Analysis of arterial blood gases in the curarized, artificially respired rat¹

LEO V. DICARA, THE ROCKEFELLER UNIVERSITY, New York, New York 10021

The present paper describes a simple and accurate technique for the artificial respiration of curarized rats that avoids the trauma involved in a tracheotomy. Success of the technique depends on the proper positioning of a special face mask on the rat. The results of an experiment on arterial blood gases in 48 curarized rats are presented to illustrate the accuracy and effectiveness of the techniques described.

This paper reports a simple technique for the artificial respiration of curarized rats by means of a face mask and a relatively inexpensive small-animal respirator. The techniques described have been used in previous experiments in this laboratory on the instrumental conditioning of visceral and glandular responses (DiCara & Miller, 1968a, b, c, d; Miller & DiCara, 1967, 1968). The results of an experiment on arterial blood gases are presented to illustrate the accuracy and effectiveness of the techniques described.

METHOD

Subjects

A total of 48 male albino Sprague-Dawley rats were used in two experiments. Rats were divided into two groups on the basis of body weight. There were 24 rats with a mean body weight of 503 ± 12.7 g in the first group and 24 rats with a mean body weight of 401 ± 10.4 g in the second.

Catheter Implantation

Each rat was anesthetized with 40 mg/kg Nembutal, and a catheter consisting of polyethylene tubing connected to a length of Vivofil tubing (i.d. 0.12, o.d. 0.25) was implanted into the lower third of the abdominal aorta; the distal end was threaded under the skin and brought out at the back of the neck (DiCara & Miller, 1968d). The catheter was filled with Ringer's solution and heat-sealed. Rats were allowed 1 week to recover from the operation.

Precurarization Procedures

A 0.25-ml sample of blood from the aortic catheter was obtained from each rat prior to curarization. The collection of blood was carried out under anaerobic conditions in a heparinized gas-tight syringe. Analysis of blood gases was carried out immediately, using a micro-pH and gas-analyzing system (Instrumentation Laboratory, Inc., Model 113-S2). This apparatus enables the operator to determine, with a minute volume of blood, P_{CO_2} determination to within ± 0.5 mm Hg and P_{O_2} determination to within ± 1.0 mm Hg. The blood-gas analyzing system was calibrated daily by means of analyzed gas mixtures. Analyses of blood gases were carried out at 37°C and have been corrected for an O_2 membrane factor and ambient barometric pressure.

Curarization and Procedures for Artificial Respiration

The rats were injected intraperitoneally with 2 mg/kg of d-tubocurarine chloride (Squibb) in a solution containing 3 mg/ml. As soon as the rat showed the first signs of difficulty in breathing, it was fitted with a specially constructed, airtight face mask.

The mask is made from the mouthpiece end of a rubber balloon placed over a plastic tube (3.0-cm diam). The rat's snout is placed in this plastic tube, and the lower lip of the mouthpiece end of the balloon is hooked behind the upper incisors. The rat's head is held in place by adjustable bars placed behind it on the occipital ridge, as shown in Fig. 1. The upper portion of the balloon is held in place on the dorsal surface of the rat's snout by elastic pressure; the fit is airtight. In extreme tests, we have respired curarized rats under water for over 15 min without detecting any air bubbles. The end of the plastic tube, over which the balloon is placed, is connected via a rubber stopper, with a hole drilled through its middle to a small-animal artificial respirator equipped with solenoid valves to prevent rebreathing of expired air (ENM Instrument Co., Model VE5AG). The total dead air-space volume between the pump and the rat is 8.3 cc.

