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Abstract

1. A. simple hot-plate apparatus, with comparatively good accuracy in the control of temperature, chiefly composed of a circular lead plate embedded with nicrome-wire heater was devised in order to improve the complications and inconveniences of the hot-plate apparatus used to date in algesimetric determination with mice. 2. The reaction times measured with this hot-plate maintained at 55°C with 500 normal mice showed an average of 9.96 ± 1.58 seconds and about 90% of the total mice exhibited reaction time of 7-13 seconds. With animals showing reaction time within this range, the daily mean reaction time did not vary with measurement once a day for consecutive days and the effect of drugs with comparatively weak analgesic effect was well reproduced with small number examples, such as 12 mice to a group.

A SIMPLIFIED HOT-PLATE APPARATUS FOR EVALUATION OF ANALGESIC EFFECT

By

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Of the numerous methods (*Haffner*, 1929; *Hesse*, 1930; *Eddy*, 1932; *Hildebrandt*, 1934; *Keil* and *Pöhls*, 1936; *D'Amour* and *Smith*, 1941; *Nomiyama*, 1942; *Uetsuka* and *Okushima*, 1947; *Green* and *Young*, 1951; *Kolle*, 1952; etc.) of testing the analgesic effect of drugs by the use of experimental animals published to date, the so-called hot-plate method of *Woolfe* and *Macdonald* (1944) is one of the most often used. The principle of this method is to measure the analgesic effect of drugs by the length of time it takes for a mouse to show the signs of discomfort by the abnormal movement of its hind legs by heat stimulus given off from a metallic hot plate. In the original method, a zinc plate on which a restraining hollow glass cylinder of 150 mm. in diameter is put, is maintained at a definite temperature and the analgesic effect of a drug is judged by the percentage of mice which remains on the hot plate for 30 seconds or longer without showing the above-mentioned reaction. This method is advantageous in that small animals such as mice can be used and statistical analysis can be made on the results obtained from a large number of animals.

The important point in this apparatus is the device which could keep the hot plate at a definite temperature and this device must be simply constructed that it would not interfere with the rapid movement of the worker. The apparatus used by a few of the workers (*Jacob* and *Szerb*, 1952; *Eddy* and *Leimbach*, 1953; *Ueshima*, 1954) keep the metal plate hot by some kinds of aliquid or a water bath. However, this method requires a fairly large volume of liquid to keep the hot plate at the exact temperature so that the device for its temperature control becomes complicated, with attendant difficulties as to

experimental procedures and observations, and some limitations are placed on the rapidity. *Eddy*, *Touchberry* and *Lieberman* (1950) used an electric bulb as the heat source for the hot plate but had to use a special temperature regulator in order to maintain the hot plate at a definite temperature.

In order to improve the defects in some of these existing apparatus, a hot-plate apparatus was devised which can be easily constructed, is easy to use, and is convenient for simple manipulation and observation. Further, good results were obtained by the use of this apparatus. The present paper records the construction of this apparatus and a few basic experiments regarding the reaction of mice to heat stimulus using this apparatus.

Experimental Apparatus and Method

Apparatus: a) *Hot plate Type 1.* This apparatus utilized the *Leitz*'s warm stage for a microscope. This stage is constructed of two circular brass plates of 130 mm. in diameter, with a heater enclosed inside. A thin, mercury thermometer, graduated to 1°C is embedded in a furrow about 5 mm. wide which circles along the brass plates. The stage is provided with a small hole in the center to permit irradiation of light but this was closed with a lead plate which fitted the hole exactly. The electric source was a 105—110 volt A. C. and the metal plate was maintained at a definite temperature by controlling the voltage of the heater wire through a transformer, Slidac. By this device, differing from automatic temperature regulator, a constant electric current will be supplied to the heater wire, which in turn will heat the hot plate to the exact tepmerature desired, compensating the heat lost by the cool air of the room. Since such constant current can be supplied by the graduation of the transformer, the procedure of temperature regulation is very simple. According to the present series of experiments, the temperature of the metal plate can be maintained at a definite level for a few hours to within ± 0.5°C accuracy with the transformer needle fixed at one point, as long as there is no intermittent loading of large electricity in nearby laboratories. A glass cylinder of 180 mm. in height and 120 mm. in diameter was

placed over this circular stage in order to limit the movement of the animal on the plate.

