metabolites. The following experiment examines the effects of Δ^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 Δ^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 Δ^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 Δ^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 Δ^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) Δ^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 Δ^9-THC à des grenouilles, nous avons calculé la durée de la perte du réflexe de redressement. La tolérance au Δ^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 Δ^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 Δ^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 Δ^9-tetrahydrocannabinol (Δ^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 Δ^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

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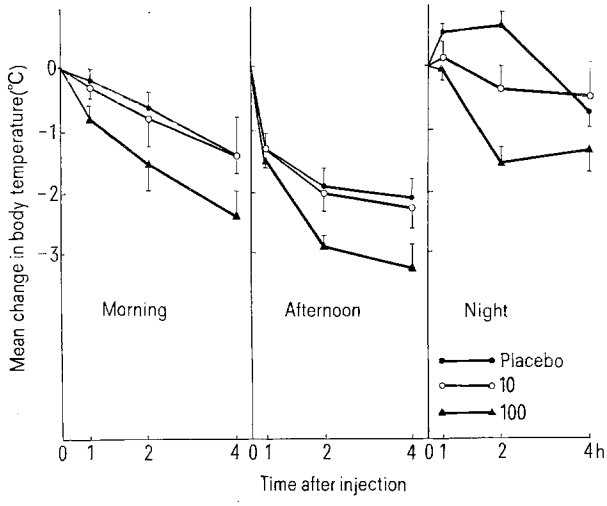
[3] F. HALBERG and A. N. STEPHENS, Fedn. Proc. *17*, 339 (1968).

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

injections are made constitutes a significant variable in determining the magnitude of drug effect. The greatest degree of effect appears to occur when animals are injected during the afternoon, whereas the smallest changes in body temperature occur when injections are administered at night. The effects of the vehicle alone are likewise greatest and smallest at these times. There is also evidence of a slight increase in the body temperatures of the vehicle-treated animals during the first 2 h after injection in the latter group. Thus, at 4 h after treatment, the body temperatures of the control animals had decreased by −1.4, −2.1 and −0.6 °C for the morning, afternoon and night treatments repectively. The changes for the 10.0 mg/kg groups was −1.4, −2.2 and −0.4 °C and for the 100.0 mg/kg groups, these were −2.4, −3.2 and −1.3 °C respectively.

Thus, these data further illustrate the importance of the chronological variable in drug-related research. Methodologically, they suggest that drug-produced effects ought not to be compared when animals are tested or injected at different times of day, especially when subtle differences are expected.

With respect to the present experiment, the most likely explanation for the data is that since mice tend to be more active during the night and least active during the afternoon, this diurnal activity pattern most likely contributed to the overall effect by increasing motor activity at night and thus masking to some extent, the well known sedative-like effects of high doses of marihuana and its derivatives in animals (e.g., CARLINI, SANTOS, CLAUSSEN et al.[5]).



Changes in body temperature of mice treated at different chronological time periods. Vertical lines indicate the standard errors of the mean. There were 9 animals in each drug group.

Résumé. La température rectale de souris ayant subi une unique injection de Δ^9THC (injection administrée soit le matin, soit l'après-midi, ou soit durant la nuit) a été mesurée 1, 2 et 4 h suivant cette injection. Chez les animaux, injectés l'après-midi, l'abaissement de température était maximal, mais il fut minimal chez les animaux injectés de nuit.

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12 June 1973.

[5] E. A. CARLINI, M. SANTOS, U. CLAUSSEN, D. BIENIK and F. KORTE, Psychopharmacologia *18*, 82 (1970).

[6] Present address: Research Institute on Alcoholism, New York State Department of Mental Hygiene, 1021 Main Street, Buffalo (New York 14203, USA).

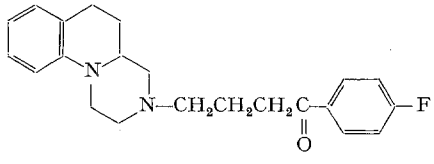
Antihypertensive and Central Nervous System Depressant Properties of 3-(γ-*p*-Fluorobenzoyl Propyl) 2,3,4,4a,5,6-hexahydro-1(H)-Pyrazino(1,2-a) Quinoline Hydrochloride (Compound 69-183, Centpyraquin)

Synthesis and preliminary pharmacological results of a series of quinoline derivatives have been reported earlier[1]. Centpyraquin (3-(γ-*p*-fluorobenzoyl propyl) 2, 3, 4, 4a, 5, 6-hexahydro-1(H)-pyrazino (1, 2-a) quinoline hydrochloride (compound 69-183, I) showed significant hypotensive and CNS depressant properties and a brief report of the important results is being presented.

Cardiovascular and autonomic effects in normotensive cats and dogs. Centpyraquin lowered the blood pressure of anaesthetized or immobilized (D-tubocurarine) cats and dogs. The minimum effective dose was 0.1 mg/kg i.v. which lowered the blood pressure by 10% for about 10 min. A dose of 1.0 mg/kg i.v. lowered the blood pressure by 47% for about 90 min. The response was still more marked with higher doses. Hypotension was accompanied by blockade of the nictitating membrane contraction due to both pre- and post-ganglionic stimulation, potentiation of the adrenaline and noradrenaline pressor responses and blockade of the tyramine pressor response. It had a positive inotropic effect in open chest, anaesthetised cats (upto 5 mg/kg i.v.) and in isolated perfused guinea-pig and rabbit hearts (100–200 μg).

Effect in hypertensive rats. Centpyraquin (10 mg/kg i.p. or oral) lowered the blood pressure of renal hypertensive rats by 20% for more than $3^1/_2$ h. With 20.0 mg/kg (i.p.), blood pressure was reduced by about 40% for 4 h. Daily administration (20 mg/kg i.p., once a day for 10 days) lowered the blood pressure by 30% and it returned to original level 3 days after the stoppage of the compound.

CNS effects in mice. The compound depressed the spontaneous motor activity of mice in doses of 30 mg/kg and above. Ptosis was a marked feature. Rectal tem-



Centpyraquin (I)

[1] V. A. RAO, P. C. JAIN, N. ANAND, R. C. SRIMAL and P. R. DUA, J. med. Chem. *13*, 516 (1970).

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[3] Thanks are due to Drs. M. L. DHAR and N. ANAND for their interest in the work.

[4] Communication No. 1600 from the Central Drug Research Institute; Lucknow.