N-Demethylation of *N*-^{14}C-Methyl-codeine in Morphine Tolerant and Nontolerant Rats and Mice[1] (35364)

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The *N*- and *O*-demethylation of codeine *in vivo* by morphine tolerant male mice has been reported by Adler (1) to be significantly increased compared to nontolerant male mice where the *N*-demethylation of morphine by morphine tolerant mice was not changed. Tolerance development in male rats has been reported to decrease the *N*-demethylating activity *in vivo* (2) as well as *in vitro* (3–6) but not in female rats (7–8). Accordingly, a comparative study in *N*-demethylation of codeine by morphine tolerant and nontolerant male and female rats and tolerant male mice was undertaken.

Methods. Sprague–Dawley male and female rats, weighing 190 to 210 g, were made tolerant to morphine with sc injection of morphine sulfate, twice daily, 10 mg/kg (as base), for 3 days, 15 mg/kg for subsquent 3 days and finally maintained on 20 mg/kg. Control rats were injected sc with equal volume of saline, 2 ml/kg. After sc injection of 20 mg/kg of morphine for at least 3 days, the rats were injected sc with 40 mg/kg (as base) of *N*-$^{14}CH_3$–codeine HCl (9) and placed in a Delmar-Roth metabolism cage. The respiratory $^{14}CO_2$ was collected and estimated by the procedure described (10).

Swiss-Webster male and female mice, weighing between 25 to 30 g, were used. The male mice were made tolerant to morphine by implantation of a morphine pellet[4] (11) in the dorsal subcutaneous tissue. Sham operation was performed without placebo tablet on the control male mice. A group of 2 to 3 mice were kept in a cage after morphine-pellet implantation. The animals implanted with morphine-pellet exhibited the typical Straub tail within 30 min which lasted for several hr. By 24 hr this effect had largely subsided and their general behavior except for sedation was similar to that of control animals.

The mice were injected sc with 40 mg/kg of *N*-$^{14}CH_3$–codeine between 66 to 68, 90 to 92, and 192 hr after morphine-pellet implantation and the respiratory $^{14}CO_2$ collected and estimated. Four mice were used in each group and the experiment conducted individually. The animal was sacrificed at the end of the 24-hr experiment; the morphine-pellet was removed, dissolved in 1 *N* HCl, centrifuged, and morphine content in the supernatant was determined spectrophotometrically at wavelength 285 mμ. The amount of morphine absorbed from the pellet by mice after 4 and 5 days implantation was 165 ± 14 (SE) mg/kg/day.

The modified hot plate method (12) was used to test for analgesia. Analgesia occured in both control male and female rats 30 min after injection of 5 mg/kg of morphine as indicated by increase in the reaction time by 15.4 ± 2.7 (mean ± SE) and 11.2 ± 2.17 sec for male and female rats, respectively, whereas the saline control group showed no significant change. Analgesia was not observed in the rats treated with morphine for 9 days, indicating tolerance developed in mor-

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[4] Morphine-pellets, kindly provided by Dr. E. L. Way, the Department of Pharmacology, San Francisco Medical Center, The University of California, consisted of morphine base, 75 mg; fumed silicone dioxide, 0.75 mg; and calcium stearate, 1.5 mg.

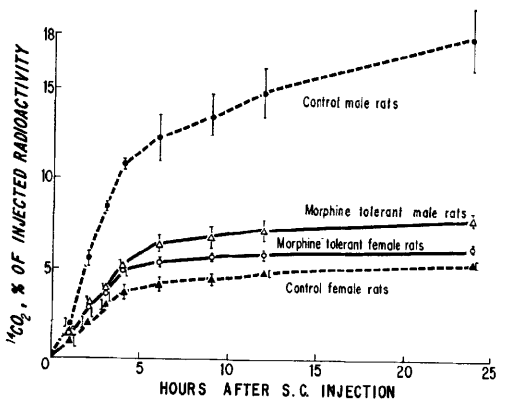

FIG. 1. Pulmonary elimination of $^{14}CO_2$ from the morphine tolerant and nontolerant male and female rats after sc injection of 40 mg/kg of *N*-$^{14}CH_3$–codeine. The points and bars represent the mean and SE, respectively.

phine-treated rats.

The mean reaction time of the mice before, and 24, 48, 72, 96, 144, and 192 hrs after morphine-pellet implantation was 6.5 ± 0.81; 13.2 ± 1.54; 19.7 ± 1.9; 14 ± 2.93; 11.5 ± 2.6; 10.2 ± 3.06; and 6.2 ± 3.5 sec, respectively, whereas the mean reaction times of control mice tested on three successive days were 6.7 ± 0.67; 6.8 ± 0.53; 6.5 ± 0.58 sec. This result is similar to that observed by Way *et al.* (13).

Results and Discussion. N-Demethylation of N-$^{14}CH_3$–codeine in the rat. In 24 hr 7.8 ± 0.42 and 18 ± 1.86% of injected radioactivity were recovered as $^{14}CO_2$ in the exhaled air from 3 morphine tolerant and 3 nontolerant male rats, respectively; and 6.4 ± 0.05% and 5.4 ± 0.05% from 3 morphine tolerant and 3 nontolerant female rats, respectively (Fig. 1). The difference in *N*-demethylation of codeine between morphine tolerant and nontolerant male and female rats was significant.

