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Analgesic Activity as Determined by the Nilsen Method

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Abstract □ The Nilsen method for determining analgesic activity (pain stimulus and electrical pulsations to the mouse tail) was compared with the hot-plate procedure for several compounds including "pure" analgesics (agonists) (morphine, codeine, and meperidine), a known "pure" antagonist (naloxone), several so-called mixed agonist–antagonists (nalorphine, pentazocine, and cyclazocine), one antagonist that appears to be naloxonelike, and two compounds of relatively unknown pharmacology. The results obtained confirm the validity of the Nilsen test for the agonists and indicate its superior predictive value (to the hot-plate, Smith D'Amour, and perhaps writhing methods) for man with those substances possessing antagonist properties. The Nilsen technique may also be complementary to other procedures now used to predict "pure" antagonism. Refinements in methodology and instrumentation are described.

Keyphrases □ Analgesic activity—determination by Nilsen method, compared to hot-plate procedure □ Nilsen method for determination of analgesic activity—compared to hot-plate procedure

Since the discovery that the narcotic antagonist, nalorphine, also has analgesic (agonist) properties (1, 2) and particularly since the emergence of the much weaker antagonist, pentazocine (3), as a clinically useful, pain-relieving agent without substantial abuse liability (4, 5), a main thrust of research to develop improved analgesics has been on structures possessing a mixture of agonist and antagonist components. The hot-plate method (6, 7) of testing for analgesia, although of excellent predictive value for compounds (*e.g.*, morphine, codeine, meperidine, and methadone) displaying almost exclusively agonist activity mediated by the CNS, is not, in general, as sensitive for the antagonist–agonists [*e.g.*, nalorphine, cyclazocine, and pentazocine (3)].

An assay method first described by Nilsen (8) and modified by Helsley *et al.* (9) was suggested as being simple, economical, and predictive for the narcotic antagonists. This procedure involves applying electrical pulsations as the pain stimulus through the tissue of the tail of suitably selected mice. This test method was installed and what are believed to be improvements in instrumentation and methodology are reported. The method's reliability, predictive value, and reproducibility for narcotic antagonists as well as for the purer agonists have also been confirmed.

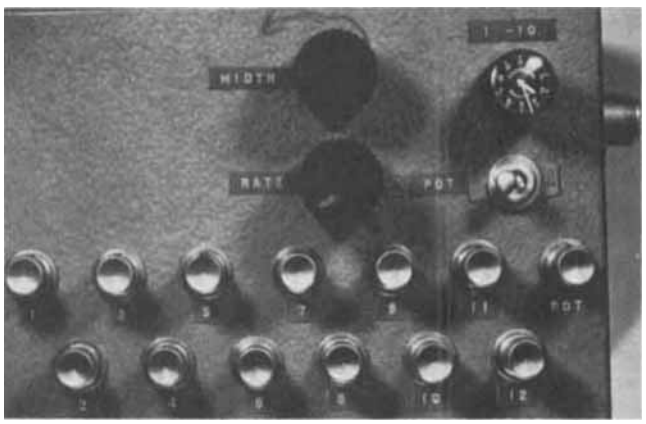


Figure 1—*Pulse control box. Pulse rate and duration may be controlled and monitored on an oscilloscope* via *coaxial connector seen on upper right-hand side of box. Voltage levels may be selected by push buttons or, alternatively, varied continuously by means of dial potentiometer in upper right corner of box.*

EXPERIMENTAL

Apparatus and Procedure—The voltage pulse (electrical stimulus) is derived from the box shown in Fig. 1. The electrical circuit is illustrated in Fig. 2. The stimulus is applied to the mouse by piercing the tail with gold-coated electrodes spaced 12 mm. apart. The electrodes are mounted in a Lucite spring clip modeled after a spring-type wooden clothespin. The clip is mounted in such juxtaposition to a mouse holder that when the mouse is in the holder the electrodes enter the tail approximately 25 mm. from the base of the tail. The electrodes are mounted somewhat off-center, so that they do not strike the bone of the tail, and at such an angle that they enter the tail with a slight compressive force toward the center. Details of the arrangement are illustrated in Figs. 3 and 4.

