sues; the incubation conditions employed had therefore apparently not altered the structures and the noradrenaline stores.

All the tissues assayed reacted to the addition of the venom with a striking reduction of the specific yellow green fluorescence; however, the concentration necessary to obtain this effect was not the same for all tissues. The addition of an extract corresponding to 0.1 pairs of venom glands/ml of incubation medium (13 µg protein/ml) was followed by complete disappearance of the specific fluorescene in all the tissues examined, with the exception of the longitudinal muscular layer of the gut. This tissue showed an almost total depletion only when incubated at a 5 times higher concentration (Figure 1). With the addition of 0.01 pairs/ml the iris, the mesentery, the inferior vena cava and the spleen capsule still showed a total or subtotal disappearance of the specific fluorescence (Figures 2, 3, 4), while at a 10 times lower concentration the depleting effect was still clearly visible in the mesentery, but only very slightly in the other tissues. These quantitative differences in the response of various tissues could be due to differences either in penetration of the venom, or in sensitivity of the nerve terminals towards it. Guinea-pig irises were sensitive to the same concentrations of venom as rat irises, although the whole organisms differ in sensitivity. The amounts of venom/ml of incubation medium which proved effective in vitro may be compared with the $LD_{50}$/g of animal body weight in vivo, which was established for mice: 0.01 gland couples corresponded to 2 $LD_{50}$'s/h.

The neurotransmitter depleting action of *Latrodectus* venom, which has already been shown not to be restricted to cholinergic nerve terminals, extends therefore to adrenergic nerve fibres and terminals of different mammalian organs, although with different degrees of susceptibility. These findings may help to explain the symptoms of *Latrodectus* poisoning.

*Riassunto.* Diversi tessuti di cavia, adatti per l'allestimento di preparati in toto per la dimostrazione delle catecolamine (per mezzo della fluorescenza specifica che si sviluppa in seguito al trattamento con formaldeide gassosa) vengono incubati in vitro in presenza o in assenza di un estratto di ghiandole velenifere del ragno *Latrodectus mactans tredecimguttatus* (Rossi). I tessuti incubati in presenza del veleno (in concentrazioni che per certi tessuti sono di 1,3 µg di proteina/ml) mostrano una scomparsa della fluorescenza, evidentemente dovuta a uno svuotamento delle riserve di noradrenalina dalle terminazioni adrenergiche.

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## Development of Tolerance to $\Delta^9$-THC in the Frog

Although 1-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) is now generally recognized as the principle active component of marihuana (*Cannabis sativa*)[1], it is still a matter of dispute whether the biological activity resides in $\Delta^9$-THC, its hydroxylated metabolites, particularly 11-hydroxy-$\Delta^9$-THC, or both[2]. The latter metabolite has been reported to be markedly more potent in the mouse than the parent compound following intracerebral administration[2]. However, it has also been noted that while $\Delta^9$-THC is extensively metabolized to 11-hydroxy-$\Delta^9$-THC by the liver, the amount of this metabolite that reenters the blood is rather negligible, the 11-hydroxy metabolite apparently being excreted rather than recirculated[3]. This suggests that the activity of the 11-hydroxy metabolite may be negligible if the parent compound is being examined. This might explain Sofia and Barry's[4] finding that SKF-525, which inhibits the microsomal enzyme systems responsible for much of the metabolism of $\Delta^9$-THC, did not attenuate the depressant effect of $\Delta^9$-THC. However, SKF-525 A has recently been shown to increase the latency of the onset of activity of $\Delta^9$-THC[5], which supports the contention that a metabolite may in fact be the active component in $\Delta^9$-THC. However, 2 aspects of the latter experiment make this result somewhat tenuous. First, SKF-525 A was shown to have a slight excitatory effect on the behavioral response being measured, (operant bar pressing), and second, the same animals were used to assess the onset of the effects of $\Delta^9$-THC alone and in the presence of SKF-525 A, thus introducing the possibility that tolerance to the effects of $\Delta^9$-THC may have been responsible for the increased latency of onset[6].

To investigate the problem somewhat differently, we examined the effects of $\Delta^9$-THC in the frog. The reason for using this animal is that the mechanism responsible for hydroxylation of drugs by the liver is not present in these animals[7]. Nor is it possible to induce these drug metabolizing enzymes in the frog by treatment with phenobarbital, although this has been shown for a variety of other species[8]. Consequently, unless other tissues are actively metabolizing the drug, any behavioral effects observed following administration of $\Delta^9$-THC ought to be due to the parent compound and not its hydroxylated

Duration of loss of righting reflex in the frog following chronic administration of $\Delta^9$-THC

| | N | Median time (sec) | | |
|---|---|---|---|---|
| | | Day 1 | Day 3 | Day 5 |
| Drug (60 mg/kg) | 7 | 2435 | 76 | 1 |
| Control | 6 | 1 | 1 | 1 |

[1] H. Isbell, C. W. Gordetzsky, D. Jasinski, U. Claussen, F. von Spulak and F. Korte, Psychopharmacologia *11*, 184 (1967). – R. Mechoulam, Science *168*, 1159 (1970).
[2] H. D. Christensen, R. I. Freundenthal, J. T. Gidley, R. Rosenfeld, G. Boegli, L. Testino, D. R. Brine, C. G. P. H. Wall and M. B. Wall, Science *172*, 165 (1971).
[3] M. E. Wall, Ann. N. Y. Acad. Sci. *191*, 23 (1971).
[4] R. D. Sofia and H. Barry, III, Eur. J. Pharmac. *13*, 134 (1970).
[5] D. W. Peterson, G. M. Cohen and S. B. Sparber, Life Sci. *10*, 1381 (1971).
[6] D. E. McMillan, L. S. Harris, J. M. Frankenheim and J. S. Kennedy, Science *165*, 501 (1970).
[7] B. B. Brodie and R. P. Maickel in *Metabolic Factors Controlling Duration of Drug Action* (Eds. B. B. Brodie and E. B. Erdös; Academic Press, N. Y. 1962).
[8] Y. Gutman and M. Kidron, Biochem. Pharmac. *20*, 3548 (1971).

metabolites. The following experiment examines the effects of $\Delta^9$-THC on loss of righting reflex and the possibility of the development of tolerance to these effects in the frog.

