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121. Keijiro Takagi and Tsutomu Kameyama : Studies on Analgesics. IV.[1] A Recording Apparatus for Pain Reaction on the Hot Plate.

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The importance of objective recording of pain responses has become urgent in order to estimate reproducible potency ratios of several analgesics, especially those with weak activity. The movement and behavior of an animal on a hot plate might indicate effect of a drug upon pain reactions and at the same time upon other central nervous system. The momentary decision of the first appearance of pain reaction, however, is extraordinary difficult, when normal pain reactions are modified by weak analgesics, belonging to antipyretics or hypnotics, and these would be the cause for erroneous results on analgesic activities of such drugs to be reported often in literature.

In this paper, an improved apparatus, which can record the movement of a hot plate on smoked drum by means of a tambour system is described.

Apparatus and Method

The heating system is the same as described in the preceding report.[1] The hot plate (A) is isolated from the cylinder (B) and rests on a thin rubber membrane (C) at 3 points, as shown in Figs. 1 and 2.

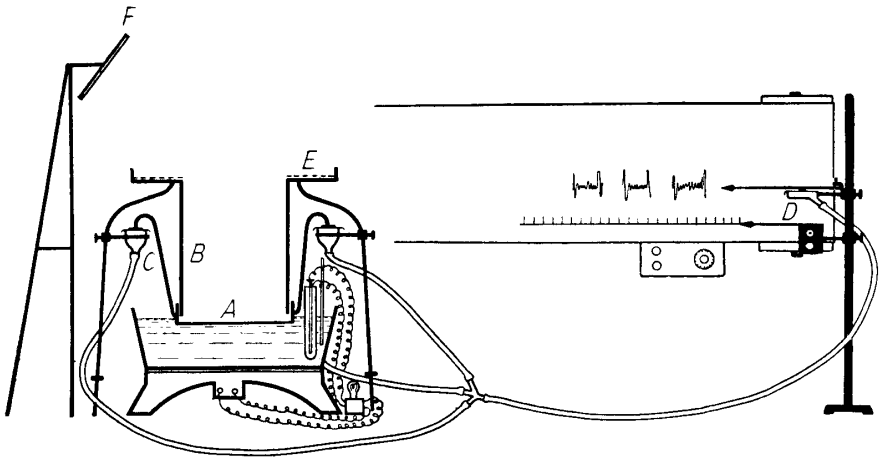

Fig. 1. Diagram of the Apparatus

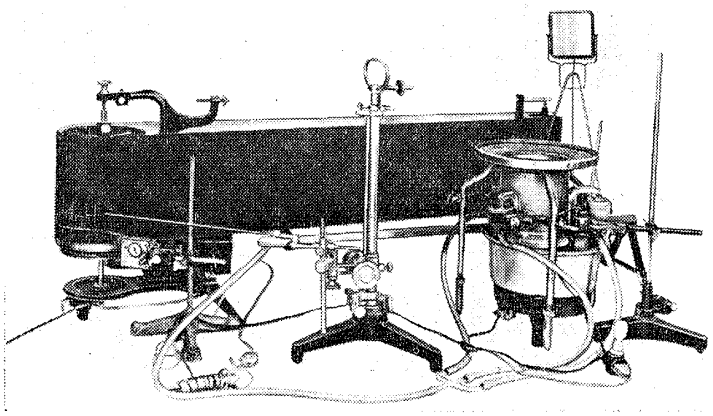

Fig. 2. Photographic Representation of the Improved Hot Plate Apparatus

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1) Part III : Yakugaku Zasshi, 78, 553(1958).

The movement of (A) is transmitted through rubber tubings to a tambour (D). The hot plate is made as light as possible in order to register its very slight movements. On an outer frame of the cylinder (B) is placed a metal net (E), which prevents an animal from slipping down when trying to jump out from the hot plate. By using a mirror (F), the behavior of the mouse and the kymograph tracing can be observed at the same time. The pain thresholds can be read from the time mark recorded during the experiment.