A "lazy-susan" type of mouse "dispenser" markedly reduces operator fatigue, speeds the processing of the mice, and reduces the chance of error in the order in which the mice are tested. This device consists of two concentric series of wells of radii of 19.5 and 24.5 cm., respectively. Each well is 8.25 cm. in diameter × 11.7 cm. deep; there are 12 wells to each circle, each fitted with expanded metal floors and all carried by a 62-cm. diameter, circular Lucite

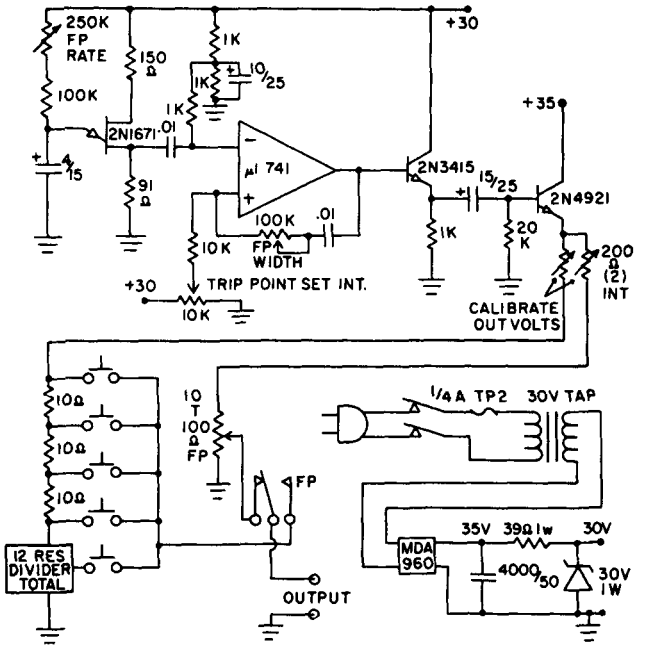


Figure 2—*Details of electrical circuit.*

disk free to rotate about its axis. Covering the assembly with a clearance of about 0.6 cm. is a similar concentric stationary disk 59 cm. in diameter which prevents the mice from escaping from the wells. The stationary disk contains two 8.3-cm. diameter portholes, each of which registers with one of the circular series of wells in the rotary disk; each well may, in turn, be brought into register with its porthole for access to the mouse being tested. A spring-loaded ball detent indexes the wells in alignment with these portholes. Either of the portholes may be kept covered while the other series of wells is being used. This device is illustrated in Fig. 5.

The testing protocol was similar to that reported by Helsley *et al.* (9). White, male, individually segregated, caesarian-derived, general purpose (CDGP) mice (7), weighing 16–20 g., were used. Only those mice giving "defined" (9) predrug vocalization for three successive pulses at 6 v. rather than 8 v. were used. This threshold was chosen as optimal after numerous trials. At a 6-v. stimulus, fewer mice (about 50%) had to be discarded than at any other voltage (from 2–12) and more uniform results were obtained with this as the base

