Urine workup and opiate analysis of Group II (tolerant subjects) was performed according to the standard procedures[2–5]. Case C showed the presence of 6% codeine relative to morphine. 0.9% codeine relative to morphine was present as the chloroform-extractable free base. Cases D1 and D2 showed presence of 8 and 10% codeine relative to morphine. Confirmation of codeine was obtained in all cases by mass spectrometry[3].

Discussion. Preliminary studies in humans have shown morphine to undergo *O*-methylation and to form codeine which is excreted in the urine as the free base and bound (presumably glucuronide). A significant increase of codeine in addict urine would indicate a strongly increased *O*-methyl transferase activity, which may be specific for addicts and thus supply a marker for addiction.

Further studies of the kinetics of *O*-methylation are planned and are in progress[8].

Zusammenfassung. Nach Morphium- oder Heroingaben wurden im Urin normaler und heroinsüchtiger Versuchspersonen freies und gebundenes Codein als neues Morphiumstoffwechselprodukt mit Dünnschichtchromatographie und Massenspektroskopie nachgewiesen.

U. Börner and S. Abbott[9]

Department of Public Health, Toxicology Chemistry Laboratory at San Francisco General Hospital, 1001 Portrero Avenue, San Francisco (California 94102, USA), and Varian Instruments, Palo Alto (California, USA), 11 July 1972.

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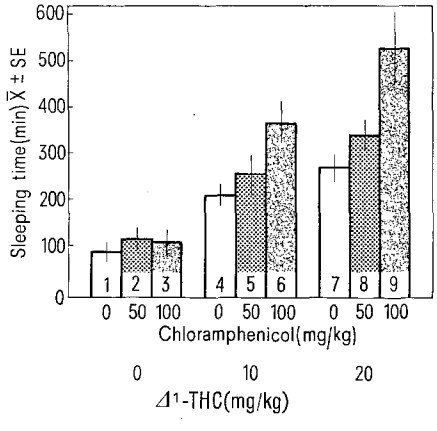
[9] Varian Instruments, Palo Alto (California, USA).

Interaction of Chloramphenicol and Δ^1-Tetrahydrocannabinol in Barbital-Anesthetized Mice

The ability of Δ^1-tetrahydrocannabinol (Δ^1-THC) to prolong the duration of barbital hypnosis in mice is enhanced by 2-diethylaminoethyl 2, 2-diphenylvalerate HCl (SKF 525-A), a recognized inhibitor of hepatic microsomal enzymes[1]. Based on this indirect evidence, it was suggested that the major central depressant effect of Δ^1-THC was due to the intact molecule rather than a metabolite, and that the microsomal-mediated biotransformation of Δ^1-THC to its 7-hydroxy metabolite was inhibited by SKF 525-A[1]. Burnstein and Kupfer[2] later demonstrated in vitro that SKF 525-A does indeed inhibit the 7-hydroxylation of Δ^1-THC in liver microsomes.

Chloramphenicol (CHPC), a widely used broad-spectrum antibiotic, has been shown to markedly prolong the duration of hexobarbital hypnosis in mice and also inhibit its rate of in vivo and in vitro biotransformation[3]. Prolongation of barbiturate anesthesia by CHPC has since been corroborated in several species of laboratory animals[4–6]. In man, the biological half-lives of several compounds have been reported to be increased by CHPC[7,8]. It has been suggested that CHPC, like SKF 525-A, inhibits the liver microsomal enzymes responsible for drug metabolism[3].



Mean duration of sleeping time after administration of barbital sodium (300 mg/kg) in mice treated with Δ^1-THC alone or in combination with CHPC.

The present report presents evidence for interaction between CHPC and Δ^1-THC in barbital-anesthetized mice.

Method. The experiment was conducted using 72 Swiss-Webster male albino mice, weighing 20 to 25 g at the time of testing, i.e., following 5 days acclimation to the laboratory environment. Following random division into groups of 8 each, all mice were injected i.p. with barbital sodium (300 mg/kg), followed 15 min later by either CHPC or its vehicle (0.3% sodium succinate-0.9% saline), and 40 min after barbital by either Δ^1-THC (supplied by NIMH) or its vehicle (10% propylene glycol-1% Tween 80-0.9% saline). Volume of all injections was 0.1 ml per 10 g of body weight. Sleeping time was measured as the time during which the righting reflex was completely absent, observed for 30 sec after the animal was placed on its back. Mean sleeping times were calculated and statistically evaluated using the Student's *t*-test.

