TABLE I.—RESULTS OF EXTRACTION OF TSH FROM BAL WITH PETROLEUM ETHER

1	2	3	4	5	6	7	8
	Before Extn.		Iodine Titer per ml. Petroleum Ether		TSH mg./ml. Petroleum		
Ampul No.	BAL mg.	TSH mg.	BAL + TSH	TSH	Ether Phase	BAL Phase	K
1	2431.6	21.1	0.988	0.127	0.592	10.1	.0586
2	2487.0	64.1	1.223	0.377	1.758	29.6	.0594
3	2443.9	122.9	1.529	0.703	3.277	56.5	.0580
4	2545.2	236.8	1.945	1.151	5.366	101.3	.0529
5	2535.4	329.5	2.217	1.449	6.755	137.7	.0489
6	Pure BAL	.....	0.868	0.000	...	...	Av. (1–4)
7	.....	Pure TSH	10.119	10.119	47.175	...	.0572

Approximately 2-ml. volumes of BAL were weighed into glass ampuls. The TSH, in varying amounts, was then weighed into the ampuls to give the desired concentrations. Exactly 2 ml. of petroleum ether was pipetted into each ampul and the ampuls quickly sealed with an oxygen-gas flame and shaken for 3 hours in a thermostated water bath at 24.5°. After removing each ampul from the bath, a 1-ml. sample of the petroleum ether phase was withdrawn as quickly as possible and pipetted into an excess of 0.1 *N* iodine; back titration was carried out with 0.1 *N* thiosulfate solution.

RESULTS

The data are presented in Table I. Columns *2* and *3* represent the quantities of BAL and TSH weighed into each ampul. The iodine titer of the petroleum ether phase, representing the sum of BAL and TSH, is given in column *4*. The iodine titer due to the TSH content of each ampul was calculated as follows. The iodine titer of the petroleum ether extract of the pure BAL (ampul 6) was multiplied by the mole fraction of BAL in the BAL phase of each ampul; the resultant corrected iodine titer due to the BAL in the petroleum ether extract of each ampul was subtracted from the corresponding titer of column *4* to give column *5*. The TSH content of the petroleum ether phase in mg./ml. is given in column *6*. The amounts of BAL and TSH in the BAL phase were then recalculated for the amount removed by extraction, and the concentration of TSH in the BAL phase is given in column *7*. The partition coefficient in column *8* is the mg. TSH per ml. petroleum phase divided by mg. TSH per ml. BAL phase. The average partition coefficient obtained, $K = 0.0572$, may be used in calculations applicable to the purification of BAL contaminated with trithiol.

The feasibility of the extraction method was verified by the multiple extraction of a typical BAL sample. A volume of petroleum ether equal to that of the BAL was used for each successive extraction step. The fraction of trithiol originally present which remains in the BAL after n extractions under these conditions is $(1 + K)^{-n}$. Therefore, 41 extractions would be expected to result in a 10-fold reduction in trithiol concentration. This was found to be the case experimentally. The percentage loss of BAL into the petroleum ether equals 0.435 × (number of volumes of petroleum ether used in the extraction). Except for convenience, little is gained in the way of efficiency by use of continuous extraction against a multiple extraction performed in this way.

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Identification of Some Barbiturates in Blood by Use of Thin-Layer Chromatography

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A simple, rapid method for the identification of mixtures of barbiturates when present in blood is described. This procedure is especially useful in differentiating between amobarbital and pentobarbital.

THE PROCEDURES involving the identification of certain barbiturates leave much to be desired. This is particularly true when it is necessary to establish the presence of barbiturates in the blood and urine as the agents causing comas in hospital patients or deaths in medical-legal cases.

Two popular approaches of identification using paper seem to be those similar to Algeri, *et al.* (1), and Stevens (2), involving either the treatment of the paper chromatogram after elution of the barbiturate or treatment of the barbiturate before spotting in order to differentiate between saturated and unsaturated groupings attached to the pyrimidine ring. Among the many investigators using thin-layer chromatography, Frahm, *et al.* (3), using piperidine: petroleum ether as the mobile phase, and Eberhardt, *et al.* (4), using isopropanol: ammonia: chloroform as the mobile phase, have reported success in the separation of several barbiturates.

We would like to report a simple, rapid method of

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TABLE I.—R_f VALUES OBTAINED USING STANDARD STOCK SOLUTIONS OF BARBITURATES[a]

Barbiturate(s)[b] Used	Untreated	Treated with 4N H_2SO_4
A	0.75	0.29
B	0.74	0.74
C	0.74	0.40
D	0.51	0.51
E	0.62	0
A + B	0.74	0.29 + 0.74
A + C	0.74	0.29 + 0.40
B + C	0.74	0.40 + 0.74
A + B + C	0.75	0.29 + 0.40 + 0.74
D + E	0.52 + 0.61	0.51
A + B + C + D + E	0.51 + 0.62 + 0.74	0.29 + 0.41 + 0.52 + 0.74

[a] Rf values given here represent the average of four determinations. [b] Code to barbiturate(s) used: (A) secobarbital, (B) amobarbital, (C) pentobarbital, (D) phenobarbital, (E) diallylbarbituric acid.

