Br. J. Pharmac. (1974), 51, 391-397

TOLERANCE TO THE EFFECT OF Δ^1-TETRAHYDROCANNABINOL ON CORTICOSTERONE LEVELS IN MOUSE PLASMA PRODUCED BY REPEATED ADMINISTRATION OF CANNABIS EXTRACT OR Δ^1-TETRAHYDROCANNABINOL

R.G. PERTWEE[1]
Department of Pharmacology, University of Oxford, Oxford OX1 3QT

1 Three injections of cannabis extract (500 mg/kg s.c. given over 3 or 5 days) diminished thymus gland weight but not the weights of spleen or liver in weanling female and adult male mice kept at room temperature.

2 Both cannabis extract (500 mg/kg s.c.) and Δ^1-tetrahydrocannabinol (Δ^1-THC; 10 and 20 mg/kg i.p.) elevated corticosterone levels in mouse plasma.

3 A pretreatment that consisted of three daily subcutaneous injections of 500 mg/kg of cannabis extract and that was shown to produce tolerance to the 'cataleptic' effect of Δ^1-THC (2 mg/kg i.v.) in mice, also produced tolerance to the effect of Δ^1-THC (10 mg/kg i.p.) on corticosterone levels in mouse plasma. However, this pretreatment did not reduce the rise in plasma corticosterone concentration produced by immobilization.

4 Tolerance to the effect of Δ^1-THC (10 mg/kg i.p.) on corticosterone levels in mouse plasma was also produced by the pretreatment of mice with a single injection of Δ^1-THC (10 mg/kg s.c.). Three daily injections of Δ^1-THC (10 or 30 mg/kg s.c.) also produced tolerance.

5 In a thermoneutral environment (30-32° C) in which cannabis extract does not produce hypothermia, the drug no longer reduced thymus gland weight. However the effect of cannabis extract and of Δ^1-THC on corticosterone plasma levels was the same at room temperature as at 30-32° C. Tolerance to the latter effect of Δ^1-THC was also produced equally readily under the two conditions.

6 It is concluded that pretreatment with cannabis extract or Δ^1-THC can produce tolerance to the effect of Δ^1-THC on corticosterone levels in mouse plasma and does so without impairing the effect of immobilization stress on corticosterone release. In addition, both the rise in corticosterone plasma levels produced by cannabis or Δ^1-THC and the development of tolerance to this effect can still take place in the absence of hypothermia.

Introduction

Δ^1-Tetrahydrocannabinol (Δ^1-THC) can elevate levels of plasma corticosterone (Kubena, Perhach & Barry, 1971) and also deplete adrenal ascorbic acid (Dewey, Peng & Harris, 1970) in rats. These effects are probably produced by a central action (Kubena *et al.*, 1971) of Δ^1-THC and are of special interest since it is probable that several of the effects of Δ^1-THC are due to increased release of adrenocorticotrophic hormone (ACTH) or adrenocorticosteroids (Drew & Slagel, 1973; Sofia, Nalepa, Harakal & Vassar, 1973).

The experiments of Kubena *et al.* (1971) and of Dewey *et al.* (1970) failed to demonstrate that repeated treatment of rats with Δ^1-THC can produce tolerance to the effects of this drug on corticosterone release or on adrenal ascorbic acid levels. However, it is not clear whether the procedures in these experiments were effective in producing any of the other numerous types of tolerance to Δ^1-THC now known (Paton & Pertwee, 1973). It is also uncertain how far the hypothermic effect of Δ^1-THC (Holtzman, Lovell, Jaffe & Freedman, 1969) may play a role in elevating corticosterone levels. The experiments described in this paper have explored both these

[1] Present address: Department of Pharmacology, University Medical Buildings, Foresterhill, Aberdeen AB9 2ZD.

issues. In the first experiments thymus gland weight was used as an index of adrenocorticosteroid release since adrenal cortical hormones are known to cause atrophy of this gland (Dougherty, 1952). Subsequently, the levels in mouse plasma of corticosterone, the principal adrenal cortical hormone in mice (Halberg, Peterson & Silber, 1959), were measured directly.

