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Analgesic Tolerance to Etorphine (M99)* and Morphine in the Mouse (33812)

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The new highly active morphine like drug, etorphine hydrochloride (M99 Reckitt), has been shown to have potent knock down properties for many animals, both wild as well as domesticated (1–5). Competitive antagonism

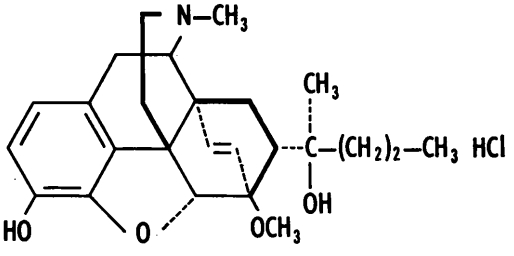

FIG. 1. 7α[1-(*R*)-hydroxy-1-methylbutyl]-6,-14-*endo*-ethenotetrahydrooripavine hydrochloride (M99).

between M99 and nalorphine (*i.e.*, nalorphine antagonizing the pharmacological effects of M99) has been shown to exist (6). Similar antagonism between M99 and cyprenorphine (M285), a new and highly potent morphine antagonist, has also been reported (6). While M99, like morphine, is a potent analgesic agent (7–9), no studies of tolerance to this drug have been published. The purpose of the present studies was to determine the relative degree of tolerance to the analgesic effects of M99 in the mouse and to study the time course of the development and persistence of this tolerance.

Methods. Young adult female Swiss Webster mice (Simonsen Laboratories, Gilroy, California) 18–27 g, were tested by means of the "caudal immersion" technique using a modification of the method of Ben-Bassat *et al.* (10). The water temperature used was 50 ± 0.2° with a 15-sec maximum immersion period after which the tail was removed from the water. A vented adjustable plexiglass tube was used to hold the mouse with tail extended into the water bath. Approximately four-fifths of the tail was immersed. Timing to 0.1 sec was by means of a stop watch. The end point was an oscillatory flick of the tail characteristic of attempted withdrawal. Drug dosage was by the subcutaneous route in Series I and by the intraperitoneal route in Series II and III. All drugs used were weighed and mixed in water, M99 as the hydrochloride and morphine as the sulfate.

While no ED_{50} determinations were made, the 15-min postinjection period utilized was shown to represent a period of near peak effect in a number of animals tested at these and other dosages (unpublished data). While potency ratios were not determined in Series I (Fig. 2), near equianalgesic doses utilizing these time intervals were estimated from the

* Received M99 from Dr. Wayne H. Linkenheimer, Manager, Nutrition and Physiology Section, American Cyanamid Co., Princeton, N. J. 08540.

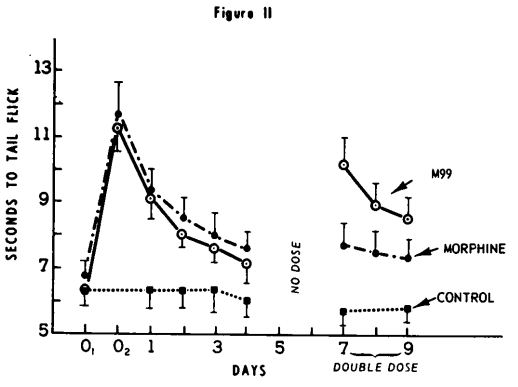

FIG. 2. Series I (subcutaneous).

peak effect in 15 min, the arbitrarily chosen time for testing. The control group was injected with 0.1 ml distilled water. After two adaptive dips, 10 consecutive observations in Series I and 5 observations in Series II and III were recorded on each mouse, each one of which was followed by a 15–20-sec rest period in which a fan blew room temperature air over the tail of the mouse for cooling purposes. Statistical treatment of the data was by use of the *t* test, the *n* value being the number of mice tested, not the number of determinations. All animals were fed Purina lab chow and water *ad libitum* during the course of the experiment.