The animals and experimental conditions used were the same as those in the previous paper.[2)]

Results

Normal Pain Reactions—Two types of pain reaction are shown in Fig. 3. Reflective and abnormal movements of hind legs, which are a primary response according to Uejima, *et al.*,[2)] began at the arrow B, and the animal jumped at C up to the outer frame E. On repeated application of pain stimuli at 15-min. intervals on the same animal at 60°, the pain reactions became shorter and shorter, especially the primary response disappeared and the animal instantly jumped out after a few seconds (Fig. 4).

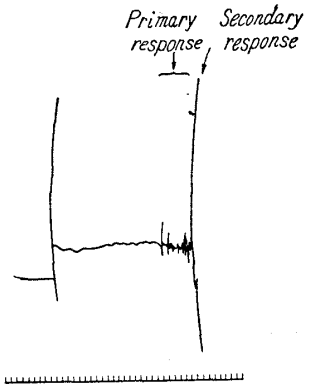

Fig. 3.
A typical Pain Response Recording on Hot-plate (60°)

Time interval, 1 sec.

Fig. 4. Results of Repeated Application of Pain Stimuli at 15-min. Intervals on the Same Animal (60°)

Pain Reaction at Various Temperatures—The animal, which had been trained several times on the hot plate of 60°, was stimulated at lower temperatures. At 50°, the animal showed slight and intermittent primary reactions on the hot plate, but did not jump out. At 52°, primary reactions increased in severity and the animal jumped out after 1 min. The jump reaction became more and more prevailing at higher temperatures and the interval between primary and jump reactions became shorter (Fig. 5).

Effect of Morphine and Codeine in elevating Pain-Reaction Threshold—Analgesic effect, that is elevation of pain reaction threshold, of morphine and codeine is shown in Fig. 6. Of the animals which jumped normally within 3 sec. on the hot plate of 60°, the pain reaction of primary type was inhibited and prolonged by subcutaneous injection of 40 mg./kg. of codeine phosphate and the jump reaction was particularly retarded. With 6 mg./kg. of morphine hydrochloride a jump reaction

2) K. Takagi, T. Kameyama: Yakugaku Zasshi, **77**, 871(1957).

