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.

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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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Release of 5-HT in ng per 30 min in control perfusates and during metrazol convulsions within various brain structures of unanesthetized cat

Anatomical region	Resting release	Metrazol (60–90 mg/kg)	Position of cannula
Caudate nucleus	< 0.2	< 0.2	AP = 19.0
	1.4	1.6	AP = 18.0
	0.7	< 0.2	AP = 16.0
	0.5	1.4	AP = 16.0
	< 0.1	< 0.1	AP = 15.0
	< 0.3	< 0.3	AP = 14.0
Amygdala	< 0.1	< 0.1	AP = 15.0
	1.6	1.4	AP = 15.0
	< 0.2	0.4	AP = 12.5
	0.5	1.5	AP = 10.5
	< 0.3	< 0.3	AP = 9.0
Hippocampus	0.4	1.0	AP = 4.0
	< 0.1	< 0.1	AP = 1.0
Border between Colliculus superior and Corpus geniculatum mediale	0.5	2.0	AP = 4.0
Colliculus superior	0.7	1.8	AP = 2.0
Mesencephalic reticular formation	< 0.2	< 0.2	AP = 2.0
Mean $\pm$ standard error	0.8 ± 0.12	$1.3^{a} \pm 0.3$	

The mean is calculated only from the sites releasing 5-HT (values ≥ 0.4 ng/30 min). [a] Not significantly different from control ($P > 0.05$). AP are the coronal levels of sites in brain structures.

shape and magnitude to that caused by 5-HT; and b) it was abolished by either methysergide or bromlysergic acid added to the bath in doses of 10 to 20 µg. Values for 5-HT were calculated in terms of the creatinine phosphate salt.

Results and discussion. From an analysis of 16 perfusion sites in caudate nucleus, amygdala, hippocampus, colliculi and mesencephalic reticular formation, 2 types of loci were identified. The first type represents the points at which 5-HT was found in amounts large enough to be detected by the biological assay (values ≥ 0.4 ng/30 min). These points constituted 50% of the total number examined. Anatomically, the sites extended as far as the head of caudate nucleus (AP = 18.0) and were scattered throughout the amygdalala caudly through the hippocampus and colliculi (Table). They were interspersed with points at which 5-HT was not detected. In these ar eas, the resting release of 5-HT varied from 0.5–1.6 ng per 30 min perfusion interval.

The second type of locus comprised 50% of all the sites perfused, and were those at which 5-HT was not released in detectable quantities. The sites at which 5-HT was not released extended from the most rostral portion of the caudate nucleus (AP = 19.0) to the mesencephalic reticular formation (AP = 2.0). There were 8 of these loci which were intermingled with the first type of sites (Table).

The average resting output was 0.8 ± 0.12 ng per 30 min in those 8 sites at which 5-HT was released. Intraperitoneal injections of metrazol (60–90 mg/kg) tended to raise the release of 5-HT from subcortical structures and colliculi at which sites this amine was spontaneously released. After metrazol, the mean rate of 5-HT release amounted to 1.3 ± 0.3 ng per 30 min ($P > 0.05$), as shown in the Table. In sites at which spontaneous release of 5-HT was not detected, the presence of this amine was not usually shown during convulsions, even when metrazol was used in the dose of 90 mg/kg.

The findings obtained in these experiments show that the spontaneous release of 5-HT in the brain structures mentioned is low or not detectable at some sites. The sites releasing, as well as those not releasing 5-HT, were widely distributed in subcortical structures, colliculi and mesencephalic reticular formation. Thus, these results strongly suggest an uneven distribution of 5-HT within the brain structures mentioned.

The results of a number of studies on the release of neurotransmitter agents in the peripheral and autonomic nervous system have shown that these substances are released from nerve terminals. The limbic structures and colliculi are known to contain nerve terminals from ascending serotonergic neurones with cell bodies situated in the lower brain stem[11]. Furthermore, in the cat, lesions interrupting brain stem ascending fibres travelling along the base of the midbrain produced a significant decrease of the hypothalamic and striatal serotonin[12]. In view of these findings, it is possible that 5-HT within the subcortical structures and colliculi mentioned originates mainly from these terminals.

In these experiments, convulsions produced by metrazol did not change significantly the 5-HT release from the subcortical and mesencephalic brain regions. The present experiments tend to confirm the earlier suggestion that 5-HT content in the whole brain is not significantly altered during convulsions.

Résumé. Nous avons étudié par la technique «push-pull» sur des chats non anesthésiés, la libération du 5-HT dans les formations souscorticales et mésencéphaliques du cerveau, et y avons trouvé deux types. Dans le premier, nous avons pu constater la présence de la 5-HT par les méthodes biologiques, mais dans le second, cette amine manquait. Le métrazol (60–90 mg/kg) n'eut pas d'effet significatif sur la libération de la 5-HT.

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