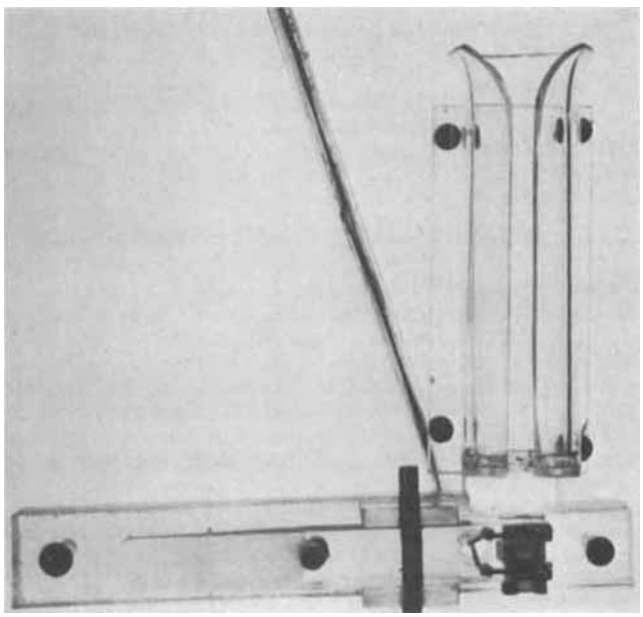


Figure 3—*Mouse holder.*

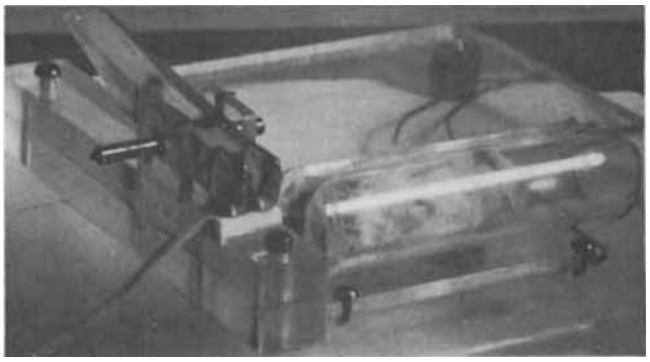


Figure 4—*Electrode assembly. The electrodes are made from the old style "fat" phonograph needles used in nonelectronic phonographs and are gold plated after assembly. The wide angle of the electrode taper expands the tail tissue to ensure firm contact. The gold plating minimizes surface reaction at the electrodes. Beneath the tail is a Teflon block, which protects the electrode tips.*

stimulus. It was also observed that better "responders" were obtained from mice weighing 16–20 g. (not more than 3 weeks old). The mice were tested at 10, 20, 30, and 60 min. following administration of drug subcutaneously. Usually 3–5 dose levels (eight mice at each level) were sufficient. When the pain (vocalization) threshold was increased by at least 2 v. for three or more consecutive pulses, the test animal was considered a respondent. A drug was considered active at a given dose level in an individual mouse if that mouse responded consecutively in at least two of the four time periods. Such a schedule also gives an indication of onset, peak, and duration of action.

Using this procedure, known analgesics [morphine, codeine (3-methyl ether of morphine), meperidine, and α-(−)-2,9-dimethyl-2′-hydroxy-5-propyl-6,7-benzomorphan (NIH 7373) (10)], known antagonists [nalorphine, naloxone, pentazocine, cyclazocine, and α-(±)-5,9-dimethyl-2′-hydroxy-2-propyl-6,7-benzomorphan (NIH 7549) (3, 11)], and new compounds designated NIH 8624 ("commercial discreet") and 8645 were assayed and the data were subjected to probit analysis. The results are given in Table I along with corresponding data obtained by the hot-plate method and, where available, the optimal analgesic dose in man. Finally, potencies as antagonists are given, if known. The structures are also given here.

RESULTS AND DISCUSSION

There is good agreement in Nilsen and hot-plate ED_{50}'s for morphine, codeine, and meperidine which have no detectable antagonist component. The same is true for the benzomorphan NIH 7373, a weak antagonist (10). The analgesic potency relationship is the same

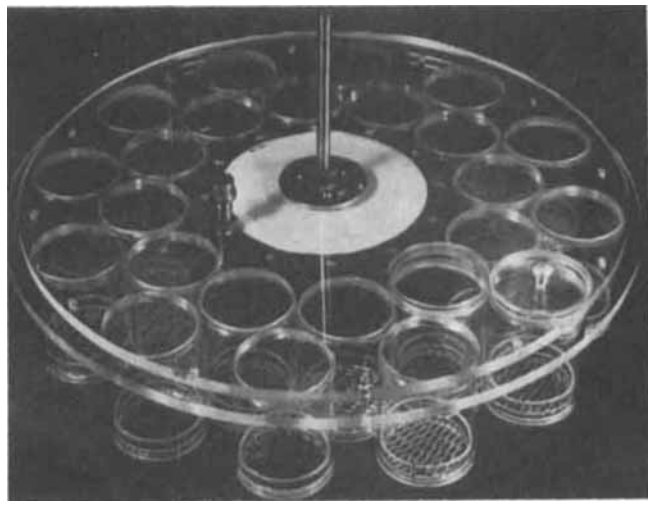


Figure 5—*Mouse dispenser.*

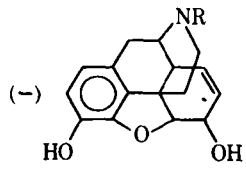
Table I—Comparative Pharmacologic Data for Agonists and Antagonists