N-Demethylation of *N*-$^{14}CH_3$–codeine measured as exhaled $^{14}CO_2$ in tolerant male rats in the present studies was similar to that of dihydromorphine (2), morphine and aminopyrine (3–6, 14–15). The *N*-demethylating activity of morphine tolerant male rats decreased to the level of nontolerant female rats. Sex-dependence has been observed in the *N*-demethylation of codeine (9), dihydromorphine (2), morphine, and aminopyrine (8). The *N*-demethylation of aminopyrine in castrated male rats did not show further decrease by the administration of morphine (8). Remmer (7) observed acceleration instead of decrease in the *N*-demethylating activity of female rats made tolerant to moderate doses of pethidine and morphine. The present result confirms these observations. Kato and Onoda (15) proposed that the administration of morphine blocked the action of male sex hormone which stimulated the activity of rat liver microsomal enzymes. Elison and Elliott (16) reported that tolerance did not affect the *N*-demethylase activity in the brain slices of rats.

N-Demethylation of N-$^{14}CH_3$-codeine in mice. In 24 hr, 22.0 ± 1.38, 22.1 ± 1.26, and 22.6 ± 1.11% of injected radioactivity were recovered as $^{14}CO_2$ in the respired air from 7 female mice, 7 control male mice, and 12 morphine-pellet implanted male mice, respectively (Fig. 2). There was no significant difference in mean recoveries of $^{14}CO_2$ in groups of 4 male mice 3 and 4 days after morphine-pellet implantation (peak analgesic time) and after 8 days when analgesic response returned to control values. These re-

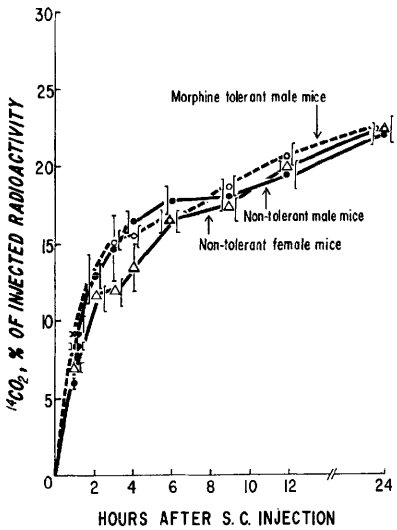

FIG. 2. Pulmonary elimination of $^{14}CO_2$ from morphine tolerant and nontolerant male mice, after sc injection of 40 mg/kg of *N*-$^{14}CH_3$–codeine. The points and bars represent the mean and SE, respectively.

sults show no significant difference in N-demethylation of codeine in normal and morphine tolerant male and female mice and confirm earlier observations on morphine (1, 15, 17). No sex difference in metabolism of hexobarbital has also been reported for mice (18) and the liver microsomal activity of male mice did not increase after treatment with testosterone and methyltestosterone (19). The findings of Adler (1, 20) that N-demethylation of codeine between male and female mice was significantly different and that N-demethylation of codeine increased in morphine tolerant mice could not be confirmed.

Summary. N-Demethylation of codeine by morphine tolerant male rats decreased significantly as compared to nontolerant male rats to the level of nontolerant female rats; whereas in the nontolerant female rats, female mice, and tolerant male mice, it was not changed.

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1. Adler, T. K., J. Pharmacol. Exp. Ther. **156**, 585 (1967).

3. Yeh, S. Y., and Woods, L. A., J. Pharmacol. Exp. Ther. **169**, 168 (1969).

3. Axelrod, J., Science **124**, 263 (1956).

4. Cochin, J., and Axelrod, J., J. Pharmacol. Exp. Ther. **125**, 105 (1959).

5. Cochin, J., and Economon, S., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **18**, 377 (1959).

6. Herken, H., Neubert, D., and Timmler, R., Arch. Exp. Pathol. Pharmakol. **237**, 319 (1959).

7. Remmer, H., "Drug Tolerance in Enzymes and Drug Action, Ciba Foundation Symposium" (J. L. Mongar and A. V. S. de Reuck, eds.), p. 276. Little, Brown, Boston (1962).

8. Kato, R., and Gillette, J., J. Pharmacol. Exp. Ther. **150**, 285 (1965).

9. Yeh, S. Y., and Woods, L. A., J. Pharmacol. Exp. Ther. **166**, 86 (1969).

10. Yeh, S. Y., Cavanaugh, J. H., and Woods, L. A., J. Pharm. Sci. **55**, 1212 (1966).

11. Gibson, R. D., and Tingstad, J. E., J. Pharm. Sci. **59**, 426 (1970).

12. Johannesson, T., and Woods, L. A., Acta Pharmacol. Toxicol. **21**, 381 (1964).

13. Way, E. L., Loh, H. H., and Shen, F. H., J. Pharmacol. Exp. Ther. **167**, 1 (1969).

14. Franklin, M., Can. J. Biochem. **43**, 1053 (1965).

15. Kato, R., and Onoda, K., Jap. J. Pharmacol. **16**, 217 (1966).

16. Elison, C., and Elliott, H. W., Biochem. Pharmacol. **12**, 1363 (1963).

17. Kato, R., Takanaka, A., and Onoda, K., Jap. J. Pharmacol. **18**, 516 (1968).

18. Quinn, G. P., Axelrod, J., and Brodie, B. B., Biochem. Pharmacol. **1**, 152 (1958).

19. Novick, W., Stohler, C., and Swagadis, J., J. Pharmacol. Exp. Ther. **151**, 139 (1966).

20. Adler, T. K., J. Pharmacol. Exp. Ther. **140**, 155 (1963).

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