To obtain the subjective evaluation of drug effects, that is of marihuana-like 'high', whether pleasent or unpleasant, we asked the subjects to rate themselves in a graph form provided for them. This rating was obtained at appropriate intervals for 6 h following drug administration. No specific instructions were given for these ratings, and each subject was free to utilize whathever criterion he wished. We found that although there were variation in rating the magnitude of 'high', the pattern of the psychological experience in time was consistently similar.

Subjects were told that initially they would be i.v. infused with a drug-free solution (normal saline), and that at some unspecified time, it would be replaced with the preparation containing either Δ^9-THC or any of its monohydroxylated derivatives. The replacement of solutions without the subjects' awareness was possible because the Harvard constant infusion pump utilized for injection was located in the observation room. The subjects were instructed to report the moment they felt the action of the drug, that is the initial perception of marihuana-like effects, and to ask for the termination of the infusion as soon as they felt they had arrived at their desired level of 'high'. The volunteers were encouraged to receive the largest amount of the drug that they could comfortably tolerate. By giving the subjects control as to the amount of drug to be injected and by the constant recording of vital signs, we insured the safety and confidence of the volunteers. Likewise, progressive administration of the drugs mimics their actual pattern of use, since they are most frequently inhaled until the user decides that he has reached his desired level of 'high'. Variable times of placebo injection were used ranging from 15–25 min, and the subjective ratings were always base line indicating that there were no placebo responses under our experimental conditions. After the placebo injection, Δ^9-THC and 11-OH-Δ^9-THC were infused at the rate of 0.2 mg/min (0.92 ml/min) until the subject decided that he had achieved his desired level. Infusion of 8β-OH-Δ^9-THC at this rate in the first subject tested failed to produce any marihuana-like effects. For this reason, the rate of administration was increased to 0.46 mg/min (2.23 ml/min) for the remaining 9 subjects. Infusion of 8α-OH-Δ^9-THC at this higher rate of administration failed to produce any effects in the first subject tested, and the rate of administration was increased to 0.92 mg/min (4.46 ml/min) for the remaining 5 subjects.

The Table illustrates the doses necessary in µg/kg to perceive the action of the drugs, to accelerate the heart rate more than 25% over the initial level, and to achieve the desired level of 'high'. The results indicate that the Δ^9-THC and its 11-hydroxylated metabolite have similar potencies; the 8β-hydroxylated metabolite is less potent, and the 8α-hydroxylated derivative appears to have no potency at the dose and rate of infusion utilized.

The subjective experience of marihuana-like 'high' was rated as equal both in duration and intensity among the subjects receiving the Δ^9-THC and its 11- and 8β-hydroxylated metabolites (Figure). At the end of the experiment, subjects infused with either Δ^9-THC or the 11-OH-Δ^9-THC reported that the 'high' had been the most intense they had ever experienced, while those infused with the 8β-OH-Δ^9-THC did not. This diminished potency of the 8β metabolite is further illustrated by the fact that 5 of the 9 subjects infused with it never reached their desired or maximum level of 'high'.

The i.v. infusion of Δ^9-THC and its 11-hydroxylated metabolite produced a marked acceleration of the heart rate and although there was a difference in the magnitude (Figure), it was not statistically significant. The tachycardia produced by the 8β-OH-Δ^9THC was of less magnitude and duration ($p > 0.01$) even though it was infused at a faster rate. The 8α-OH-Δ^9THC did not accelerate the heart when infused at 4 times the rate of that of the Δ^9-THC. This failure to accelerate the heart parallels the absence of psychological effects and demonstrates the lack of pharmacological activity of the 8α-hydroxylated metabolite.

The finding that hydroxylation at the 11-position did not significantly increase the potency of Δ^9-THC casts reasonable doubt on the hypothesis that the 11-hydroxy derivative is the active form of the parent compound. Thus, it if were necessary for Δ^9-THC to be metabolized to the 11-hydroxy compound to exert its marihuana-like action, the injection of the 11-hydroxy derivative at the same rate should have produced more intense effects more quickly than those produced by the parent compound, but this did not occur. Furthermore, after the i.v. injection of Δ^9-THC, we have found that the levels of the 11-OH-Δ^9-THC were never more than 2% of the total cannabionoids present in the plasma (results will be published elsewhere). It is unlikely that this minute amount of the 11-hydroxy metabolite is responsible for the effects of Δ^9-THC.