Results. The Figure graphically depicts the results of this study. The first 3 bars reveal that neither dose of CHPC (50 mg/kg-bar 2; 100 mg/kg-bar 3) significantly altered barbital sleeping time (bar 1). However, administration of Δ^1-THC alone significantly increased barbital sleeping time when given either at 10 mg/kg (bar 4; $t = 6.71$; df $= 14$; $p < 0.001$) or 20 mg/kg (bar 7; $t = 8.60$; df $= 14$; $p < 0.001$). Barbital sleeping time was not reliably longer with 20 mg/kg of Δ^1-THC than with 10 mg/kg ($t = 2.08$; df $= 14$; $p > 0.05$).

However, the prolonging effect of both doses of Δ^1-THC was significantly augmented by pretreatment with 100 mg/kg of CHPC. With 10 mg/kg of Δ^1-THC plus 100 mg/kg of CHPC (bar 6), sleeping time was signifi-

[1] R. D. Sofia and H. Barry, III, Eur. J. Pharmac. *13*, 134 (1970).
[2] S. H. Burnstein and D. Kupfer, Chem. Biol. Interact. *3*, 316 (1971).
[3] R. L. Dixon and J. R. Fouts, Biochem. Pharmac. *11*, 715 (1962).
[4] H. R. Adams, J. Am. vet. med. Ass. *157*, 1908 (1970).
[5] H. R. Adams and B. N. Dixit, J. Am. vet. med. Ass. *156*, 902 (1970).
[6] B. N. Dixit and I. G. Sipes, Indian J. Physiol. Pharmac. *13*, 34 (1969).
[7] L. K. Christensen and L. Skovsted, Lancet *2*, 1397 (1969).
[8] B. Petitpierre and J. Fabre, Lancet *1*, 789 (1970).

cantly longer than after 10 mg/kg of Δ^1-THC alone (bar 4; $t = 3.16$; df $= 14$; $p < 0.01$). In addition, the mice given 20 mg/kg of Δ^1-THC plus 100 mg/kg of CHPC (bar 9) were anesthetized significantly longer than those mice given only 20 mg/kg of Δ^1-THC (bar 7; $t = 3.00$; df $= 14$; $p < 0.01$). The lower dose of CHPC, however, did not reliably alter the prolonging effect of either dose of Δ^1-THC (bar 5 and 8).

Neither dose of CHPC nor of Δ^1-THC given alone or in combination induced loss of the righting reflex.

Discussion. The CNS depressant effects of barbital are due almost exclusively to the parent compound since the drug is excreted almost 100% as the unchanged molecule[9,10]. Modification of the pharmacologic activity of barbital is therefore not attributable to alteration of its metabolism. Thus, prolongation of barbital hypnosis by Δ^1-THC is most likely due to the central depressant activity of Δ^1-THC. Present findings indicate that CHPC enhances the ability of Δ^1-THC to prolong barbital sleeping time, yet the antibiotic has no effect itself on barbital activity. SKF 525-A similarly augments the Δ^1-THC induced prolongation of barbital hypnosis[1] as well as inhibits the hepatic microsomal-mediated oxidation of Δ^1-THC to ts 7-hydroxy metabolite[2].

CHPC added in vitro inhibits the oxidation of aminopyrine, codeine, acetanilid, and hexobarbital in the $9{,}000 \times g$ supernatant fraction of homogenized murine livers[3]. Ethylmorphine N-demethylase activity of the $100{,}000 \times g$ rat liver microsomal fraction is likewise inhibited by CHPC, whether added in vitro or administered in vivo[11]. Present observations suggest that CHPC also inhibits the in vivo biotransformation of Δ^1-THC in mice and, thus, present additional evidence of the apparently nonspecific inhibitory influence of CHPC on the drug metabolizing functions of hepatic microsomal enzymes. By inference, present data also support the previous conclusion[1] that the CNS depressant effect of Δ^1-THC, at least with respect to its interaction with barbital anesthesia, is due to the parent molecule rather than a biotransformation product[12].