TABLE II.—R_f VALUES OF BARBITURATES EXTRACTED FROM BLOOD[a]

Sample No.	Barbiturate(s)[b] Present	Untreated	Treated with 4N H_2SO_4
1	A + B	0.75	0.28 + 0.74
2	A + D + E	0.52 + 0.62 + 0.74	0.27 + 0.41
3	C + E	0.62 + 0.74	0.40
4	A + B + D + E	0.52 + 0.62 + 0.75	0.29 + 0.51 + 0.74
5	A	0.75	0.29
6	A + B + D	0.51 + 0.74	0.30 + 0.51 + 0.74
7	A + C + D	0.51 + 0.74	0.29 + 0.40 + 0.52
8	A + B + C + D	0.50 + 0.75	0.28 + 0.40 + 0.51 + 0.74
9	C	0.74	0.40
10	A + B + C + D + E	0.50 + 0.62 + 0.75	0.27 + 0.39 + 0.50 + 0.74

[a] Rf values given here represent the average of four determinations. [b] Code to barbiturates present: (A) secobarbital, (B) amobarbital, (C) pentobarbital, (D) phenobarbital, (E) diallylbarbituric acid.

identification of five barbiturates more commonly encountered either alone or in a mixture during the toxicological studies in the Coroner's Laboratories of Cook County, Ill. The procedure is based on the differences in the R_f values obtained after treatment of the barbiturates with 4N H_2SO_4.

EXPERIMENTAL

Apparatus.—The apparatus used was essentially the one designed by Stahl (5), using 8 × 8-in. glass plates.

Adsorbent.—Silica gel G (Merck) was used.

Mobile Phase.—Acetone:*n*-butyl alcohol:ammonium hydroxide, concentration 9:9:2 was employed.

Reagents.—Silver acetate, A.R., 1% aqueous solution; diphenylcarbazone, A.R., 0.1% in 95%; ethyl alcohol; sulfuric acid, 4*N*; chloroform, reagent grade; sodium hydroxide, A.R., 0.45*N*; ammonium chloride, A.R., approximately 16%; Florisil, 60/100 mesh were employed.

Standard Solution of Barbiturates.—The following barbiturates were used in an alcoholic solution of a concentration of 2 mcg./μl.: secobarbital U.S.P., diallylbarbituric acid N.F., phenobarbital U.S.P., amobarbital U.S.P., pentobarbital U.S.P.

PROCEDURE

Each plate was covered to a thickness of about 250 μ with a paste consisting of 4 Gm. silica gel in 12 ml. distilled water. Precautions were taken to prevent air bubbles. The chromatoplates were dried in air for 15 minutes followed by a drying period of 30 minutes at 105°.

The plates while still hot were spotted in duplicate with 3 μl. of the alcoholic solution of the barbiturates. Immediately after application of the barbiturates, one spot of each duplicate was treated with 5 μl. of 4N H_2SO_4, and the chromatoplates were heated in an oven at a temperature of 125° for 1 hour. After cooling in air to room temperature, the chromatoplates were placed into a chamber containing the mobile phase. The solvent front ascended to the proper height within 1 hour. After drying at room temperature in a stream of air for 15 minutes, the barbiturates were developed as purple-blue spots using the standard method of spraying with silver acetate followed by the diphenycarbazone (1).

Table I shows the typical results obtained using the standard barbiturate stock solutions.

Extraction and Identification from Blood.—Five milliliters of blood containing barbiturate(s) were extracted with 50 ml. of chloroform and the extract evaporated to dryness in a stream of air. The residue was taken up in 10 ml. of chloroform, passed through a Florisil column, and the barbiturate(s) eluted with 10% methyl alcohol in chloroform as described by Stokes, *et al.* (6). The methanol-chloroform eluate was evaporated to dryness in a stream of air and taken up in 100 μl. of ethanol. Six to twelve microliters of the alcoholic extract was spotted on a silica gel chromatoplate and the barbiturates identified as described above.

When quantitative as well as qualitative results were desired the 0.45*N* NaOH extract obtained in the method of Stokes, *et al.* (6), was acidified to a pH of 2–3 and extracted with 50 ml. of chloroform. The residue left after evaporation to dryness in a stream of air was taken up in 100 μl. of ethanol and treated as described above.

Urine when extracted and purified as described by Stokes, *et al.* (6), produced comparable results.

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