Resumen. Se hizo un estudio comparativo de la capacidad del Δ^9-tetrahidro-cannabinol y de sus metabolitos monohidroxilados en producir effectos similares a los de la marihuana cuando son inyectados intravenosamente a humanos. Se encontro que la hidroxilacion en la posición 11 no cambio la potencia, en la posición 8β la redujo, y en la posición 8α la abolio por completo.

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The Activating Action of Acetylcholine and Pilocarpine on the Oxidation of Luminol

The chemiluminescent oxidation of luminol (3-aminophtalhydrazide) with aqueous alkaline hydrogen peroxide and an 'activating' agent[1] or 'co-oxidant'[2] is a very complex multistep reaction. A number of detailed mechanisms have been proposed for this reaction by various workers. In most of them there are two common points: 1. the first step of the reaction involves the oxidation of the anion of luminol by the activator and 2. the key

intermediate is a peroxide. WEBER et al.[3] have proved that the mechanism of this reaction follows Michaelis-Menten kinetics.

The activator in this reaction is either a strong oxidizing agent such as hypochlorite[4], ferricyanide[4], persulfate[2], or a metal salt or complex which, on reacting with hydrogen peroxide, yields the hydroxyl radical[5,6]. The organophosphorous anticholinesterase agents have been shown by GOLDENSON[7] to activate the chemiluminescent oxidation of luminol too. In our previous work[8,9] the activating action of two other parasympathomimetic agents – acetylcholine chloride (Ach) and pilocarpine hydrochloride – was recorded.

The pharmacological action of these two drugs on parasympathetic receptors differ, Ach being a pure agonist and pilocarpine a partial agonist, which in addition exerts a non-competitive auto-inhibition at higher doses. When combined in low doses ($10^{-5}M$) with a pure agonist, pilocarpine acts sinergistically with low doses,

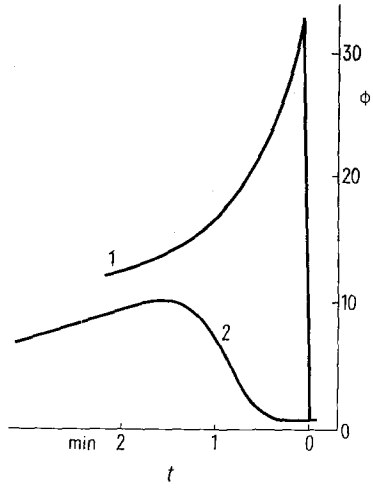

Fig. 1. The curves of luminescence of Ach $1{,}32\times10^{-2}$ M (1) and pilocarpine $3{,}5.10^{-2}$ M (2).

[1] E. H. WHITE, in *Light and Life* (Eds. W. D. MCELROY and B. GLASS; John Hopkins Press, Baltimore 1961), p. 183.
[2] M. M. RAUHUT, A. M. SEMSEL and B. G. ROBERTS, J. org. Chem. *31*, 2431 (1966).
[3] K. WEBER, Lj. HUIĆ and M. MRAZOVIĆ, Arh. Hig. Rada *9*, 325 (1958).
[4] A. O. ALBRECHT, Z. phys. Chem. *136*, 321 (1928).
[5] K. WEBER and M. KRAJČINOVIĆ, Ber. Dt. chem. Ges. *75B*, 2051 (1942).
[6] K. WEBER, A. REŽEK and V. VOUK, Ber. Dt. chem. Ges. *75B*, 1141 (1942).
[7] J. GOLDENSON, Analyt. Chem. *29*, 877 (1957).
[8] E. KUNEC-VAJIĆ and K. WEBER, Croat. chem. Acta *37*, 211 (1965).
[9] E. KUNEC-VAJIĆ and K. WEBER, Experientia *23*, 432 (1967).