Results and Discussion. Series I. Approximately equianalgesic doses of morphine and etorphine, as earlier described, were approximately 4 mg/kg and 2 μ/kg, respectively. Thus M99 was nearly 2000 times as potent as analgesic by this test, using peak effect in 15 min, as was morphine. The results are included in Fig. 2. No significant difference exists between day 0_1 in the control group and day 9 of the same group, so changes noted in drug groups are expected to be drug-induced changes. Significant differences exist between the control group and M99 as well as morphine in all observations from day 0_2 through day 4 except for M99 on day 4 where 2 μg/kg is no longer effective to produce significant analgesia. Following a 2-day rest (no injection period) the mice were again tested, this time with twice the dose, *i.e.*, 4μ/kg of M99 and 8 mg/kg of morphine. A similar tendency towards a drop in potency was noted in M99 between days 7, 8, and 9 with morphine showing decreased potency to the extent that day 9 was not significantly different from control. From these data we may conclude that M99, like morphine, induces acute analgesic tolerance in the mouse and that this tolerance occurs very rapidly (within 24 hr) in M99 and within 48 hr with morphine, since a significant difference exists between day 0_2 and day 2 for morphine.

Series II. The results are shown in Fig. 3. No significant difference between the M99 and the control group were noted at day 0_1. The increased threshold noted in the M99 dosed animals on day 0_2 is above the corresponding peak in Series I (Fig. 2), 13.4 vs 11.2 respectively, which is significantly differ-

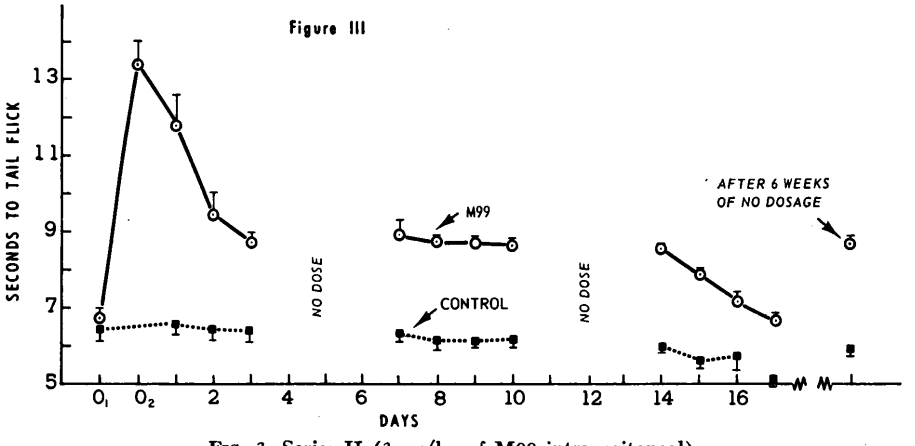

FIG. 3. Series II (3 μg/kg of M99 intraperitoneal).

ent at the 5% level. No significant difference (between Series I and II) was noted at day 0_1, thus a significantly increased potency may be detected between 2 and 3 μg/kg of body weight. It is apparent that some tolerance to the drug was retained even after a period of 2 months (since the first highly effective dose, *i.e.*, day 0_2). In Series II all animals were injected but not tested on day 6. Days 7, 8, 9, and 10 indicated little change in drug effectiveness, while a distinct fall in threshold (increased sensitivity) was noted during the 14, 15, 16, and 17 day interim. Another test was made on these same animals after 6 weeks. The final points indicate a return to essentially the same level as on days 7, 8, 9, and 10.

Series III. Results are found in Fig. 4.

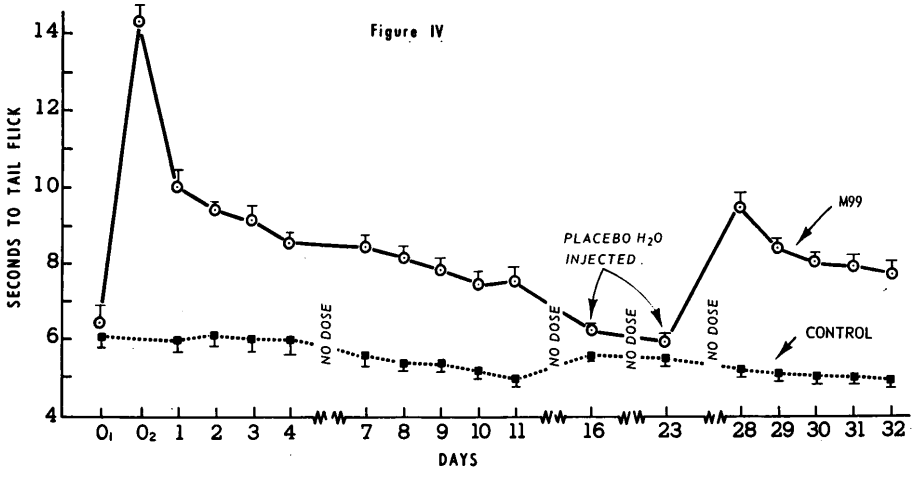

FIG. 4. Series III (5 μg/kg of M99 intraperitoneal).