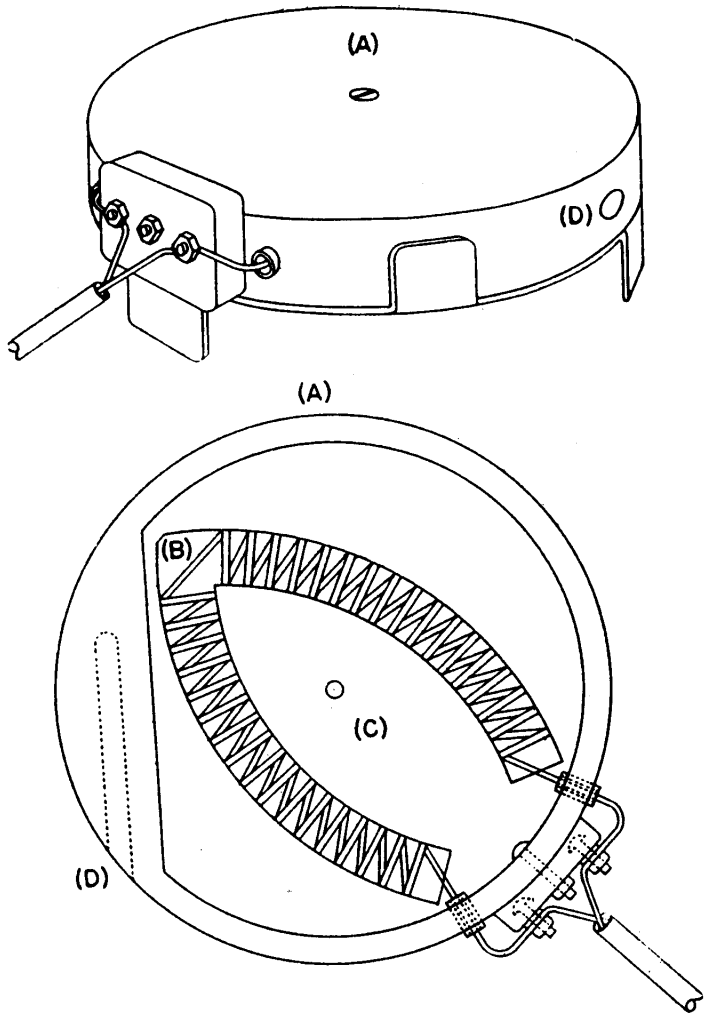

Fig. 1. Diagrammatic views of hot-plate device Type 2 for algesimetric studies. Lower diagram is an inside view. See text for detail.

b) *Hot plate Type 2.* A new apparatus was constructed in this laboratory according to the principle of Type 1 and its construction diagram is shown in Fig. 1. The main part of this apparatus is a lead disc (A), 150 mm. in diamater and 25 mm. in thickness, with a hollow (C) on the underside, in which was embedded a horse-shoe shaped mica plate, wound with a 180-

watt nichrome wire (B), covered with asbestos. Through this lead plate, avoiding the heater unit, is a hole (D) of about 50 mm. in depth, with a bore that exactly fits a thermometer which goes into this hole to measure the temperature of the lead plate. A glass cylinder which goes over it is 200 mm. in height and 140 mm. in diamter. The temparature control of this plate is also made through a transformer and it was found possible to keep the plate at a constant accuracy of the same order as that of Type 1.

Method: One mouse was placed on a hot plate maintained at a definite temperature and the time (in seconds) elapsed until the reaction appeared was measured with a stop watch. The criterion of the reaction were the bending or raising and shaking of the hind limbs, licking of the hind paws, and the dancing about or attempts to jump out of the cylinder observed in some of the mice. The mice which had urinated on the hot plate or given doubtful signs of foregoing discomfort were not used for the next 5 minutes or so. In order to avoid the heating of the air inside the glass cylinder, the cylinder was raised on every experiment to allow fresh air to be enclosed.

Experimental Results

Using 200 healthy mice of both sexes of more than 10 g. body weight, distribution of normal reaction times was measured with the hot plate Type 1, at 50°, 55° and 60°C. The results are shown graphically in Fig. 2, indicating normal distribution. The distribution range of the reaction time (in seconds) is comparatively extended at 50°C but is narrowed at 60° C. In the latter case, however, the reaction time becomes generally short and the mutual proportion of changes becomes greater which makes it inconvenient to treat the observed values, and accuracy of the measurement of reaction time cannot be expected. It was thought better, therefore, to use the intermediate 55°C for the determination of the effect of analgesic agents.

Ueshima (1954) classified such signs of discomfort shown by the mice on a hot plate into two classes: differentiating the shaking or bending and raising of the hind limbs as the first reaction, and the licking of the paws that follows and the jump-

ing motion seen in some of the mice as the second reaction. From the results of general observations on the effects of various agents on these reactions, he concluded that the first reaction is due chiefly to the spinal reflex, though it is affected to some extent by a stimulus of higher centers, while the second reaction is mainly the effect from higher centers, its disappearance showing depression of the higher centers, so that the evaluation of analgesic effect should depend on the prolongation of average time of the second reaction. Therefore, distribution of these two reaction times was examined with the hot plate Type 1 (55°C), using two stop watches.

