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THE MEASUREMENT OF ANALGESIC PROPERTIES OF INHALATION DRUGS GIVEN IN SUB-ANAESTHETIC CONCENTRATIONS

BY

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An electrical method of testing for analgesia in mice has been developed for use with inhalation anaesthetics given in sub-anaesthetic concentrations. The anaesthetic drugs are administered in a gas chamber designed for the testing of analgesia without removal of the animals from the chamber.

Although most of the well-known anaesthetic drugs have been tested for analgesic properties in sub-anaesthetic doses in man, no equivalent experiments seem to have been performed on animals. As it was intended to test new compounds for such activity, a method suitable for experimental animals was developed. Various methods have been employed to produce pain in human work on anaesthetics: these methods include radiant heat (Chapman, Arrowood & Beecher, 1943), mechanical pressure (Seevers, Bennett, Pohle & Reinardy, 1937; Dundee, Nicholl & Black, 1962) and exercise of ischaemic muscles (Chapman *et al.*, 1943; Hewer & Keele, 1948). None of these methods was considered suitable for animal work on anaesthetic drugs, when the animal had to be confined in a gas chamber which frequently contained highly inflammable vapours or gases. An electrical method of testing for analgesia was therefore developed. This has the advantage that when the animals are once placed in the gas chamber they can be left untouched for the whole experiment, and removes the possibility of an explosion which might occur with radiant heat.

Mice were chosen for the experiments as they are small and convenient to use, and, in later experiments when it was planned to use new compounds, it was known that only very small quantities of anaesthetic would be available.

Apparatus

A diagram of the complete experimental arrangement is shown in Fig. 1. Two mice at a time were placed in the gas chamber, each having a pair of electrodes attached to its tail. When required, electric shocks produced by the stimulator were given to the mice through a control box. This box contained merely a selector switch, the operation of which directed the electric shocks to the mouse being stimulated. An oscilloscope was used to monitor the electric pulses.

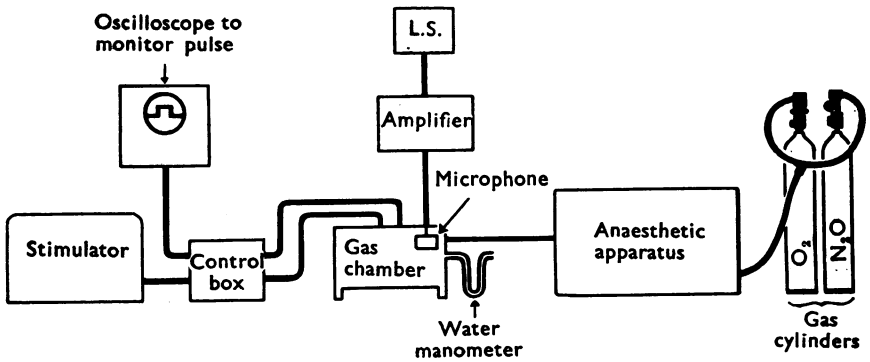

Fig. 1. Block diagram of entire apparatus. Two mice, each with a pair of electrodes on its tail, are placed in the gas chamber. Shocks from the stimulator are directed to each mouse in turn by a switch on the control box. Squeaks from the mice are amplified and are heard from the loudspeaker (L.S.).

The anaesthetic mixture was passed through the gas chamber at such a rate as to prevent accumulation of carbon dioxide. This avoided the necessity of using soda-lime for the absorption of carbon dioxide. In general, flow rates of 0.5 to 2 l./min were used.

A microphone in the gas chamber was connected to an amplifier and loudspeaker system, and this made it possible to hear the squeaks of mice clearly outside the chamber. The various parts of the apparatus are next described in greater detail.

