

TABLE III—NUMBER OF $C_{12}E_7$ MOLECULES ADSORBED PER CELL AT 100% HEMOLYSIS

| Run | 27°C. | 37°C. |
|-----|-------------------|--------------------|
| 1 | 1.3×10^8 | 1.1×10^8 |
| 2 | 1.2×10^8 | 1.0×10^8 |
| 3 | 1.0×10^8 | 0.86×10^8 |

amount adsorbed is clearly seen with a rise in temperature.

As the slope of the adsorption isotherms at 37° was generally steeper than that at 27°, the rise in temperature would result in an increased tendency of the adsorption of nonionic agent molecules onto the red cell surface from aqueous solution owing to the temperature-dependent hydration of the molecules, thereby promoting the hemolysis. A similar adsorption behavior of the polyoxyethylene glycol monoalkyl ethers has been reported (9). The increased release of lipids from the red cells mentioned earlier may also contribute to a certain extent to the enhancement of the hemolysis at elevated temperatures.

As to the attacking site of the polyoxyethylated nonionics molecules, Mima and co-workers have concluded, based on the monolayer experiments, that the cholesterol portion in the cell membrane would

be predominant (10). There still remains some doubt on this conclusion. In view of the limited scope of this study, however, any conclusion on the attacking site of the nonionics cannot be drawn.

REFERENCES

- (1) Glassman, H. N., *Science*, **111**, 688(1950).
- (2) Deibler, G. E., Holmes, M. S., Campbell, P. L., and Gans, J., *J. Appl. Physiol.*, **14**, 133(1959).
- (3) Hudgins, P. C., and Patnode, R. A., *J. Exptl. Med.*, **107**, 43(1958).
- (4) Pethica, B. A., and Schulman, J. H., *Biochem. J.*, **53**, 177(1953).
- (5) Kondo, T., and Tomizawa, M., *J. Pharm. Soc. Japan*, **86**, 251(1966).
- (6) Ohba, N., to be published.
- (7) Kondo, T., and Tomizawa, M., *J. Colloid Interface Sci.*, **21**, 224(1966).
- (8) Thron, C. D., *J. Pharmacol.*, **145**, 194(1964).
- (9) Corkill, J. M., Goodman, J. F., and Tate, J. R., *Trans. Faraday Soc.*, **62**, 979(1966).
- (10) Mima, H., Yashiki, T., Nakatani, H., Shintani, S., and Usui, T., *J. Pharm. Soc. Japan*, **82**, 1171(1962).



Keyphrases

Hemolysis—nonionic surfactants
 Polyoxyethylene glycol monododecyl ethers—
 hemolysis
 Temperature effect—hemolysis
 Erythrocytes—surfactant adsorption
 Spectrophotometry—analysis

Simple Method for Resuscitating Rats from Ether Overdosage

By L. F. SANCILIO, P. KRAUS, C. MYERS, and L. WAGNER

A simple method using compressed air for resuscitating rats from ether overdosage is described. Exposure to ether vapors for 55 and 90 sec., respectively, was lethal to 70 and 100 percent of the animals. The compressed air technique afforded protection to animals exposed to the anesthetic for 55, 90, 120, and 150 sec., while the conventional hand technique was ineffective against the 55- and 90-sec. exposure.

DURING THE PERFORMANCE of various experimental techniques in rats, *e.g.*, intrapleural injection (1), cotton pellet implantation (2), and adrenalectomy (3), using ether as the anesthetic agent, marked respiratory depression leading to death sometimes occurs unless the animal is immediately hyperventilated. This study describes a simple and effective technique used in our laboratory for reviving rats manifesting respiratory arrest following exposure to this anesthetic agent.

METHOD

Compressed air is required in performing this technique. In our laboratory, the air is pressurized at 60 p.s.i., and the tapered outlet (labcock) contains a valve to regulate its flow. The depressed animal is placed in a prone position in the palm of the hand with the head held gently but firmly between the thumb and index finger. The valve is partially opened (approximately 25%), and the air directed

toward the rat's nostrils. The animal is moved back and forth rapidly across the stream of air to allow for inflation and deflation of the lungs. Spontaneous breathing is usually restored within 30 sec. An excess of pressurized air must be avoided as this will inflate the gastrointestinal tract.

The following study was undertaken to demonstrate the superiority of the compressed air to the manual technique. In the latter method, intermittent pressure was applied to hyperventilate the animal by placing the thumb on one side and the index and ring fingers on the other side of the rib cage.

Charles River CD rats (100–150 Gm.) of either sex were etherized in the following manner. One hundred milliliters of ether was poured into a 41-oz. glass dressing jar (Aloe Medical), the base of which was immersed in a water bath maintained at 39°. Ether was added periodically to replace that which had evaporated. The animal was placed on a wire mesh platform 7.5 cm. (3 in.) from the floor of the jar. After covering the jar, the exposure time to the ether vapors was determined with a Universal timer.

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 pharyngeal areas (4).

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

REFERENCES

- (1) Sancilio, L. F., and Rodriguez, R., *Proc. Soc. Exptl. Biol. Med.*, **123**, 707 (1966).
- (2) Winter, C. A., and Porter, C. C., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 515 (1957).
- (3) Ingle, D. J., and Griffith, J. Q., "The Rat in Laboratory Investigation," 2nd ed., Hafner Publishing Co., New York, 1962, pp. 444, 445.
- (4) Oberholzer, J. H., and Tofani, W. O., in "Handbook of Physiology," vol. II, American Physiological Society, Washington, D. C., 1960, p. 1125.



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

By JANELLE BARRETT, RAMONA PUCKETT, and R. D. POE

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.

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