TABLE I—EFFECTIVENESS OF THE COMPRESSED AIR AND HAND TECHNIQUES FOR RESUSCITATING RATS FROM ETHER OVERDOSAGE

Ether Exposure Time, Sec.	No. Animals	% Survival—Treatment—No Treatment	Compressed Air Technique	Hand Technique
		Experiment No. 1		
55	10	20	—	—
	10	—	90	—
90	10	0	—	—
	10	—	100	—
120	10	—	100	—
150	10	—	40	—
		Experiment No. 2		
55	10	30	—	—
	10	—	—	20
90	10	0	—	—
	10	—	—	0

The animal was then removed and either placed in a prone position with its head turned to one side, or subjected to the compressed air or hand techniques. Return of the righting reflex indicated resuscitation from the effects of the anesthetic.

RESULTS AND DISCUSSION

Exposure to ether vapors for 55 sec. was lethal to 70 and 80% of the animals, and for 90 sec. all the animals succumbed to the anesthetic (Table I). Those dying from the 90-sec. exposure showed no visible respiratory movements following removal from the jar, and death ensued shortly as indicated by no palpable heart beat. The compressed air technique resuscitated all animals subjected to the ether vapors for 90 and 120 sec. and partially revived those exposed for 150 sec. In contrast, the hand method afforded no significant protection to animals exposed to the anesthetic for either 55 or 90 sec. (Table I).

These results clearly demonstrate the effectiveness of the compressed air technique and its superiority to the hand method. The compressed air method hyperventilates the animals and perhaps, activates those stimulatory reflexes present in the nasal and pharnygeal areas (4).

The simplicity of this technique is clear, and it requires equipment commonly found in the laboratory.

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Keyphrases

Resuscitation method—rats
Ether vapor—respiratory arrest
Compressed air—resuscitation

A Spectrophotometric Method for the Analysis of Strychnine Phosphate in the Presence of Magnesium Stearate

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A spectrophotometric technique for the analysis of strychnine phosphate in the presence of certain tablet excipients is reported. Tablets containing the alkaloidal salt are well dispersed in water, basified, and extracted with chloroform. The chloroform solution of the free base is then reacted with bromothymol blue in pH 7.65 buffer and the resultant colored product is quantified by the spectrophotometric technique.

SEVERAL METHODS are available for the analysis of strychnine phosphate, but most of them have disadvantages for use in tablet analysis, such as a large sample size, lack of precision, tedious procedure, or interference from other components present in the tablet. Among these methods are a picric acid method (1), a vanadate method (2), a titration method (3), a bromophenol blue method (4), a reduction and nitrite method (5), and a silicotungstic acid method (6).

In some of the above methods, magnesium stearate gives a negative interference. This has been overcome by a bromothymol blue extraction procedure using chloroform, in which an aliquot of methanol is added to the extract prior to making to volume. This procedure is simple and gives accurate results when applied to tablet assays.

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EXPERIMENTAL

Reagents—Chloroform A.R., methanol A.R., phosphate buffer pH 7.65 were used. Mix 50 ml. of 0.1 *M* sodium dihydrogen phosphate with 450 ml. of 0.1 *M* disodium hydrogen phosphate. Bromothymol blue: Prepare 0.018% in pH 7.65 buffer and wash twice with chloroform before use. Ammonium hydroxide, 10% aqueous. Strychnine phosphate standard: the commercially available salt should be thoroughly investigated by the usual

TABLE I—RECOVERY OF STRYCHNINE PHOSPHATE FROM LOT 1 BY VARIOUS METHODS

Method[a]	Average Percentage[b] Found
1	73%
2	87%
3	82%
4	74%
5	90%[c], 88%[d]

[a] Code number explanation: 1, bromothymol blue method with no methanol, 2, modified bromothymol blue method with methanol added, 3, bromophenol blue, 4, ammonium vanadate method, and 5, picric acid method. [b] Averages of four to six samples. [c] Calculated from absorbances at the 400-mμ peak. [d] Calculated from absorbances at the 360-mμ peak.

techniques of melting point, infrared, ultraviolet spectroscopy, and thin-layer chromatography to assure its purity and identity as a "house standard."

