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SHORT COMMUNICATION

A TRIPHASIC VENTILATOR FOR REPTILES

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In connection with studies of the spinal motor-control system (Hasan & Sasaki, 1986), the need arose to maintain freshwater turtles (*Pseudemys scripta elegans*) in a decapitated state for several days. Although a commercially available ventilator has been used in a similar situation (Rosenberg, 1972), such ventilators, apart from being expensive, are not designed to suit the reptilian respiratory pattern. In particular, they do not usually accommodate low frequencies of respiration, nor do they allow the apnoeic pauses (during which the lungs remain inflated while the glottis is constricted) that are observed in normal reptilian breathing (Wood & Lenfant, 1976; Jackson, 1979; Randall, Burggren, Farrell & Haswell, 1981). A simple, inexpensive ventilator was therefore constructed, which mimics the reptilian pattern. Its design is described here for possible use by others who may have similar requirements.

It was considered inadvisable to employ a pressure source for inspiration, since turtle lungs are highly compliant (Jackson, 1979), exhibiting an intrapulmonary pressure fluctuation of only a few centimetres of water (Gans & Hughes, 1967) though the tidal volume (normalized) exceeds $10\,ml\,kg^{-1}$ (Jackson, 1971; Glass, Burggren & Johansen, 1978). Accordingly, a source with a constant rate of air flow was used, derived from an air-supply tap available in most laboratories. (It may, in general, be safer to employ a tank of compressed air, in view of the possibility of oil contamination in the laboratory air supply.)

The air, after humidification by bubbling in water (which also served as a trap for any particulate contaminants), passed through a thin tube (resistance tube: Fig. 1) (inside diameter = 1 mm, length = 1·4 m). The air-inflow valve was adjusted so that the upstream pressure monitored by the mercury manometer was 100 mmHg. Approximate constancy of flow was assured as long as this pressure did not change, because the pressure at the downstream end of the resistance tube could be expected to fluctuate by no more than a few centimetres of water, which would be small in comparison with the upstream pressure. A flow rate of approximately $3\,ml\,s^{-1}$ was obtained, though other rates could be achieved readily. The mercury manometer served not only as a visual indicator of the flow rate, but also as a safety valve in case of an accidental rise in pressure.

The ventilator cycled repeatedly through three phases in sequence, which will be referred to as the E- (for expiration), I- (for inspiration) and H- (for holding) phases,

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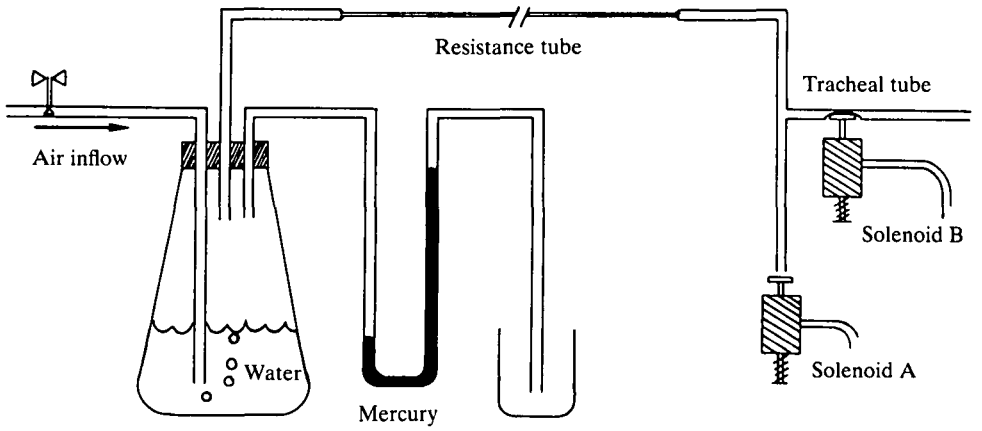

Fig. 1. The air-flow source and the switching solenoids. An approximately constant air flow through the resistance tube is either directed into the trachea or is allowed to escape, depending upon the state of solenoid A. Glottal closure is simulated by activation of solenoid B.

the latter being a phase in which the airway was blocked with the lungs inflated. In the E-phase, expiration occurred passively, and its duration affected the residual lung volume. The duration of the I-phase determined the tidal volume, whereas the duration of the H-phase could be chosen so as to match the normal breathing frequency of the animal.