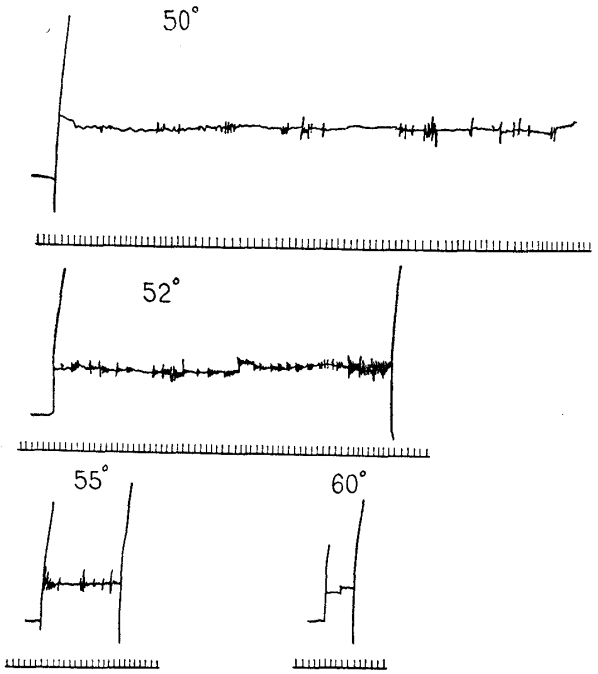

Fig. 5.
Pain Reaction on the Hot-Plate of Various Temperatures

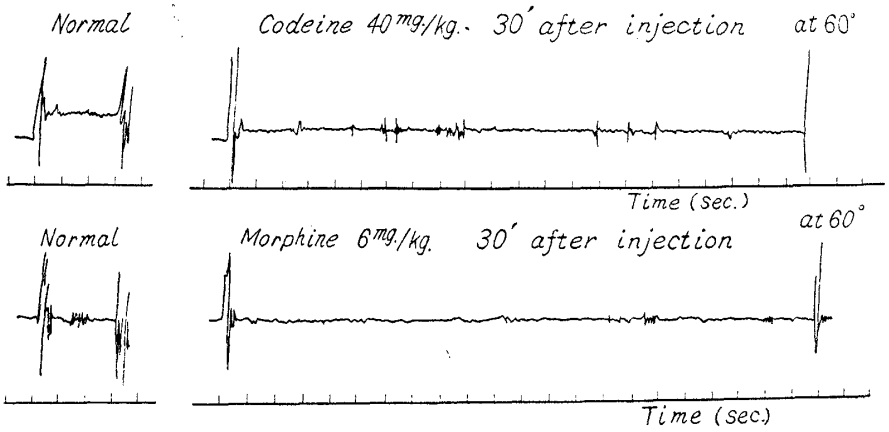

Fig. 6. Analgesic Effect of Morphine and Codeine

threshold was almost the same as that with 40 mg./kg. of codein phosphate but the animal seemed to be more insensitive to heat pain before jumping. The strong inhibitory action of morphine on the primary type of pain reaction is thus clearly demonstrated. When a very large dose of morphine (30 mg./kg.) was given, some stimulating effect was seen. The mouse walked about on the hot plate regularly to the same direction, but the pain reaction was completely depressed (Fig. 7).

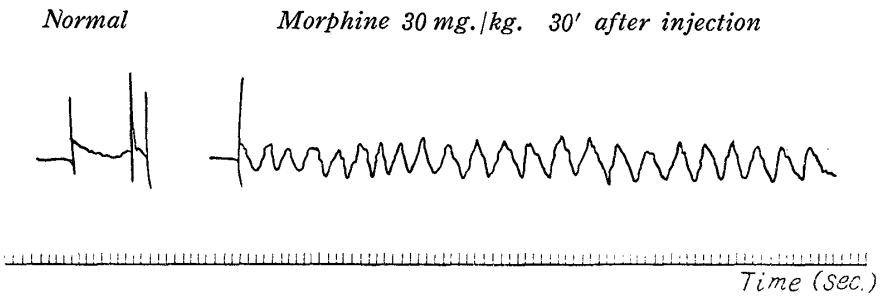

Fig. 7. Stimulating Effect of Large Dose of Morphine Hydrochloride (30 mg./kg., subcutaneously)

Potency Ratio of Codeine Phosphate vs. Morphine Hydrochloride—Analgesic activity of morphine and codeine was compared according to 4-point assay, using the jump reaction time as a pain response (Table I). The potency ratio of codeine phosphate was 0.152 and fiducial limits were 0.1295 and 0.1778 ($p=0.95$).

Table I. Four-point Assay of Morphine and Codeine

Drug / Dose (mg./kg.) / Mice	Morphine		Codeine	
	3	6	20	40
Response	2.0	15.0	4.9	17.6
	13.0	18.7	3.4	25.4
	7.2	23.4	7.8	28.0
	6.7	26.8	7.1	17.1
	13.0	21.9	9.6	24.3
	7.2	20.5	7.6	24.5
	8.5	17.6	8.8	21.5
	8.7	14.8	6.7	23.4
	5.3	15.5	9.3	14.2
	6.0	13.8	8.6	18.2
Mean	7.8	18.8	7.4	21.4

Analgesic Effect of Aminopyrine—A dose of 125 mg./kg. of aminopyrine given subcutaneously did not change the normal reaction time. 150 mg./kg. of aminopyrine prolonged the jump reaction time, but rather intensified the primary pain reactions, which were again inhibited by larger doses of aminopyrine (Fig. 8). Such a situation is shown at 180 mg./kg. On each of 3 doses of aminopyrine (125, 150, 180 mg./kg.) 10 mice were allotted at random and mean responses were proved to increase linearly with the log dose (Fig. 9 and Table II).