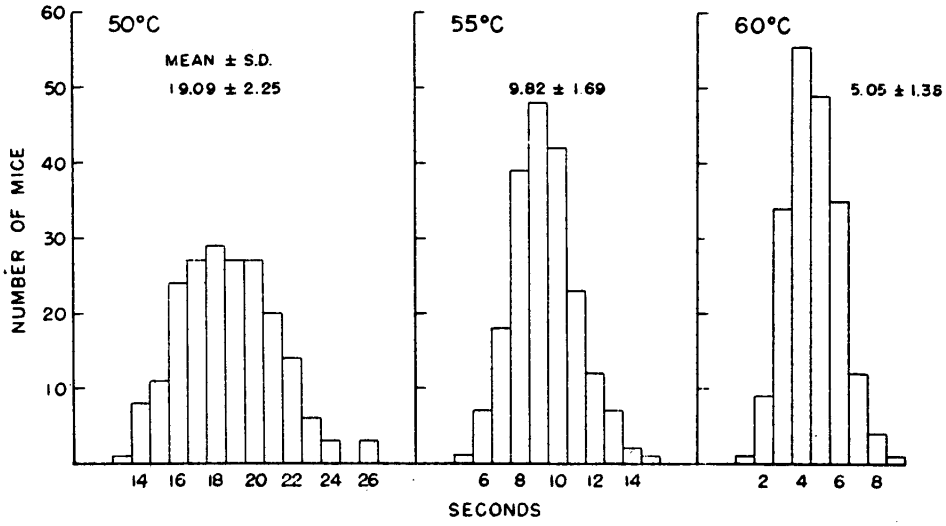

Fig. 2. Distribution of normal reaction times for 200 mice at 50°, 55° and 60°C.

The results obtained are shown in Fig. 3. The distribution of reaction times was measured with 500 mice for the first reaction and 300 mice for the second reaction. The reaction times for the second reaction in a few of the cases exceeded 40 seconds but the observations were terminated within this time to avoid inflicting burns on the paws, and these cases were all recorded as 40 seconds. Of the mice tested, 12.3% exhibited the second reaction from the beginning. In such a case, they were recorded as having exhibited the first and second reactions at the same time so that the reaction times recorded in such a case for the first reaction would be the same as shown before, taking the

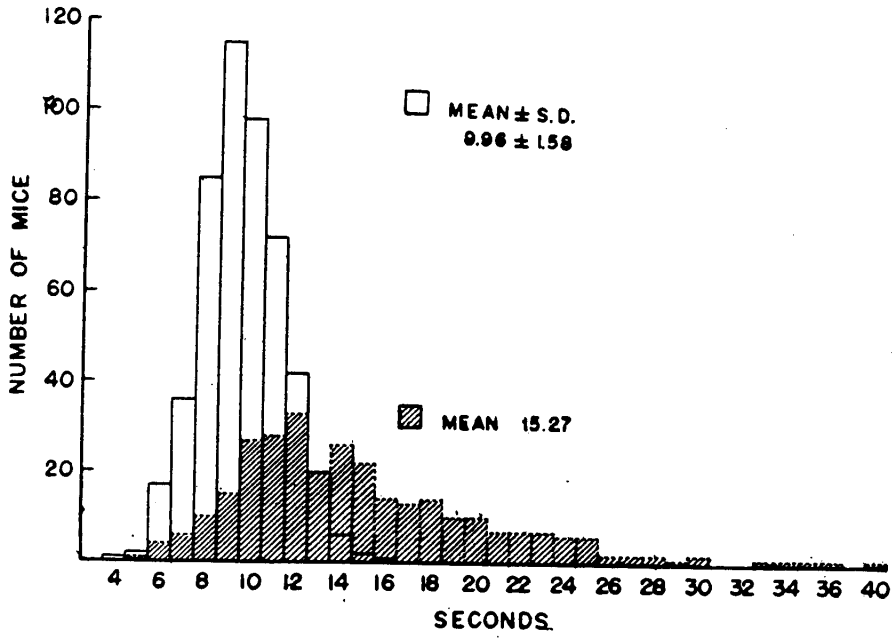

Fig. 3. Distribution of normal reaction times at 55°C. Open columns: *Ueshima*'s first reaction (500 mice); shaded columns: his second reaction (300 mice).