The typical peak on first injection (day 0_2) was followed by the expected decline. The 2-day interim of no injection (days 5 and 6) was followed by a continuation of the fall (days 7, 8, 9, 10 and 11). Two placebo (water injected) runs were made on days 16 and 23 in both control and experimental groups. The results indicate that these groups were essentially together, although an unexplained significant difference did exist on day 16 but not on day 23. On day 28 the same injection routine was reinstituted, which resulted in a small peak followed by a return to a flat plateau. This series indicates a moderate loss of tolerance during the 16-day interim followed by a return to an effective analgesic level. A highly significant difference exists ($p < 0.001$) between the control and M99 groups in every case where the drug was used.

Summary and Conclusion. The mouse given M99 or morphine exhibited an acute tolerance to the analgesic effects of both drugs. The acute tolerance was built up rapidly following a single injection of M99 or two daily injections of morphine (24 or 48 hr, respectively). Discontinuation of drug dosage for up to 6 weeks resulted in only a slight decrease in tolerance, which returned to its earlier state after a very few daily doses. M99, based upon 15-min peak analgesic effects in these tests, was approximately 2000 times as potent as morphine.

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Delayed Hypersensitivity Induced in Guinea Pigs with Tuberculoprotein from *M. bovis* (BCG)* (33813)

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No reports have appeared in the literature showing tuberculoproteins to be capable of inducing delayed hypersensitivity in animals. Gell and Benacerraf (1) state that attempts to produce delayed tuberculin sensitivity with tuberculoproteins alone have consistently failed. Other recent reviews dealing with delayed hypersensitivity (2, 3) make no reference to the ability of tuberculoproteins to act in this manner. Raffel (4) demonstrated that tuberculoprotein alone was incapable of provoking delayed hypersensitivity. Boyden (5) studied the effect of unheated tuberculin upon guinea pigs and concluded that when this material was injected subcutaneously in either saline or oil, only immediate sensitivity was induced. The guinea pigs were sensitized with 0.1 ml of tuberculin and tested 42 days later by intradermal injection of 20 μg of heated or unheated tuberculoprotein.

Previously, we have shown that protoplasms of acid-fast bacilli were able to elicit delayed reactions in sensitized guinea pigs and rabbits (6, 7) although they failed to induce delayed hypersensitivity in such animals (8). The two present experiments demonstrate that a fraction derived from *M. bovis* (BCG) protoplasm and labeled "C protein" is capable of inducing a state of delayed hypersensitivity in guinea pigs when it is incorporated in incomplete Freund's adjuvant and injected subcutaneously into the foot pads.

Materials and Methods. Animals. Female Hartley strain guinea pigs weighing about 300 g were used. These were obtained from a supplier in Hamilton, Montana.

Antigens. Old tuberculin (OT) was generously supplied by Parke Davis and Company, Detroit, Michigan. Protoplasm was obtained from viable BCG bacilli by methods previously described (7). The fraction, "C protein," was prepared from this protoplasm by the following method. This procedure is a modification of the technique described by Seibert and Affronti (9). All manipulations were carried out at 4° and all materials and equipment were cooled to this temperature prior to their use.

Whole BCG protoplasm in phosphate buffered saline (PBS), pH 7.2, was adjusted to pH 4.5 with 1 *M* acetic acid. After 5 min of stirring, the solution was centrifuged at 12,100*g* for 10 min and the resulting brownish translucent precipitate was dissolved in PBS. The pH was adjusted to 7.6 with 1 *N* NaOH and ethanol was rapidly added to a final concentration of 40%. After 10 min of stirring, the suspension was centrifuged at 12,100*g* for 10 min. The clear but slightly opalescent suerpnatant was adjusted to pH 4.5 with 1 *M* acetic acid. After 5 min, the suspension was again centrifuged at 12,100*g* for 10 min and the resulting precipitate was dissolved in PBS.

Sensitization. Animals in Experiment 1

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