*Method.* Frogs received an injection into the lymph sac of either 0, or 60 mg/kg $\Delta^9$-THC suspended in bovine serum albumin. Control subjects received only the vehicle. The effect of the drug was measured 2 h after injection by the time for recovery of the righting reflex. The subjects in the 0 and 60 mg/kg groups were then injected every other day after injection until tolerance was evident. The control group then received a single injection of 60 mg/kg and the time for recovery of the righting reflex was measured as before. A 10 min maximum was set for each observation.

*Results and discussion.* The data are shown in the Table. The drug obviously suppressed the righting reflex in these animals. It is also apparent that tolerance to this effect was evident by the 2nd injection and by the 3rd injection there was no difference between experimental and control animals. Evidence that tolerance had in fact occurred is indicated by the loss of righting reflex in the control group that received the single injection of 60 mg/kg $\Delta^9$-THC. The second half of the Table shows that there was no effect of the drug on reflex leg withdrawal when the frog leg was inserted into a solution containing 0.2 *N* HCl. This suggests that the loss of righting reflex was the result of an effect of the drug at some level higher than the spinal cord.

These findings are of considerable interest in terms of the problem of biological activity of the parent compound versus the hydroxylated metabolite. The fact that frogs do not readily hydroxylate drugs[7] suggests that the parent compound is in fact biologically active. KAYMAKÇALAN and DENEAU[9] have recently come to the same conclusion upon observing that the analgesic effect of $\Delta^9$-THC was significantly greater in hepatectomized rats than in sham-operated controls. Given this conclusion, it follows that tolerance to this drug can occur without the involvement of a metabolic transformation of the drug. The actual mechanism, however, is still to be elucidated.

Latency of leg withdrawal from water and 0.2 *N* HCl in the frog following injection if $\Delta^9$-THC on day 1

| | Median latency (sec) | | |
|---|---|---|---|
| | *N* | Water | 0.2*N* HCl |
| Drug (60 mg/kg) | 7 | 10 | 1 |
| Control | 6 | 10 | 1 |

All animals were tested 2 h after injection of placebo (bovine serum albumin) or drug (60 mg/kg) $\Delta^9$-THC suspended in bovine serum albumin. On day 1, foot withdrawal was tested prior to testing of righting reflex. On day 3, following testing, the control group was injected with 60 mg/kg of drug and then 2 h later, was tested for loss of righting reflex. Duration of loss of righting reflex (median time) = 2240 sec.

*Résumé.* Après injections bi-journalières de $\Delta^9$-THC à des grenouilles, nous avons calculé la durée de la perte du réflexe de redressement. La tolérance au $\Delta^9$-THC fut évidente après la seconde injection et, après la troisième, aucun effect ne fut visible. Ceci suggère que la tolérance se développe par le $\Delta^9$-THC, et non par ses métabolites, puisque les grenouilles n'ont pas de système enzymatique d'hydroxylation.

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[9] S. KAYMAKÇALAN and G. A. DENEAU, Acta med. turc. Suppl. 1, S (1972).

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## Chronopharmacology of $\Delta^9$-Tetrahydrocannabinol Hypothermia in Mice

It is becoming increasingly clear that biological responses to many drugs can be markedly affected by the time of day at which they are administered. For example, the duration of sleep in rats induced by sodium pentobarbital is as much as 50% longer when injections are given at 06.00 h than if given at 18.00 h (PAULY and SCHEVING[1]). The toxicological effects of given doses of many drugs such as alcohol and oubain have likewise been shown to vary in lethality as a function of chronological time of drug treatment (HAUS and HALBERG[2]; Halberg and STEPHENS[3]). In light of the ever-growing body of literature involving the effects of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) on human and sub-human species, the following experiment was conducted to determine whether the thermogenic effects of the drug (see review by ABEL[4]) would likewise be affected by the chronological time of injection.

81 male Dublin mice (25–30 g) were divided into 3 main treatment groups depending on the time of their injection. These main treatment groups were then subdivided into 3 drug groups of 9 animals each and subjects injected were i.m. (right fore-leg) with either 10.0 or 100.0 mg/kg $\Delta^9$-THC dissolved in dimethyl sulfoxide (DMSO) or with the vehicle alone (1 cm³/kg) immediately after their rectal temperatures had been determined (Yellow-Springs Telethermometer, Model 34TD). Body temperatures were again determined 1, 2 and 4 h after injection. Each main group (27 animals) received their injections at either 10.30 h, 15.45 h or 20.00 h and are designated as morning, afternoon and night-time treatments respectively. The ambient room temperature during the experiment was 23.0 ± 1.0 °C.

The results are shown in the Figure which depicts the drug-induced changes in body temperature as a function of pre-drug body temperature. It is readily apparent from inspection of the Figure that the time of day at which

[1] J. E. PAULY and L. E. SCHERING, Int. J. Neuropharmac. *3*, 651 (1964).

[2] E. HAUS and F. HALBERG, J. appl. Physiol. *14*, 878 (1959).

[3] F. HALBERG and A. N. STEPHENS, Fedn. Proc. *17*, 339 (1968).

[4] E. L. ABEL, in *Cannabis and Its Derivatives* (Ed. W. D. M. PATON and J. CROWN; Oxford Univ. Press, London 1972).