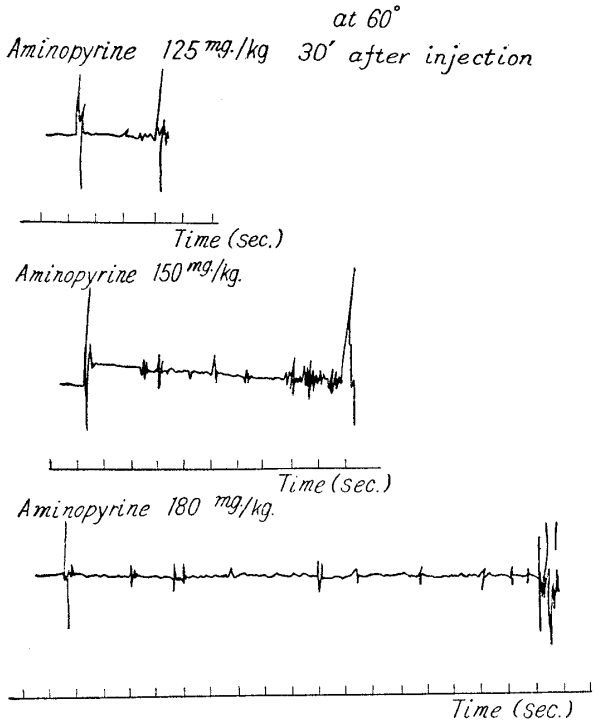

Fig. 8. Analgesic Effect of Aminopyrine

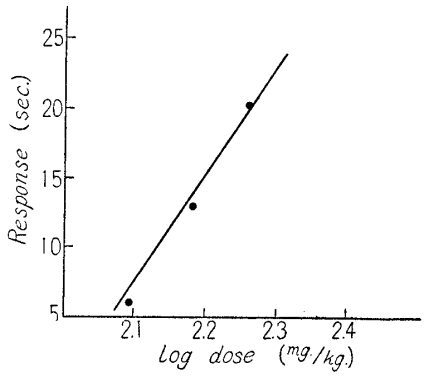

Fig. 9. Dose-Response Curve of Aminopyrine

Table II. Analysis of Variances of the Analgesic Effect of Aminopyrine

Nature of variation	d. f.	Sum of squares	Mean of squares
Regression	1	1059.94	1059.94*
Deviation from regression	1	1.63	1.63
Treatment	2	1061.57	530.79*
Error	27	766.42	28.39
Total	29	1827.99	

* Significant at 0.01 probability level

Discussion and Conclusion

In the present work it was shown that the behavior of mice on the hot plate can be illustrated on kymographic records, which reproduce the observations reported in the preceding paper.[1] The most striking fact is that the effect of lowering the temperature of the hot plate is similar to the effect of analgesics. At lower temperatures the jump reaction threshold is increased in the first place, even when the primary reaction is not inhibited.

The primary reaction is rather intensified in the case of weak analgesics, for example, with 150 mg./kg. of aminopyrine (Fig. 9), although the jump reaction time is certainly prolonged, but by increasing the dosage the primary reaction is also inhibited (180 mg./kg. of aminopyrine, Fig. 9). Comparison of the effect of 150 mg./kg. of aminopyrine with 180 mg./kg. reveals that the onset of the primary reaction was not different, but that violence and frequency of the reaction at the high dose of aminopyrine was far more depressed than those at the lower dose. For this reason the analgesic effect of antipyretic analgesics cannot usually be detected by primary reactions, unless higher dose is applied.

The jump reaction is proved to be more sensitive to the action of narcotic and antipyretic analgesics. It was already reported[3] that the weak analgesic activity of hypnotics could only be detected in the prolonging of a jump reaction. By the combined use of this analgesimetric method depending on heat pain with the method which can record the pressure pain threshold of mouse tail on a smoked drum,[1] it might be possible to make a comparatively accurate estimate of analgesic activity of test compounds without prejudice.

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3) K. Takagi, *et al.*: Yakugaku Zasshi, **72**, 787(1952).