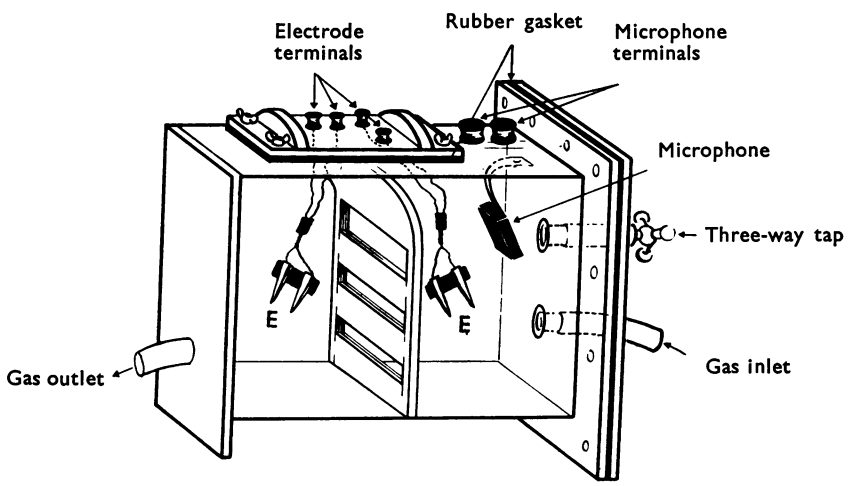

Fig. 2. Details of gas chamber. The chamber is divided into two by a perforated wall. A mouse is placed in each half and a pair of electrodes (E) taped on to the mouse's tail, with zinc oxide plaster. A microphone is mounted in the roof of the chamber. The lid is made gas-proof by a rubber gasket and is secured by four wing-nuts. One end of the chamber is removable to enable a copper hot-plate to be inserted if required.

Gas chamber

The details of the gas chamber are shown in Fig. 2. The chamber was made of Perspex and measured 23 × 15 × 15 cm with a volume of approximately 5 l. One end was made removable to allow for the insertion or removal of a copper hot-plate. The joint was made gas-proof by a rubber gasket. A crystal microphone, mounted on the inside top surface, was connected to terminals also on the roof of the gas chamber.

The lid of the box had four terminals. These were used to connect the stimulator leads to the electrodes inside the box. The lid was fixed down firmly by means of four wing-nuts. In addition to a gas inlet and outlet, an additional tube was connected to a three-way tap. By means of this tap gas samples could be bubbled through a solution containing methyl red indicator to permit colorimetric determination of carbon dioxide. For the remainder of the time, the tap connected a water manometer to the chamber, thus ensuring that pressurization did not occur.

Method of administering anaesthetic

The apparatus used was basically that described by Raventos (1956). Additional features were the flowmeters for oxygen, nitrous oxide and cyclopropane, and the gas chamber specially devised to facilitate the method of testing for analgesia. The anaesthetic apparatus is shown diagrammatically in Fig. 3.

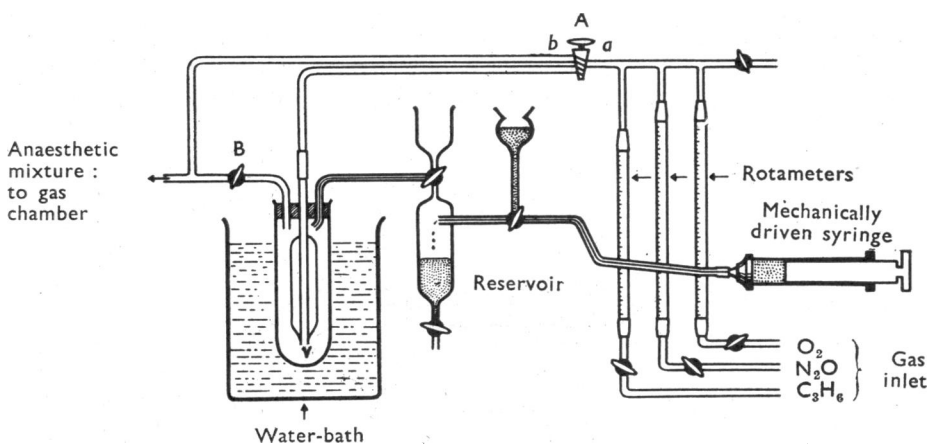

Fig. 3. Apparatus for supplying a known concentration of anaesthetic. Mercury injected at a constant rate from the syringe displaces anaesthetic from the reservoir into the vaporizer (*v*) which is maintained at the boiling point of the anaesthetic. The vapour formed is carried away by a stream of oxygen into the gas chamber. The concentration is regulated by altering the mercury injection rate and/or the oxygen flow.

Mercury was injected into the anaesthetic reservoir at a constant rate, displacing the anaesthetic on to the inner surface of the vaporizer, which was surrounded by water at the boiling point of the anaesthetic. The vapour was then carried away by oxygen entering at the bottom of the vaporizer. The resulting mixture was led to the gas chamber. The concentration was controlled by varying the oxygen flow and/or the anaesthetic injection rate.