Previous experiments have demonstrated that if rats are artificially respired at 1-to-1 I/E ratio, 70 breaths/min, and a peak inspiration pressure of 20 cm H_2O , they maintain normal heart rate,

Fig. 1. Apparatus and face mask used to hold and artificially respire a curarized rat.

temperature, and peripheral vasomotor responses. In order to obtain more extensive information about the relationship between arterial blood gas composition and artificial respiration in curarized rats, each rat in the present experiment was respiration for 60 min at each of three different levels of peak inspiration pressure: 10, 15, and 20 cm H₂O. Inspiration/expiration ratio and rate were held constant at a 1-to-1 ratio and 70 cycles/min for all rats. The peak inspiration

pressure was changed at the end of each 60-min period during curarization so that, in each group of rats, all combinations of the three peak inspiration values used occurred twice.

The tidal volume of air delivered to 400- and 500-g curarized rats at peak inspiration pressures of 10, 15, and 20 cm H₂O was determined by recording the pressure changes produced in an airtight body plethysmograph. Rate and I/E ratio were maintained, respectively, at 70 cycles/min

Fig. 2. Arterial P_{CO_2} as a function of peak inspiration pressure at a 1-to-1 I/E ratio and 70 cycles/min.

and a 1-to-1 ratio. As the animal was respiration, the pressure in the plethysmograph varied directly with the volume of tidal air. The pressure changes of the system were transmitted to a Statham pressure transducer and were amplified by a Grass polygraph (Model 5). In order to calibrate the system, the rat respiration was killed by asphyxiation after an injection of Nembutal and an airtight syringe connected to this system, and the pen deflection produced by moving known volumes of air in and out at 70 cycles/min was recorded. Under these conditions, the gas compression was approximately adiabatic.

RESULTS

The main results are presented in Figs. 2 and 3. In Fig. 2, arterial blood P_{CO_2} is shown as a function of peak inspiration pressure. Values of arterial blood P_{CO_2} measured before curarization and after 1 h of artificial respiration are shown for the two groups of rats. Fig. 3 presents similar measurement of arterial P_{O_2} . The points marked by arrows in both figures represent the mean arterial blood gas obtained during the second and third hours of artificial respiration. The close agreement between each set of points indicates that the sequence in which the peak inspiration pressure was changed during curarization did not have any systematic effect on level of arterial blood P_{CO_2} or P_{O_2} . This was confirmed by a statistical analysis of variance.

As can be seen from Figs. 2 and 3, normal noncurarized rats have an arterial blood P_{CO_2} of 25.4 mm Hg and an arterial blood P_{O_2} of 75.1 mm Hg. Although the P_{O_2} levels are below the normal range for man, they are quite normal for rats, as are the P_{CO_2} values (Jones, MacGrath, & Aculthorpe, 1950; King & Bell, 1966). Normal arterial blood P_{CO_2} and P_{O_2} can be maintained in curarized rats by artificial respiration at a peak inspiration pressure of approximately 17-19 cm H₂O at a rate of 70 beats/min and a 1-to-1 I/E ratio.

Figure 4 illustrates the relationship between peak inspiration pressure and tidal volume in rats of different body weights. Each point on the curves represents the mean of 36 observations obtained on six

Fig. 3. Arterial blood P_{O_2} as a function of peak inspiration pressure at a 1-to-1 I/E ratio and 70 cycles/min.

rats. There is a linear relationship between pressure and tidal volume, and, as expected, heavier rats showed a higher tidal volume at a given peak pressure.

DISCUSSION

In addition to confirming the results of previous experiments on blood-gas composition in the normal rat (King & Bell, 1966), the present experiment describes a simple and accurate technique for the artificial respiration of curarized rats. The technique avoids the surgical trauma involved in a tracheotomy. Success

of the technique depends on the proper positioning of the face mask. For instance, the rat should be artificially ventilated as soon as it shows the initial effects of curarization and held in place in the mask, with the head extended, by hand if necessary, until total paralysis is achieved. This will minimize the possibility of bronchoconstriction, laryngospasm, obstruction of the glottis, and subsequent passage of air into the rat's stomach.

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Fig. 4. Peak inspiration pressure and tidal volume in curarized rats of different body weights.

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NOTE

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