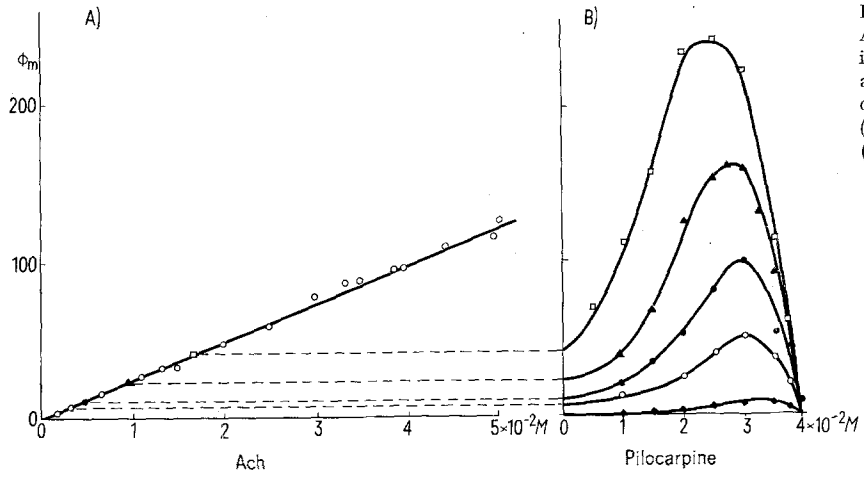

Fig. 2A) Maximum luminescence intensity vs. Ach concentration. B) Maximum luminescence intensity vs. pilocarpine concentration (✦–✦) and in the presence of various concentrations of Ach: $3{,}3\times10^{-3}$ M (○–○), $4{,}95\times10^{-3}$ M (●–●), $9{,}9\times10^{-3}$ M (▲–▲), $1{,}65\times10^{-2}$ M (□–□).

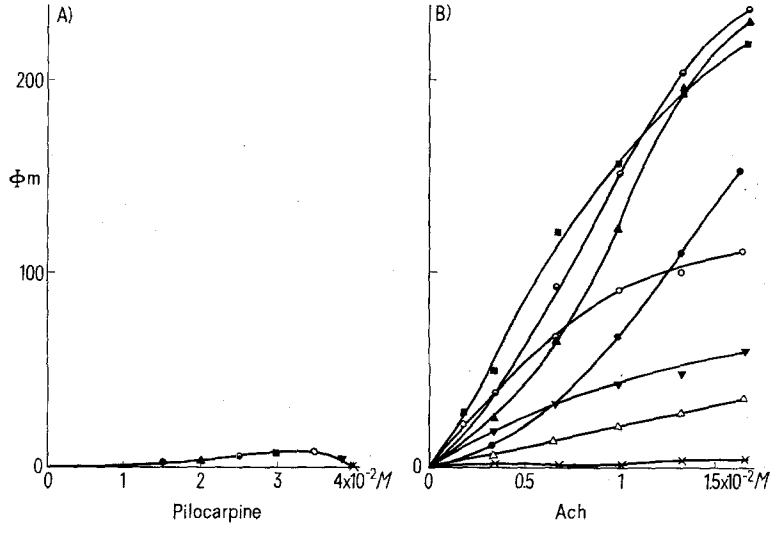

Fig. 3A) Maximum luminescence intensity vs. pilocarpine concentration. 3B) Maximum luminescence intensity vs. Ach concentration (△—△) and in the presence of various concentrations of pilocarpine: $1{,}5\times10^{-2}$ M (●—●), 2×10^{-2} M (▲—▲), $2{,}5\times10^{-2}$ M (◒—◒), 3×10^{-2} M (■—■), $3{,}5\times10^{-2}$ M (○—○), $3{,}75\times10^{-2}$ M (▼—▼), 4×10^{-2} M (×—×).

but competitive antagonistically with high doses of the agonist. In higher doses pilocarpine always acts as a competitive, and in very high doses as a non-competitive antagonist[10,11]. As to the site of action of these drugs, it is generally accepted that an anionic site on the surface of the muscarinic receptor is required for the interaction of the parasympathomimetic drug with its receptor.

The work presented here reports experiments in which the combined action of Ach and pilocarpine on the oxidation of luminol was investigated. Furthermore the effect of a pure parasympatholytic drug – atropine sulfate – on the activating action of Ach and pilocarpine was studied.

Luminescence intensity was recorded as a function of time with a luminophotometer described earlier[12]. The reaction mixture in a glass of 100 ml was placed above the photocell, and the recorder was started with activator addition. The maximum readings were plotted against activator concentration. The total volume of the reaction mixture was always 10 ml, except in the experiments with atropine, in which it was 50 ml. The final concentrations of the reagents in all the experiments were as follows: luminol $4\times10^{-4}M$, hydrogen peroxide $1.76\times10^{-2}M$ and NaOH $4.5\times10^{-2}M$. The reagents were of analytical grade. Acetylcholine chloride (Hoffmann-La Roche) and pilocarpine hydrochloride (Medika) solutions were always freshly prepared prior to use.