abnormal movement of the hind legs as the criterion of the reaction. As can be seen from Fig. 3, the second reaction generally

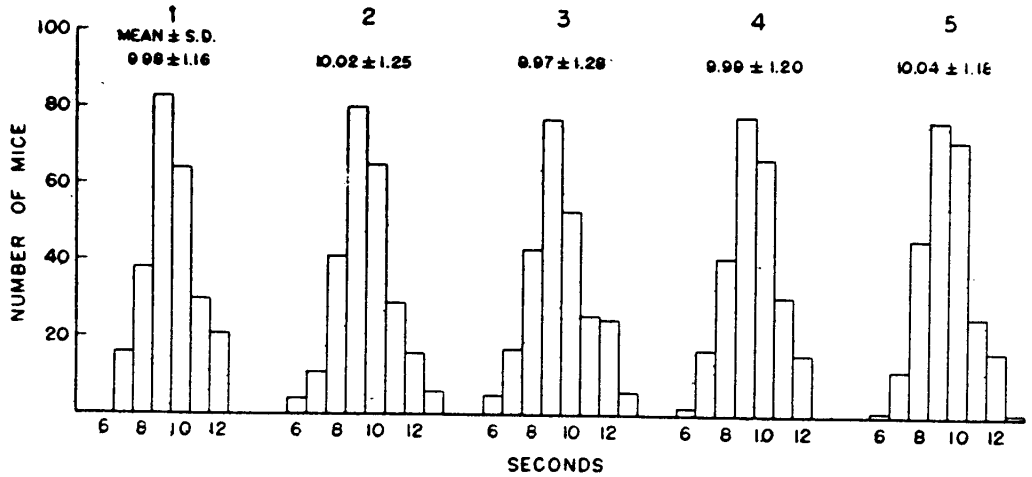

Fig. 4. Relatively constant response in mean reaction time on daily heat stimulus of 55°C for 252 mice exhibiting a reaction time of 7—13 seconds in preliminary test.

appeared later than the first reaction and its reaction time is distributed over a wide range. On the other hand, the reaction time for the first reaction is comparatively concentrated in the area centering around 9—10 seconds (average 9.96 ± 1.58 seconds), showing fairly ideal normal distribution pattern. It follows, therefore, that the first reaction is far easier to use as the criterion of judging the effect.

Next, healthy male mice of 15 ± 3 g. body weight were examined twice with an interval of over 1 hour and those showing reaction time of less than 7 seconds and/or more than 13 seconds were discarded. With the remainder of 252 mice, the

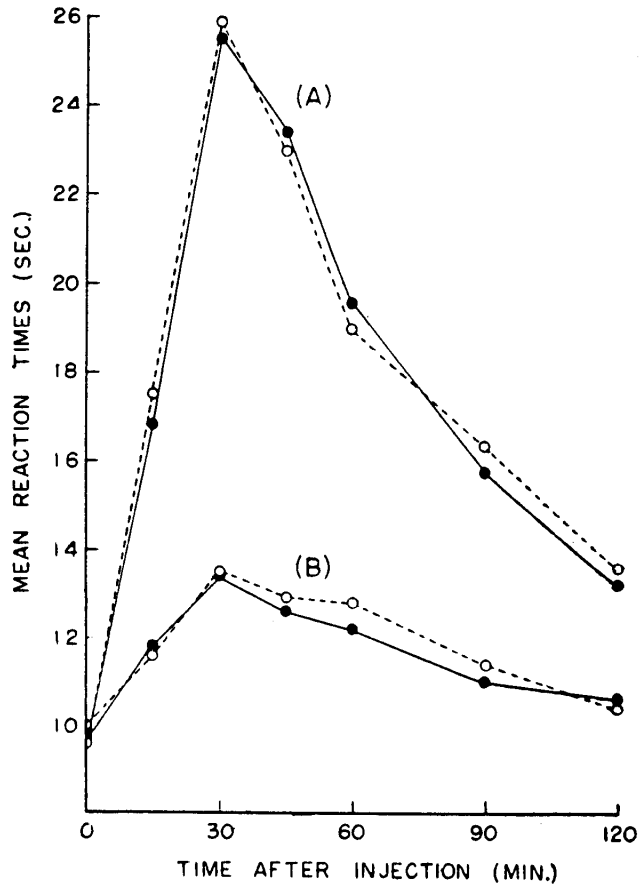

Fig. 5. Effect of subcutaneous injection of (A) morphine hydrochloride 10mg./kg. and (B) of aminopyrine 50mg./kg. on the mean reaction time of mice. Dotted lines : Hot-plate Type 1 ; solid lines : the Type 2. Each for a separate group of 12 mice.

reaction times were measured once every day under the same conditions. As shown in Fig. 4, there was no marked variation in the average time measured during the consecutive 5 days. Such experimental results were shown to be repeated almost wholly with the hot plate Type 2.