Compound[a]	Analgesic Action, ED_{50}, mg./kg. s.c. — Nilsen Method	Analgesic Action, ED_{50}, mg./kg. s.c. — Hot-Plate Method	Parenteral Human Dose	Antagonistic Action[b]
Morphine	0.8 (0.6–1.2)	1.2 (0.9–1.3)	10[c]	—[d]
Codeine	4.5 (2.7–7.6)	7.5 (6.8–8.3)	60–120[c]	—[d]
Meperidine	3.5 (2.3–5.4)	4.7 (4.2–5.4)	75[c]	—[d]
NIH 7373	0.64 (0.39–1.03)	0.80 (0.60–1.10)	—	0.2
Nalorphine	4.8 (2.7–8.5)	36.3 (27.1–48.7)	10[e]	1.0
Cyclazocine	0.08 (0.04–0.10)	23.1 (16.7–31.9)	0.25[e]	4–6
Pentazocine	4.7 (2.9–5.1)	12.3 (9.3–16.3)	20–40[e]	0.02
Naloxone	—[d]	—[d]	—[f]	7.0
NIH 8624[g]	—[d]	—[d]	—	3.0
NIH 7549	—[d]	—[d]	—	1–2
NIH 8645[h]	—[d]	—[d]	—	0.1

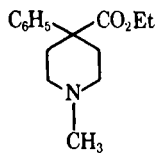
[a] Administered as a water-soluble (usually HCl) salt. [b] Ratio (nalorphine = 1) based on precipitation of abstinence phenomena in nonwithdrawn, morphine-dependent monkeys (J. E. Villarreal, University of Michigan, private communcation). [c] Eddy *et al.* (12). [d] Inactive. [e] Jacobson *et al.* (5). [f] Slight effect at 1.0 mg., little or no effect at lower or higher doses; essentially inactive (13). [g] "Commercial discreet" compound. [h] M. Takeda, private communication.

for these four compounds in both animal methods and essentially the same for morphine, codeine, and meperidine in man, so NIH 7373 is expected to be a strong analgesic in man.

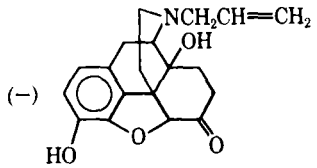
All other compounds in Table I have antagonistic action and show greater analgesic potency by the Nilsen method than by the hot-plate method, if they evoke activity at all. They may be divided into two groups. The first group includes nalorphine, cyclazocine, and pentazocine, which are much more effective by the Nilsen method than by the hot-plate method. Nalorphine and cyclazocine are strong antagonists; pentazocine is weak as an antagonist. All are effective analgesics in man with potency ratings that are fairly consistent with their analgesic activities as determined by the Nilsen method. Thus, the order of strength both as antagonists and for the relief of pain in man is cyclazocine > nalorphine > pentazocine. Nilsen ratings would be cyclazocine > nalorphine ≡ pentazocine, and hot-plate potencies are pentazocine > cyclazocine > nalorphine. The second group, consisting of naloxone, NIH 7549, NIH 8624 ("commercial discreet"), and NIH 8645, are all ineffective in the Nilsen and hot-plate methods and are strong-, to medium-, to low-potency antagonists. Naloxone is devoid of analgesic activity in man, and the other three would be expected to be devoid also. They may be good candidates for "pure" antagonists similar to naloxone.



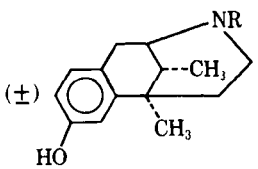
R = CH_3, morphine
R = $CH_2CH{=}CH_2$, nalorphine



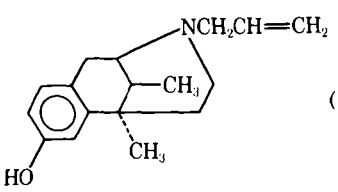
meperidine



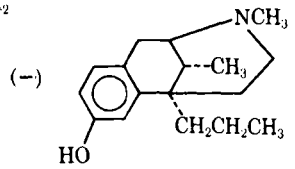
naloxone



R = CH_2—△, cyclazocine
R = $CH_2CH{=}C(CH_3)_2$, pentazocine
R = $CH_2CH_2CH_3$, NIH 7549



NIH 8645



NIH 7373

The data of Table I indicate that the Nilsen method gives good predictability of clinical analgesic action with centrally acting analgesic agents. It would appear to be equal or superior to presently available tests, including the phenylquinone procedure (14) for detecting analgesia in compounds having dual agonist-antagonist action. Operational procedures and apparatus in this modification of the Nilsen method are relatively simple.

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