Current Comment

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Control of Concentration of Volatile Agents in Open In Vitro Systems

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Research involving the effects of volatile anesthetic agents on isolated tissue has generally been hampered by the lack of a suitable method of metering into the system quantities of agent which remain uniform and can be reproduced from one experiment to the next. In a previous publication [1] we have described an electronic device for reproducibly controlling the force of contraction of isolated rat auricles suspended in a bathing medium. This device consisted of a feedback principle in which the force of contraction of the auricle (via the strain gauge) served as the signal to permit more or less anesthetic to enter the system. This report deals with a much more simplified method of accomplishing the same result.

METHODS

Male rats, 140–275 g., were killed by decapitation. The intact auricles (both right and left lobe with connecting bridge) were removed and suspended in a modified Krebs-Henseleit solution at 27° C. and stimulated 180 times per minute at twice threshold voltage. Force of contraction was recorded at 15-minute intervals. The first hour served as equilibration period for all experiments. Eleven control auricles were run for two additional hours to determine stability of the preparation. Administration of chloroform was begun at the 60-minute period and continued until the 180-minute period. An attempt was made to keep the force of contraction as close as possible to 50 per cent of the 60-minute force. This was achieved using a Tektronix stimulator and relay to trigger the opening of a solenoid valve permitting access of 95 per cent O_2–5 per cent CO_2 to anesthetic in a vaporizer which was connected to the bathing medium. The rate and duration of opening of the solenoid valve were set by the frequency and duration dials on the stimulator. Manual adjustments of the stimulator were made as necessary to keep the force of contraction depressed 50 per cent. At the end of the two hour experimental period administration of anesthetic was discontinued and the preparation allowed to recover.

One-half milliliter samples of bathing medium containing anesthetic were taken at 15-minute intervals throughout the experimental period and mixed with 1½ ml. tetrachlorethylene. This resulted in better than 99 per cent extraction of anesthetic (partition coefficient of

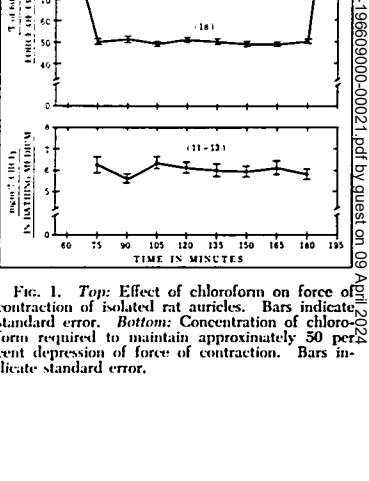

FIG. 1. *Top:* Effect of chloroform on force of contraction of isolated rat auricles. Bars indicate standard error. *Bottom:* Concentration of chloroform required to maintain approximately 50 per cent depression of force of contraction. Bars indicate standard error.

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TABLE 1. Mean Concentrations of Chloroform in Bathing Medium Necessary to Maintain Approximately 50 Per Cent Depression of Contractile Force

Experiment	Chloroform Mg./100 ml.
C-8	6.24 (7)*
C-9	5.61 (7)
C-10	6.94 (8)
C-11	6.22 (7)
C-12	5.46 (8)
C-13	5.59 (8)
C-14	6.22 (8)
C-15	5.85 (7)
C-16	7.78 (7)
C-17	6.59 (8)
C-18	5.98 (8)
C-19	4.23 (8)
Mean	6.06
S.D. ±	0.87
S.E. ±	0.25

* Figures in parentheses indicate number of determinations made at 15-minute intervals during each experiment from which the corresponding mean values were calculated.

chloroform between tetrachlorethylene and water was found to be 576. Ten microliters of the tetrachlorethylene fraction were injected into the port of a Beckman GC 2A gas chromatograph with thermal conductivity detector. The column used contained 30 per cent apiezon L, 5 per cent flexol 8N8 on C-22 firebrick, mesh 42 60 and was 6 feet long. Current was set at 330 milliamperes and temperature at 160° C. Recorded peak heights were measured and concentration determined from a calibration curve. Standards of anesthetic in tetrachlorethylene were made up fresh daily.

The degree of control of the force of contraction achieved by our method can be seen in figure 1. The force of contraction was measured every fifteen minutes in each experiment. Eleven auricles not exposed to chloroform showed a slight gradual decline in force of contraction over the two-hour period, at the 180-minute period the force was 93 per cent of the 60-minute period. Auricles exposed to chloroform demonstrated a mean decrease in force of contraction of approximately 50 per cent which was well maintained, over the experimental period. Maximum recovery of contractile force occurred in 5–10 minutes.

Figure 1 also shows the concentrations of chloroform in the bathing medium required to keep the force of contraction depressed 50 per cent (samples taken at fifteen minute intervals). Table 1 shows the mean chloroform concentrations of 7–8 samples taken for each muscle during each experiment. The mean chloroform concentration for all samples for all experiments was 6.06 with a standard error of ± 0.25.

DISCUSSION

To our knowledge this is the first report of a relatively simple method which makes it possible to reproducibly affect the function of living tissue (*in vitro*) easily with agents that are difficult to work with due to their volatile nature. This approach may also make work with volatile agents on tissues other than heart possible. All that is needed is a suitable parameter that is affected by the volatile agent. If this becomes difficult it would be feasible to include an isolated heart preparation such as an auricle or papillary muscle in the same container with the tissue to be studied. The heart preparation would then be used to monitor the anesthetic concentration. Depressing the force of contraction of the heart preparation to a certain level would give a constant anesthetic concentration (the actual value to be determined by gas chromatography or some other means) in contact with the tissue to be studied. This can significantly broaden the entire spectrum of *in vitro* work with volatile agents on many types of isolated tissue. Metabolic, ion flux, action potential studies among others, which would otherwise have been difficult can now more easily be performed.

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REFERENCE

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