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# Automatic apparatus for recording duration of narcosis in mice

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An apparatus for automatically recording the duration of narcosis in mice is described. Following induction of anaesthesia the animals may be left unattended for the remainder of the experiment.

THE effects of compounds on the duration of drug-induced narcosis in mice are commonly studied during their preliminary examination for possible actions on the central nervous system. The test, often referred to as the "sleeping time" test, was first described by Winter (1948) and is based on findings that central depressants prolong and central stimulants reduce the duration of anaesthesia caused by a standard dose of hypnotic (usually pentobarbitone or hexobarbitone sodium).

As usually carried out the test is subject to a number of disadvantages. Each experiment requires the undivided attention of the experimenter to record the time that elapses from the moment of induction of anaesthesia to that of recovery for each mouse. In addition, errors arising during determination of the time of recovery, often the return of the righting reflex is used as the end-point, adversely affect precision. These arise because of the premature disturbance of the mice, by the handling necessary for the assessment of the end-point and also by the recovered animals running across their neighbours before capture. Furthermore, as determination of the end-point is a subjective assessment it varies with the skill of the observer. It was of interest therefore to devise an apparatus* that recorded the duration of anaesthesia automatically using an objective end-point the mice being kept in a uniform environment free from extraneous influences. We describe here the construction of such an apparatus and compare the results obtained with its use with those obtained using the established, conventional technique.

## Method

The principle of the method is that the duration of anaesthesia in each mouse is recorded separately by a timing device, started by closure of a microswitch under the weight of the mouse placed on a small spring loaded shelf. The timer is switched off by the mouse falling from the shelf during recovery from the central depressant effects of the barbiturate.

The design of the shelf and its position relative to the micro-switch is indicated in Fig. 1. Following intravenous induction of anaesthesia each animal is placed on a hinged perspex shelf S, pivoted at P the animal's weight depressing the shelf against the spring loaded contacts of the micro-switch M. The incoordinated movements that occur during the preliminary stages of recovery from the barbiturate are used as the end-point

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for recovery. These cause the mouse to fall from the shelf, which is only just large enough to support it, allowing the microswitch to reopen, thus breaking the circuit. Small side walls are fitted to each shelf so that the mouse does not fall because of its respiratory activity and has to give a very positive movement in order to fall off.

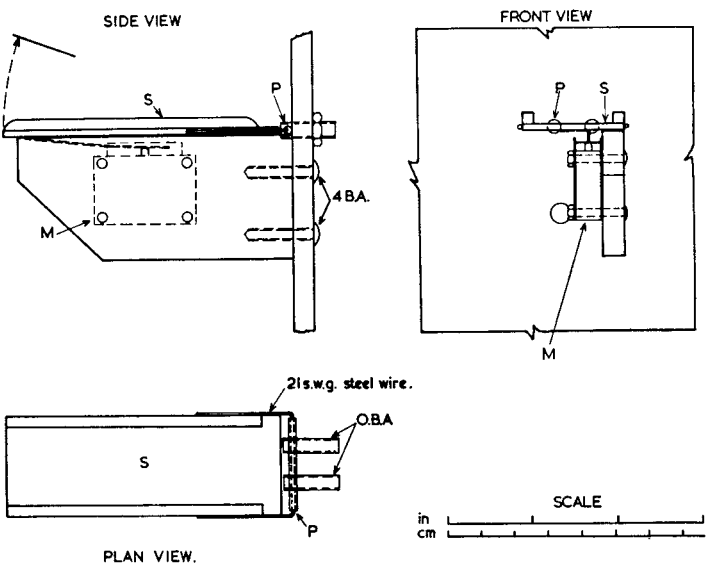


FIG. 1. Working diagram of a shelf and its accompanying microswitch. The shelf S pivoting at the point P is illustrated in its working position depressing the lever of the microswitch M.

The shelves and their accompanying microswitches are mounted on a perspex stand 9 cm apart, in rows of 5. Each row is 15 cm above and 9 cm in front of the row below. This arrangement minimises the disturbance to other animals caused by a mouse falling from its shelf. A gauze net placed beneath the shelves prevents injury. The entire unit is installed in a thermostatically controlled incubator, as the precision of the test is increased by carrying it out in a uniform and constant temperature (Riley & Spinks, 1958).

