardization of ergot preparations. To briefly summarize the literature, Burn (2), Linnell and Randle (3), Burn and Ellis (4), Nelson and Pattee (5), Pattee and Nelson (6), Thompson (7), and Swanson (8) apparently agree that the Epinephrine-Reversal Uterus Method (Broom and Clark) is equal to or more accurate than the U. S. P. Cock's Comb Method in determining the potency of ergot. Thompson (7) recommended that the Epinephrine-Reversal Method be adopted as the official method in the next Pharmacopœia in place of the now official U. S. P. Cock's Comb Method for the biological assay of ergot and its preparations.

It is the purpose of the writers to report in this article further (1) comparative study of the Epinephrine-Reversal and Cock's Comb Methods, and (2) the p_H or hydrogen-ion concentration in relation to deterioration and stabilization of ergot and its preparations.

EPINEPHRINE-REVERSAL METHOD.

Pattee and Nelson (6) state that much valuable time can be saved by external examination of the vaginal orifice in the selection of a uterus. Long and Evans (9) investigated the œstrus cycle of various animals and found that the rabbit is an exception in that the ovum is present only at the time of copulation. Knude and Proud (10) observed that there is no regularity in the appearance or disappearance of the different types of epithelial cells or leucocytes in the lumen of the vagina of normal rabbits. Our own experience showed that by vaginal smears studied under a microscope no definite œstrus cycle could be determined in rabbits.

The selection of a muscle with the Epinephrine-Reversal Method as previously reported requires some care and experience. The segregation of young female rabbits until they reach maturity (2.5 Kg. to 3 Kg.) is helpful in obtaining a suitable uterus. This also eliminates the influence of pregnancy, multiparous parturition and postpartum factors. The size of the individual uterus, regardless of the age of the rabbit, is variable. However, this is not so great

* Scientific Section, A. PH. A., Miami meeting, 1931.