Fig. 5 shows the average values of the effect of subcutaneous injection of 0.1 mg./10 g. of morphine hydrochloride and 0.5 mg./10 g. aminopyrine in two groups of mice, composed of 12 healthy males showing normal reaction time of 7—13 seconds, examined with these two types of hot plate (55°C). These results indicate the good reproducibility of this method.

Discussion

The hot-plate method reported by *Woolfe* and *Macdonald* (1944) for algesimetry seems to be an excellent method in that a heat stimulus of a definite intensity can be given to animals in almost free state and the most economically advantageous mice can be used as the experimental animals, thereby permitting statistical treatment of the experimental results.

The hot-plate apparatus devised by the present writers is not only far more simple in construction than those reported by various workers, but also extremely easy in controlling the temperature and possesses good precision that it can be recommended for this kind of work. The appratus devised to date necessitated comparatively complicated accessories which proved to be a hindrance to experimental procedures to some extent and this point has been vastly improved in the present apparatus. It is also convenient to carry about and can be manufactured at a low cost, which should prove of value in its extensive use.

Eddy and *Leimbach* (1953) measured the reaction times of 2000 mice on a hot plate maintained at 55.0°—55.5°C by a formate-acetone constant temperature bath devised by them. They found the mean of the normal reaction times to be 9.05 ± 1.02 seconds, that these reaction times showed normal distribution, and that more than 90% of these mice exhibited pain reaction within a range of 6—13 seconds. The mean reaction time (with S. D.) examined in 500 mice with a hot plate maintained at 55°C

in the present series of experiments was 9.96 ± 1.58 seconds and 93.2% of the total mice exhibited the pain reaction in a range of 6—13 seconds, which are in good agreement with the earlier work. *Jacob* and *Szerb* (1952) carried out statistical analysis of the reaction time with mice picked out at random and with those showing a definite reaction time by preliminary tests, on a hot plate maintained at 58 ± 0.2°C, and concluded that the reaction time should be tested four times with an interval of a few hours before actual experiment and the animals showing reaction time of over 13 seconds should be excluded. According to the present experiments with the new apparatus, mice showing a reaction time of 7—13 seconds comprised 89.8% of a mixed breed of mice obtained on the market. *Eddy* and *Leimbach* (1953) measured the reaction times of 2000 mice, twice with each animal, and found that 19.6% of the total mice showed the same reaction time, 42.6% of them reacted with a difference of 1 second, 27.7% with a difference of 2 seconds, 8.1% with a difference of 3 seconds, and only 2.0% with a difference of more than 3 seconds. The present series of experiments also showed that repetition of tests under the same conditions, once a day for 5 consecutive days, with mice exhibiting a reaction time of 7—13 seconds in preliminary tests, there was only a slight difference in the distribution of reaction time. These results indicate that repetition of tests does not cause modification of results by conditional reflex and that the measurement of daily change of reaction time by continuous administration of drugs is possible. However, such measurements must be made with male mice alone in order to avoid the introduction of factors related to pregnancy and estrus cycle.

If mice selected for the foregoing range of reaction time are used, the effect of drugs could be measured with a fair reproducibility even with small numbers, such as 12 animals to a group. Further, if the effect is measured, not by the percentage ratio of the numbers exhibiting reaction time of over 30 seconds as was used by *Woolfe* and *Macdonald* (1944), but by the average prolongation of normal mean reaction time, it is possible to detect the effect of weak analgesic action, such as that of a small amount of aminopyrine.

The second reaction advocated by *Ueshima* (1954) shows

a wide distribution that it would be difficult to make the comparison of the effect unless a fairly large number of mice are used.

Summary

1. A simple hot-plate apparatus, with comparatively good accuracy in the control of temperature, chiefly composed of a circular lead plate embedded with nicrome-wire heater was devised in order to improve the complications and inconveniences of the hot-plate apparatus used to date in algesimetric determination with mice.

2. The reaction times measured with this hot-plate maintained at 55°C with 500 normal mice showed an average of 9.96 ± 1.58 seconds and about 90% of the total mice exhibited reaction time of 7—13 seconds. With animals showing reaction time within this range, the daily mean reaction time did not vary with measurement once a day for consecutive days and the effect of drugs with comparatively weak analgesic effect was well reproduced with small number examples, such as 12 mice to a group.

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