For inspiration, the constant air flow was directed into the tube leading to the trachea (Fig. 1). This was achieved by closing the escape route for air (*via* the straight portion of the T-tube) by activation of solenoid A, and opening the tracheal tube by inactivation of solenoid B. The inspiratory I-phase was followed by the apnoeic H-phase in which the tracheal tube was closed by activation of solenoid B and the flowing air was allowed to escape by inactivation of solenoid A. In the ensuing E-phase, expiration occurred as the tracheal tube was opened to the atmosphere by inactivation of both solenoids. In this phase, the incoming air simply escaped through the straight portion of the T-tube and did not enter the trachea. The escaping air in the H- and E-phases served to cool the solenoids.

The activation times of the solenoids were controlled by the electronic circuit shown in Fig. 2. The main criteria underlying the design of this circuit were: (1) the ability to adjust the duration of each of the three phases independently, and (2) the use of a single power supply for operating the circuit as well as the solenoids. Since most commercially available solenoids require a power supply voltage of 24 V, the circuit was designed to operate from the same voltage, which ruled out standard logic circuits that require a 5 V supply. The circuit utilized a readily available IC chip (consisting of four comparators), two transistors, three capacitors and associated variable resistors for selecting the durations of the three phases, and a number of fixed resistors and silicon diodes. The transistors were chosen in view of the currents (0·25 A each) drawn by the solenoids that were employed (Magnetec SCA 3607-02). The two light-emitting diodes (LED A and B) shown in Fig. 2 provided a visual indication of solenoid activation. The switch (SW), if turned on, kept the ventilator

in the H-phase, and thus prevented the switching of the solenoids. This feature was useful for the avoidance of electrical artifacts during periods of sensitive neural recording.

Briefly, the operation of the circuit was as follows. Solenoid B (S_B) remained turned on until the capacitor C_H charged, *via* R_H, to the threshold voltage on pin 4 (i.e. 12 V). At this moment, the output of the comparator (pin 2) went high, turning off the transistor driving S_B. When S_B turned off, C_E discharged *via* R_E, which determined the E-phase duration. When C_E was discharged to threshold, S_A turned on, initiating the I-phase. The I-phase was terminated when C_I charged to threshold *via* R_I. At this moment, pin 14 went to zero voltage, and remained there until C_I was discharged (approximately 3 s). During this time, C_H was also discharged, S_B stayed on, and S_A stayed off. The H-phase continued during the subsequent charging of C_H. The circuit allowed the following ranges for the durations of the three phases: H-phase, 3–60 s; E-phase, 1–18 s; and I-phase, 0–20 s. These ranges can be altered for other applications by changing the capacitors in the circuit.