The curves of luminescence obtained with Ach differ from those obtained with pilocarpine in shape as well as in intensity (Figure 1). Luminescence formed in the presence of Ach reached peak intensity immediately after adding the activator, while with pilocarpine the maximum reading took about 90 seconds to develop. On plotting the maximum readings against Ach concentration, a straight line is obtained (Figure 2A). Pilocarpine exerts its activating action in a very narrow concentration range. Maximum effect was achieved with $3.5\times10^{-2}M$ of pilocarpine, while with higher concentrations of this drug an auto-inhibition (concentration inhibition) is apparent (Figure 3A). The combined effects of Ach and low doses of pilocarpine ($2.10^{-2}M$) are much higher than those expected by simple addition (Figure 2B and 3B). The maximum height of the concentration-effect curve of pilocarpine combined with Ach is shifted to lower concentrations of pilocarpine with increasing concentration of Ach (Figure 2B). Pilocarpine in higher doses shows a non-competitive auto-inhibition similarly to its action in isolated organs described by VAN ROSSUM[11] (Figure 3B).

In the reactions of inhibition with atropine sulfate, the values of percentage inhibition were plotted as a function of inhibitor concentration. The curves thus obtained were used for estimating the 50% inhibition values ($c_1/_2$). The reaction mixture in these experiments contained $2.75\times10^{-3}M$ of Ach and $3.5\times10^{-2}M$ of pilocarpine resp. Atropine was added to the reaction mixture before adding the activator. The results of these experiments are given in the Table, and the curve of inhibition for Ach is shown in Figure 4.

In summary, the present work points to the following analogies between the activating action of the investigated parasympathomimetic drugs and their pharmacological action: 1. the synergism between low doses of pilocarpine and Ach, 2. the non-competitive auto-inhibition of high doses of pilocarpine, 3. the inhibition of the action of both drugs by atropine sulfate. To these, another analogy derived from the above-mentioned data may be added: the first step of the activating as well as pharmacological action takes place between the parasympathomimetic drug and an anion[13].

The present experiments confirm the author's previous suggestion[14] of the possible interrelation between the pharmacological and activating actions of cholinergic drugs.

Zusammenfassung. Folgende Analogien zwischen der Aktivierung der Chemilumineszenz des Luminols durch Parasympathomimetika und ihrer pharmakologischen Wirkung wurden festgestellt: 1. Synergie zwischen kleinen Dosen von Pilocarpin und Ach; 2. nichtkompetitive Autoinhibition grosser Dosen von Pilocarpin; 3. Inhibition der Wirkung beider Medikamente durch Atropin-sulfat.

50% inhibition values ($c_1/_2$) of atropine sulfate

Concentration of the activator in the reaction mixture	$c_1/_2$
Ach $2{,}75\times10^{-3}$ *M*	$9{,}0\times10^{-5}$ *M*
Pilocarpine $3{,}5\times10^{-2}$ *M*	$1{,}6\times10^{-3}$ *M*

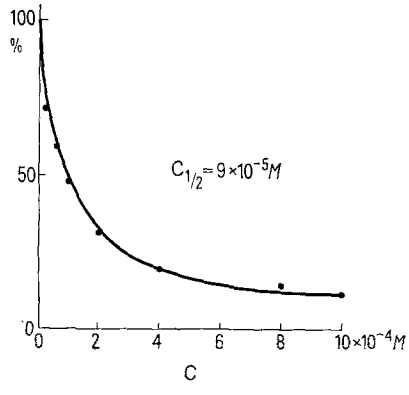

Fig. 4. Maximum luminescence intensity as a function of atropine sulfate concentration. Activator: Ach, $2{,}75\times10^{-3}$ *M*.

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29 January 1973.

[10] J. M. VAN ROSSUM, Experientia *16*, 373 (1960).
[11] J. M. VAN ROSSUM, Archs. int. Pharmacodyn. Thér. *140*, 592 (1962).
[12] K. WEBER, Dt. Z. ges. gericht. Med. *57*, 410 (1966).
[13] E. KUNEC-VAJIĆ, Experientia *27*, 29 (1971).
[14] For generous supplies of acetylcholine chloride the author is indebted to the firm Hoffmann-La Roche.