secretion is not primarily mediated through Hm release[18]. The possibility that the absence of PG-induction of HdD could be due to high tissue levels of Hd causing interference in the in vitro assay system has been ruled out. Similarly, we have shown that the transient ether anesthesia required for pylorus-ligation does not prevent PG-induced activation of HdD[19].

It is now well established in the rat, that exogenously supplied Hm can be taken up by the OGA, and PGA[19,20]. In addition, large loading doses of the immediate precursor amino acid Hd can produce a similar increase in Hm stores in the OGA[6], but not in the PGA, since this tissue is low in Hd decarboxylating activity[4]. It is not certain however into which pool this exogenously supplied histamine is going.

In the OGA uptake is at least partially into the PG-sensitive pool, since some reduction can be produced by this hormone (Table). Storage of Hm following the Hd load probably occurs in addition in the enterochromaffin-like cell system[21]; these cells have the capacity to take up precursor amino acids and decarboxylate them in situ to the corresponding amine. It is almost certain that, speculatively, the enterochromaffin-like cell Hm pool, and the PG-sensitive pool, are not within the same unit, since uptake of preformed amine by the enterochromaffin-like cells does not occur[21,22].

Zusammenfassung. Nachweis, dass Pentagastrin (ICI-50123) 200 µg/ml/kg/h die Magensaftsekretion in der Shay-Ratte 2 h nach Ligatur des Pylorus erhöht. Die Histaminspeicher des Rattenmagens wurden reduziert und Histidindecarboxylase-Aktivität gesteigert. Verabreichung von 1000 mg/kg Histidin erhöht den basalen Histaminspiegel, jedoch wurde die Histidindecarboxylase-Aktivität bei normaler Magensaftsekretion nicht durch Pentagastrin induziert.

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New Observations in the Metabolism of Morphine. The Formation of Codeine from Morphine in Man

During opiate screening of urines from heroin addicts, spots were frequently noted on the thin-layer chromatograms (TLC) corresponding in Rf and iodoplatinate color to codeine. It was believed that in man, codeine might be formed as the *O*-methylated metabolite of morphine, which had previously been observed in rats and dogs by ELISON and ELLIOTT[1].

In our study, urines from 75 heroin addicts applying for admittance into the Acute Detoxification Study Unit at San Francisco General Hospital were analyzed for opiates according to the method of PARKER et al.[2]. 85% of these urines which were found to be morphine positive also contained codeine. The presence of codeine was established by mass spectrometry[3]. The codeine concentration was determined in 5 samples by TLC[4] and gas-liquid chromatography (GLC)[5]. Codeine was found to be present in amounts between 12 and 15% relative to morphine.

To check whether the codeine in the addict urines was arising from codeine-contaminated street heroin, the street heroin currently available in the San Francisco Bay Area was analyzed by TLC, GLC, and mass spectrometry. The samples showed almost exclusively absence of codeine or acetylcodeine.

These findings make it reasonable to assume that codeine is formed as a metabolite of morphine via-*O*-methylation. To demonstrate whether the quantity of codeine formed is related to chronic morphine administration, the following study was performed on morphine tolerant and non-tolerant humans.

Group 1, non-tolerant subjects. Route of administration of morphine: oral-subjects A1, A2, 2 male volunteers, each receiving 50 mg morphine sulfate. intravenous-subjects B1, B2, 2 aortic-bypass patients, receiving as i.v. drip over 30 min., 180 and 195 mg morphine sulfate.

Group II, tolerant subjects. Route of administration of morphine: oral-subject C, male cancer patient, receiving for the sixth day, 220 mg morphine sulfate in Schlesinger solution as daily dose. Intravenous-subjects D1, D2, 2 known heroin addicts, who volunteered to inject heroin. Heroin is known to rapidly deacetylate to 6-monoacetylmorphine and morphine[6]. The dose injected was unknown. Urine samples supplied before injection and samples of the heroin injected served as control for absence of codeine.

The urines of all subjects were collected for 24 h after heroin and morphine administration. In Group 1 (non-tolerant subjects), urinalysis for opiates was performed according to the above procedures[2–5]. To recover the small amounts of codeine observed, the extraction method was modified[7] and the extract analyzed as before. Codeine was found to be present in all urines of Group 1 in amounts of 0.7–0.9% relative to morphine. No significant differences between oral and i.v. administration were noted.

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[3] Varian CVA-5 mass spectrometer. Direct injection into membrane separator-quadrupole MS system. Membrane temperature 180 °C; electron energy 70 ev: ion source temperature 250 °C; ion monitoring at *m/e* 285, 299 for morphine, codeine and molecular fraction: J. Am. chem. Soc. *89*, 4494 (1967).
[4] Thinlayer chromatography was performed on 20 × 10 cm glass plates with 0.30 mm Silica Gel GF 254 (according to STAHL). Mobile phase was a mixture of 1.4-dioxane, chloroform, ethylacetate 6:2,5-1 and ammonia atmosphere. Sprayreagent: iodoplatinate.
[5] Varian Aerograph, Model 204 B gas chromatograph was used, equipped with 6 foot $^1/_8$ inch diameter, 5% SE-30 column, and flame ion detector. Inlet temperature 260 °C, column temperature program 180–280 °C at 8 °C/min. 60 ml/min He carrier gas; 30 ml/min H_2 to flame ion detector. Analysis according to the one-column acetylation procedure of MULE (Analyt. Chem. *36*, 1907 (1964).
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[7] Modified extraction method: urine was hydrolized as in[2], then adjusted with 16 *N* KOH to pH 14 and extracted 4 times with benzene. The combined extracts were filtered through sodium sulfate, evaportated and chromatographed.

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.

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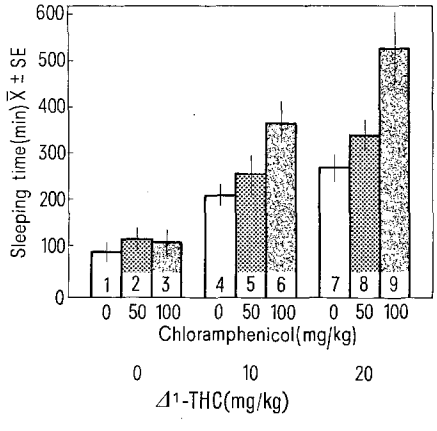
Interaction of Chloramphenicol and Δ^1-Tetrahydrocannabinol in Barbital-Anesthetized Mice

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-

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