Résumé. L'augmentation de l'anesthésie produite par le barbital chez la souris traitée au Δ^1-tétrahydrocannabinol (Δ^1-THC) à 10 ou 20 mg/kg par voie i.p. est accentuée d'une manière significative par le traitement préalable au chloramphénicol (CHPC) à 100 mg/kg, mais non pas par 50 mg/kg de ce composé injecté par voie i.p. Le CHPC seul n'a aucun effet. Le CHPC étant un inhibiteur des systèmes enzymatiques des microsomes hépatiques, on en a conclu que l'effet du Δ^1-THC sur le prolongement de la durée du sommeil au barbital est dû au composé parent plutôt qu'à l'un des produits de transformation biologique.

H. R. ADAMS[13] and R. D. SOFIA[14]

Department of Pharmacology, School of Pharmacy, University of Pittsburgh Pittsburgh (Pennsylvania 15213, USA), 31 July 1972.

[9] J. J. BURNS, C. EVANS and N. TROUSOF, J. biol. Chem. *277*, 785 (1957).
[10] E. W. MAYNERT and H. B. VAN DYKE, J. Pharmac. exp. Ther. *98*, 184 (1950).
[11] H. R. ADAMS, Ph. D. Thesis, University of Pittsburgh, Pittsburgh, Pa. 15213 USA (1972).
[12] This work was supported by research contract No. HEW, FDA 71–68 and No. HEW, FDA 72–312.
[13] Present address: Department of Pharmacology, University of Texas Southwestern Medical School, Dallas (Texas 75235, USA).
[14] Present address: Wallace Laboratories, Cranbury (New Jersey 08512, USA).

Spontaneous Release and During Metrazol Convulsions of 5-Hydroxytryptamine in Some Brain Regions of Conscious Cat[1]

The release of 5-HT from the superfused caudate nucleus and septum[2] and perfused cerebral ventricles has already been studied[3]. Recently, the release of this amine from the brain tissue of conscious animals received more attention[4,5]. Furthermore, the data on the release of 5-HT during convulsions are still lacking, though the reports on the 5-HT tissue content during convulsions are contradictory[6–8]. Thus, in the light of these findings, it was of interest to investigate the spontaneous release, and that during metrazol convulsions, of 5-HT within various subcortical and mesencephalic structures of unanesthetized animals by newly developed 'push-pull' technique.

Materials and method. The experiments were performed on 11 adult cats of either sex, weighing between 2.4–4.0 kg. Following aseptic surgical procedures described previously[9], 4–8 'push-pull' cannulae were implanted so that the tips rested in one or more subcortical and mesencephalic regions. To perfuse the tissue surrounding the tips of the 'push-pull' cannulae, a multichannel infusion withdrawal pump was used. Postoperatively, a 5-day interval elapsed before the first experiment. A Locke or Krebs solution at a pH of 7.3 was perfused at a cannula tip at a rate of 30 to 50 µl/ml min over an interval of 30 min. Samples were accepted for assay if the volume of the effluent was clear and devoid of tissue fragments. During the course of a control perfusion, the animal was held gently and showed no untoward signs of disturbance or discomfort. However, when the perfusion was carried out during convulsions, the cat was held into a restraining box so the head was outside the box. The effluents were collected on ice and if not tested on the same day, were kept at −10 °C until assayed. Ordinarily, 1–2 days elapsed between a control perfusion and a perfusion carried out during metrazol convulsions. At the conclusion of each experiment the perfusion site was verified following standard histological procedures.

The content of 5-HT in each 30 min sample was determined by the sensitive method of VANE[10], the isolated rat stomach fundus strip. The contractile activity of a perfusate was considered to be due to 5-HT only if a) the contraction produced by a sample was of similar

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[2] R. B. HOLMAN and M. VOGT, J. Physiol., Lond. *223*, 243 (1972).
[3] W. FELDBERG and R. D. MYERS, J. Physiol., Lond. *184*, 837 (1966).
[4] R. D. MYERS, A. KAWA and D. B. BELESLIN, Experientia *25*, 705 (1969).
[5] D. B. BELESLIN and R. D. MYERS, Brain Res. *23*, 437 (1970).
[6] S. GARATTINI, A. VALSECCHI and L. VALZELLI, Experientia *13*, 330 (1957).
[7] G. BERTACCINI, J. Neurochem. *4*, 217 (1959).
[8] E. M. GAL and P. A. DREWES, Nature, Lond. *189*, 234 (1961).
[9] R. D. MYERS, Physiol. Behav. *2*, 373 (1967).
[10] J. R. VANE, Br. J. Pharmac. Chemother. *12*, 344 (1957).