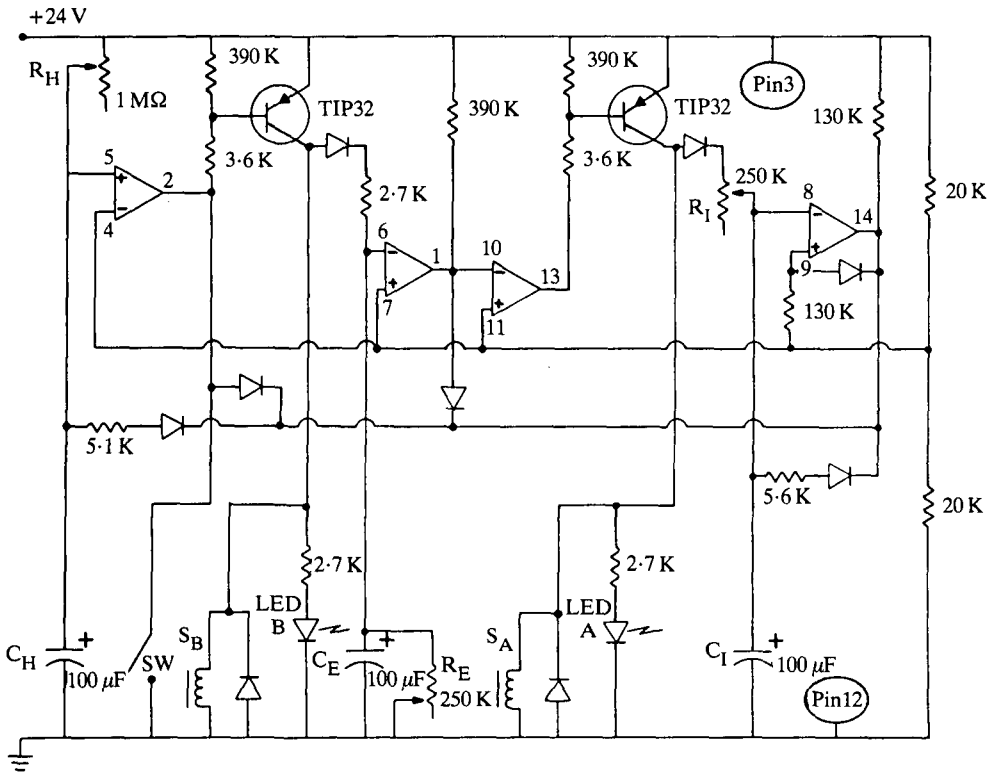

Fig. 2. Circuit for the operation of the two solenoids (S_A and S_B) in accordance with the durations chosen for the three phases (H, E and I). The respective durations are determined by the resistors (R) and the capacitors (C) denoted with the appropriate subscripts. The four voltage comparators (triangles) comprise the integrated circuit (LM339), for which the pin numbers (1–14) are indicated. LED, light-emitting diode; SW, switch.

Table 1. *Blood gas analysis in decapitated turtles under artificial ventilation*

Time after decapitation (h)	P_{O_2} (mmHg)	P_{CO_2} (mmHg)
5	88	22
23	132	23
52	133	14
77	125	10

The I-phase duration (typically 5 s) was adjusted to achieve a tidal volume of 15 ml kg^{-1}, which is appropriate for *P. scripta* (Jackson, 1971). The volume was calibrated by allowing the air to displace water in an inverted test tube instead of inflating the lungs. The duration of the E-phase was set to be 8 s, and that of the H-phase (typically 20 s) was chosen to achieve a frequency of somewhat less than 2 breaths min^{-1} (Jackson, 1971).

With the use of these parameters of ventilation, a decapitated turtle could be maintained for 3 days or more. Longevity appeared to be improved by intravenous infusion (*via* a flow pump) of Ringer's solution at the rate of 2–4 ml h^{-1}, supplemented occasionally with 5 % dextrose.

The neurological state of the animal was assessed by the ability to elicit limb movements in response to either electrical stimulation of the spinal cord or natural stimuli (Lennard & Stein, 1977; Bakker & Crowe, 1982; Hasan & Sasaki, 1986). However, since the reptilian nervous system is quite tolerant of anoxia, the respiratory state was assessed by blood gas measurements in four turtles. Blood samples drawn from the left ventricular region of the exposed heart were analysed at 37 °C for their O_2 and CO_2 content. The mean partial pressures 5 h after decapitation were $P_{O_2} = 88$, $P_{CO_2} = 22$ mmHg (Table 1). These pressures, however, may have been influenced by the procedure of keeping the animals in crushed ice for 2–3 h prior to decapitation and 1–2 h afterwards, before starting artificial ventilation and allowing the animal to warm to room temperature (22–25 °C).

Long-term use of the ventilator led to a gradual decline in P_{CO_2} (Table 1). However, with the possible exception of the measurement at 77 h, the other measurements were within the normal range for reptiles, especially in view of the large fluctuations that occur diurnally (Howell & Rahn, 1976). The P_{O_2} readings appear to be within the rather wide range of natural fluctuation (Wood & Lenfant, 1976).

While it is clear that the normal state cannot be maintained indefinitely by artificial ventilation, the deterioration in the absence of ventilation is remarkable. In one animal that was not ventilated for 5 h following decapitation, P_{O_2} was observed to be only 53 mmHg, and P_{CO_2} was elevated to 51 mmHg. (The heart rate in this animal was 46 min^{-1}, as compared to the range of 24–32 min^{-1} observed in the ventilated animals.) Thus, the ventilator is efficacious in prolonging the normal respiratory status of the blood. Whether the triphasic pattern of ventilation is better in this respect than the more conventional diphasic pattern was not investigated. The extremely low cost of the design presented here, in comparison with the cost of

conventional ventilators, may make its use attractive for artificial ventilation of reptiles.

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