THE ELECTRONIC CIRCUIT

The simple electronic circuit is shown in Fig. 2. The contacts P of a Palmer time clock are adjusted to close briefly once every 5 sec allowing relay A to energise, closing momentarily contacts $A_1$. When the mouse is placed on its shelf the microswitch contacts M adopt the position indicated in the diagram. The manually operated switch S is then momentarily closed energising coil "a" of the magnetic latching relay B so closing contacts $B_1$ and $B_2$. This allows passage of the electrical impulses caused by closing contacts $A_1$ to pass through contacts $B_1$ triggering a Post Office electromagnetic counter C. When the mouse falls from the shelf, the

contacts M change over causing the next impulse to pass through the "suicide" contacts $B_2$ to the reverse wound coil "b" opening contacts $B_1$ and $B_2$. The counter thus indicates the number of 5 sec periods that

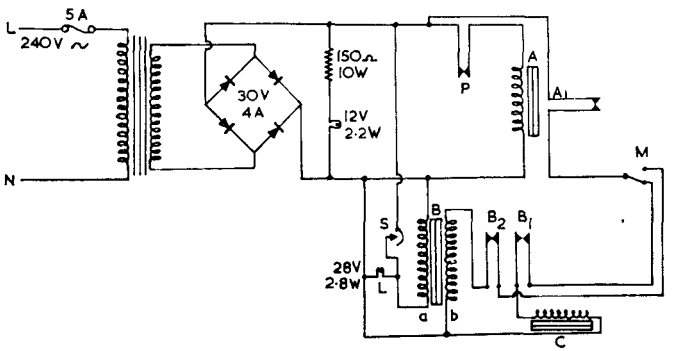


FIG. 2. Simplified circuit diagram. The circuit utilising the components L, S, B, C and M is repeated in parallel for each shelf in the apparatus. The current supplied by the full wave selenium rectifier is sufficient for up to 50 shelves and their associated electromagnetic counters. P are the contacts of a Palmer Timer type B.116. A is a P.O. 3,000 type relay having a 2,000 Ω coil; contacts $A_1$ rated at 10 A close on energising. B is a magnetic latching (remanence) relay having two coils a and b of 400 Ω and 1,000 Ω respectively causing opposing magnetic fluxes. C is a P.O., four digit, electro-magnetic counter (2,000 Ω coil). M is a Bulgin microswitch type 'M' list No. S520/W. S is a "push to make" switch, L a pilot light.

elapse during anaesthesia. Until S is again closed to reset the circuit, any subsequent closure of the microswitch does not activate the counter preventing resumption of recording should a recovered mouse climb back on to a vacated shelf.

## Results

The relative accuracy of data obtained using the apparatus has been assessed by comparing the results from its use with those obtained from similar experiments carried out concomitantly under conventional conditions. The latter were carried out as follows. Groups of mice received either one of a series of doses of the drug under test, or saline as a control, 30 min before administering intravenously an anaesthetising dose of barbiturate. Immediately following induction of anaesthesia the animals were placed in rows on a thermostatically controlled heated tray and the return of the righting reflex taken as the end-point for recovery.

Statistical analysis was separately carried out on the data obtained using each method. On all comparable sets of data the within drugs (residual) term was used as a measure of experimental variability. Fig. 3 shows that using either the automatic apparatus or the heated tray the narcosis times are log normally distributed, as the cumulative distributions conform closely to straight lines when plotted on logarithmic probability paper, confirming the results of Winter (1948). The ratio of the geometric means of the narcosis times indicated by the automatic apparatus,

for the drug-treated and control groups respectively, provides therefore an adequate initial measure of the degree of central effect.

To compare the variability of the results obtained from the automatic apparatus with those obtained using the tray, analyses were carried out

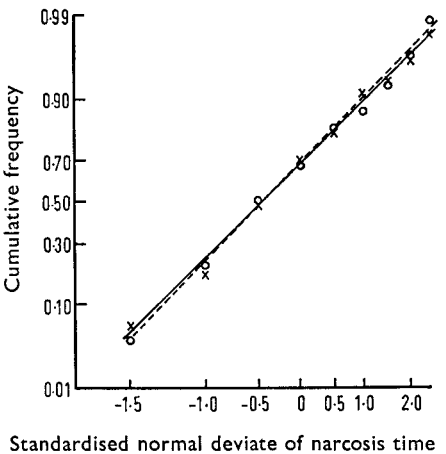


FIG. 3. Cumulative frequency distributions of narcosis times obtained using either the heated tray or the automatic apparatus. ×—× Heated tray. ○ - - - ○ Automatic apparatus.

on automatic apparatus and tray results separately and the residual mean squares used as an estimate of the within experiment variances. These were then tested for significance (Table 1) and in only one instance (60 mg/kg leptazol followed by 70 mg/kg pentobarbitone sodium) was a significant difference obtained ($P = 0{\cdot}05$), the automatic apparatus variance being less than the tray variance. Individual variances, calculated from all data obtained using either the automatic apparatus or the heated tray, were separately tested for homogenity, in order to obtain an overall estimate of the experimental variance using either technique. The variance obtained from the experiment using 2·5 mg/kg strychnine followed by 65 mg/kg pentobarbitone sodium was found to be significantly different from the others and therefore omitted from the overall variances. The ratio of the two overall variances (1·06) indicates that there is no significant difference between variation using the automatic apparatus and variation using the tray.

Table 2 shows that comparable levels of significance are obtained from an analysis of variance of figures obtained using either method and gives comparisons of the mean square ratios for the source of variation between doses. The results appear to be reasonably consistent indicating that the apparatus can distinguish between doses as well as the heated tray. It also indicates that although small groups of mice (7–10) are sufficient for screening purposes, the size of the group must be increased for accurate comparisons of centrally acting drugs.