When gaseous anaesthetics only were being used, tap A was operated to connect *a* to *b* and tap B was closed (see Fig. 3). This removed the vaporizer from the circuit.

Gas flows were measured by rotameters. Special 2 l./min, 200 mm long tubes were obtained for oxygen and nitrous oxide. For cyclopropane a 100 mm long 50 ml./min rotameter was used. Using these instruments, very accurate measurement of gas flow was possible.

Animals

A hybrid strain of male white mice weighing 20 to 25 g and bred in the Animal House of Guy's Hospital Medical School was used.

Stimulation

The mice were stimulated by DC rectangular wave pulses from a constant voltage stimulator (R.A.F. Type V, SS Electronics Ltd.) at intensities between 10 and 27.5 V. The stimulus frequency was 0.5 shocks/sec and the pulse duration was 100 msec. The pulses were directed to the appropriate mouse by means of the selector switch on the control box. (Two mice at a time were exposed to the anaesthetic.)

The shocks were applied to the tails of the mice through a pair of crocodile clips, as described by McKenzie & Beechey (1962), the clips being mounted on an insulating bar 3 cm long. The teeth were filed off and the jaws were bent to the contour of the mouse tail. The springs were replaced with much weaker coil springs. In our experiments the electrodes had to remain on the mouse's tail for up to 1 hr, and this meant that the springs had to be rather weak to prevent mechanical damage or local ischaemia of the tail. Because of this it was found necessary to tape the electrodes to the mouse tail by means of 0.5-in.-wide zinc oxide tape. The positive electrode was always placed at the proximal end of the tail.

The electrode contact resistance was reduced as much as possible by the application of Cambridge electrode jelly. The tail was clipped lightly and then rubbed between the electrode jaws so as to abrade slightly the skin surface. Measurements of electrical resistance showed this procedure to be effective in reducing the contact resistance to a minimum.

Measurement of analgesia

The method for measuring the degree of analgesia was derived from that of McKenzie & Beechey (1962). About twenty mice were used for each concentration of anaesthetic. They were exposed for periods of up to 1 hr and, in general, tested for analgesia every 10 min.

Before being placed in the gas chamber the approximate voltage threshold of each mouse was determined as follows. The electrodes were clipped on the mouse's tail about 2 cm from the base (they were not taped on at this stage). Three shocks were then given at each voltage starting at 10 V and increasing by 2.5 V increments. The voltage causing the mouse to squeak at least twice out of the three times was taken as the threshold. When two mice had been obtained with thresholds between

10 and 27.5 V, they were taped to the electrodes and placed in the gas chamber. To test for analgesia each mouse was given a series of ten shocks at its threshold voltage. The number of times the mouse did not squeak was counted (negative reponse). This was taken as proportional to the analgesic effect of the drug. The results using anaesthetics were compared with control values using pure oxygen. The response had to be a value between 0 and 10; the bias resulting from these limits was ignored in the statistical analysis.

Mice that became anaesthetized during the experiment were excluded from the results from the time they became anaesthetized.

Assessment of results

The results were tested statistically to discover whether the negative response of the treated mice was significantly different from the negative response of the controls. This was done by one of two methods: (1) Student's *t*-test; (2) a modified form of Student's *t*-test (Bailey, 1959).

The choice of method depended on whether the variances had been shown to be significantly different. If this was so the modified Student's *t*-test was used. When the variances of the control and test groups were not significantly different the unmodified Student's *t*-test was used.

DISCUSSION

The method and apparatus described have been found to work quite well under the conditions required for testing inhalation agents. Various other methods were tried, namely: (a) measuring the voltage thresholds at various times and using the change in threshold as a measure of analgesic effect; (b) using constant current instead of constant voltage; (c) using the original McKenzie & Beechey (1962) method; and (d) using different pulse durations and frequencies. However, the method described was found to be just as good or better than any of the other methods tried. It was definitely more sensitive than the method of McKenzie & Beechey (1962) under the conditions used.

Although there is the theoretical disadvantage in using DC current that polarization at the electrodes occurs, the polarization voltage is, in fact, so small compared to the stimulating voltage as to be negligible. Although the pain is supposed to be caused by the current flowing through the tail, and this current must be governed by the resistance in the circuit, a constant voltage stimulator, in practice, seems to work just as well as a constant current machine.

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