Preparation of Standard—Accurately weigh approximately 43.2 mg. of dry strychnine phosphate standard. Dilute to 200.0 ml. with water and mix well. Pipet 4.0 ml. of the standard into a 125-ml. separator.

Preparation of Sample—Stir two tablets (0.432 mg. strychnine phosphate per tablet) in 7 ml. of water until well disintegrated. Transfer quantitatively with a small amount of water to a 125-ml. separator.

Procedure—Add 0.5 ml. of the ammonium hydroxide to each separator. Extract four times with 20 ml. of chloroform, combining the extracts in 150-ml. beakers. Evaporate the extracts to about 5 ml. on the steam bath. Cool and transfer to a 125-ml. separator, washing each beaker with about 5 ml. of chloroform, and adding these washings to the corresponding separator. Add 10 ml. of pH 7.65 buffer and 10 ml. of bromothymol blue reagent to each one and to a third separator containing 10 ml. of chloroform. Shake well and allow to separate. Transfer the chloroform layers to 50-ml. volumetric flasks. Add 15.0 ml. of methanol to each volumetric flask.

Extract the buffer layers again with two further 9-ml. portions and one 5-ml. portion of chloroform, and drain the chloroform extracts into the volumetric flasks each time. Make to volume with chloroform. Mix well and determine the absorbance against the reagent blank at approximately 410 mμ on a suitable spectrophotometer, using 1-cm. cells. Calculate against the standard.

DISCUSSION AND RESULTS

Two lots of a tablet containing strychnine phosphate have been analyzed using the above method. The first lot was also analyzed by several other procedures. Comparison of the results by the different procedures is listed in Table I. The second lot, analyzed by the modified bromothymol blue method, gave an average recovery of 100% for three assays.

To ascertain whether lot No. 1 was actually low, or whether all of the techniques were inapplicable to the tablet in question, individual samples of the other ingredients (calcium glycerophosphate, dibasic potassium phosphate, lactose, starch, and acacia) were weighed to approximately the amounts present in the tablet. These samples were added individually to aliquots of the standard and were assayed against the standard. (The procedure used was the bromothymol blue without the addition of methanol.) Only magnesium stearate gave any appreciable difference. The others gave an average recovery of 99%. A precipitate was noticed in the magnesium stearate extract. The addition of methanol cleared the extract.

TABLE II—RECOVERY OF STRYCHNINE PHOSPHATE IN THE PRESENCE OF MAGNESIUM STEARATE

Amount of Magnesium Stearate Added, mg.	Percentage of Strychnine Phosphate Found
2	103
4	102
8	100
12	100
	Average 101

Further work indicated that the addition of methanol to blank, standard, and sample gives good results.

The results in Table II were obtained using the methanol modification of the bromothymol blue method for analysis of strychnine phosphate in the presence of varying amounts of magnesium stearate.

Investigations comparable to the above were conducted using calcium stearate, zinc stearate, and stearic acid. The recovery of strychnine phosphate averaged 101% in the presence of 2–10 mg. of zinc stearate. The recoveries in the presence of calcium stearate and stearic acid were not quantitative by the procedure described above. However, addition of 1.5 ml. of ammonium hydroxide in lieu of the stated 0.5 ml. and filtration (Whatman No. 40) after the dye-alkaloid extraction resulted in an average recovery of 100% in the case of calcium stearate but only 92% in the case of stearic acid.

Although the picric acid method and the bromophenol blue method gave comparable results, the modified bromothymol blue method was found to be preferable for the tablet being analyzed because it was less tedious than the bromophenol blue method and more precise than the picric acid method. (One or more washing steps were absolutely essential to remove interference in the picric acid method.)

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Keyphrases

Strychnine phosphate tablets—analysis
Magnesium stearate interference—strychnine PO_4 analysis
Colorimetric analysis—spectrophotometry
Bromothymol blue—reagent