TABLE 1. NARCOSIS TIMES OF MICE AFTER ADMINISTRATION OF CENTRALLY ACTIVE DRUGS COMPARING DATA OBTAINED USING THE HEATED TRAY METHOD WITH THAT USING THE AUTOMATIC APPARATUS. (GROUPS OF 7–10 ANIMALS)

| Drug | mg/kg s.c. | mg/kg i.v. pentobarbitone sodium | Log mean narcosis time (sec) | | | | Residual mean square | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Automatic apparatus | | Heated tray | | Automatic apparatus | Heated tray | Ratio |
| | | | Control group | Drug group | Control group | Drug group | | | |
| Leptazol .. .. .. | 60 | 70 | 3·7376 | 3·6781 | 3·6187 | 3·6460 | 0·00645 | 0·01608 | 2·49 |
| ,, .. .. | 120 | 70 | 3·6963 | 3·6887 | 3·7493 | 3·6015 | 0·01669 | 0·01432 | 0·86 |
| ,, .. .. | 240 | 70 | 3·8520 | 3·6838 | 3·8313 | 3·6048 | 0·00920 | 0·01449 | 1·57 |
| Bemegride .. .. .. | 10 | 75 | 3·5479 | 3·5962 | 3·6508 | 3·6126 | 0·02038 | 0·01765 | 0·87 |
| ,, .. .. .. | 10 | 75 | 3·6336 | 3·6231 | 3·7335 | 3·6087 | 0·01130 | 0·00768 | 0·68 |
| ,, .. .. .. | 15 | 75 | 3·5898 | 3·5299 | 3·7376 | 3·6780 | 0·00836 | 0·00913 | 1·09 |
| Strychnine nitrate .. .. | 2·5 | 65 | 3·6525 | 3·2970 | 3·6830 | 3·4679 | 0·06435* | 0·06127* | 0·95* |
| ,, ,, .. .. | 2·5 | 70 | 3·6940 | 3·6377 | 3·6936 | 3·6356 | 0·01580 | 0·01354 | 0·86 |
| ,, ,, .. .. | 2·5 | 75 | 3·7669 | 3·7344 | 3·8352 | 3·8573 | 0·03105 | 0·02444 | 0·79 |
| Chlorpromazine HCl .. | 5·0 | 60 | 3·3530 | 3·7466 | 3·3680 | 3·8753 | 0·00582 | 0·00798 | 1·37 |
| Dibenamine HCl .. .. | 10·0 | 65 | 3·6970 | 3·8047 | 3·8741 | 3·9214 | 0·01387 | 0·02269 | 1·64 |
| | | | | | Overall variance excluding* | | 0·013903 | 0·014801 | 1·06 |

Analysis of the log transformed data was also made to determine whether any differences in sleeping time occurred when the mice were placed on various shelves or in various locations on the tray. This was done comparing all positions individually, and also as a rows and columns

TABLE 2. MEAN SQUARE RATIOS FOR BETWEEN DOSES WITH SIGNIFICANCE LEVELS

| Drug | mg/kg s.c. | Pento-barbitone sodium mg/kg i.v. | Automatic apparatus | | Heated tray | |
|---|---|---|---|---|---|---|
| | | | Residual degrees of freedom | Mean square ratio | Residual degrees of freedom | Mean square ratio |
| Leptazol .. .. | 60 | 70 | 16 | 2·47 N.S. | 17 | 0·22 N.S. |
| ,, .. | 120 | 70 | 14 | 0·01 N.S. | 18 | 7·62 S. |
| ,, .. | 240 | 70 | 14 | 12·10 H.S. | 17 | 16·77 V.H.S. |
| Bemegride .. .. | 10 | 75 | 16 | 0·51 N.S. | 18 | 0·42 N.S. |
| ,, .. | 10 | 75 | 16 | 0·04 N.S. | 18 | 10·13 H.S. |
| ,, .. | 15 | 75 | 16 | 1·93 N.S. | 18 | 1·95 N.S. |
| Strychnine nitrate .. | 2·5 | 65 | 16 | 8·84 H.S. | 18 | 3·78 N.S. |
| ,, .. | 2·5 | 70 | 15 | 0·85 N.S. | 17 | 1·18 N.S. |
| ,, .. | 2·5 | 75 | 16 | 0·15 N.S. | 18 | 0·10 N.S. |
| Chlorpromazine HCl | 5·0 | 60 | 16 | 198·65 V.H.S. | 18 | 161·27 V.H.S. |
| Dibenamine HCl .. | 10·0 | 65 | 16 | 3·77 N.S. | 18 | 0·49 N.S. |

N.S. = Not significant $P > 0.05$
S. = Significant $P < 0.05$
H.S. = Highly significant $P < 0.01$
V.H.S. = Very highly significant $P < 0.001$

analysis. No significant differences were found, indicating that the results obtained are not affected by the location of the mouse within the cabinet or on the tray.

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## References

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