Methods

Drugs were administered to mice intraperitoneally, subcutaneously or intravenously. Both adult mice (23-27 g) and weanling female mice (3 weeks old; 8-13 g) were used. The mice (Tuck No. 1 strain) received food and water *ad libitum* and were maintained on a circadian cycle of 12 h light (07 h 45 min to 19 h 45 min) and 12 h darkness. Cannabis was supplied as an ethanolic tincture containing 50 mg residue per ml. The tincture was found to contain 1.8 mg/ml of Δ^1-THC, 0.4 mg/ml of cannabinol and 1.3 mg/ml of cannabidiol (D. Harvey, unpublished observations). Cannabis extract and Δ^1-THC were prepared for administration by dispersion in a mixture of Tween 80 and 0.9% w/v NaCl solution (saline). The resin was prepared by removing solvent *in vacuo* from the cannabis tincture. Δ^1-THC was extracted from the petrol-ether soluble fraction of crude resin. The solution of cannabis extract contained 2.5 parts of drug to one part Tween 80 by weight; the solutions of Δ^1-THC contained two parts Tween 80 to one part Δ^1-THC. Control injections contained doses of Tween 80 equal to or greater than those used in the appropriate drug injections. In all experiments the volume injected was either 0.25 ml/25 g (i.p. or s.c.) or 0.20 ml/25 g (i.v.).

The effects of pretreatment with cannabis or Δ^1-THC on thymus gland weight or on the capacity of cannabis or Δ^1-THC to elevate corticosterone plasma levels were studied on the day following the final injection of the pretreatment phase. Tissue weights were measured immediately after killing mice with carbon monoxide.

Weights have been expressed as wet weight of tissue per unit body weight. (The statistical significance ($P <$ or > 0.05) of changes produced in tissue weight was found to be the same when tissue weight was expressed as weight per unit body weight as when it was expressed in absolute units.)

In the experiments concerning measurement of plasma corticosterone levels, all injections were made between 10 h 00 min and 11 h 00 minutes. The corticosterone was assayed fluorometrically by a method based on procedures described by Guillemin, Clayton, Smith & Lipscomb (1958) and by Silber (1966). Each sample of plasma analysed was derived from the blood of two adult male mice. The blood was collected in heparinized tubes immediately after decapitation. The samples of plasma (0.1 to 0.5 ml aliquots) were each made up to 2 ml with deionized water, washed by shaking with 4 ml of redistilled petroleum spirit (BP 60-80° C), and centrifuged. The corticosterone was extracted from the aqueous phase with 5 ml of methylene chloride. After centrifugation the solvent phase was washed with 0.1 N NaOH (0.5 ml) and again centrifuged. Next, 4 ml of the

Table 1 Weights (means with s.e. mean) of thymus gland, spleen and liver dissected from groups of 5 (female) or 6 (male) mice that had received three injections of cannabis extract (CAN), Tween 80 or 0.9% w/v NaCl solution (saline)

Mice	Drug	Dose (mg/kg s.c.)** Days 1, 2 and 3	Body weight (g) Day 4	Organ weights (mg/g body weight) Thymus Day 4	Spleen Day 4	Liver Day 4
Weanling (female)	CAN	100	11.9 ± 0.7	2.7 ± 0.5	5.3 ± 0.3	52.9 ± 4.4
	CAN	500	11.3 ± 0.7	1.6 ± 0.3*	4.1 ± 0.5	51.5 ± 1.4
	Tween 80	200	12.4 ± 0.6	3.8 ± 0.3	7.1 ± 2.0	53.6 ± 6.3
	Saline	–	12.4 ± 0.8	3.8 ± 0.4	5.4 ± 0.6	47.7 ± 3.0
Adult (male)	CAN	500	25.1 ± 0.7	1.5 ± 0.2*	5.3 ± 0.4	58.6 ± 2.4
	Tween 80	200	26.5 ± 0.8	2.5 ± 0.2	6.3 ± 0.9	57.3 ± 1.7

* Shown by Student's t test to have fallen significantly ($P < 0.05$) below values found in mice that had received only Tween 80 and were of the same sex.

** Female mice were injected between 11 h 00 min and 12 h 00 min and male mice between 15 h 00 min and 16 h 00 minutes.

methylene chloride phase was mixed with 1.5 ml of a reagent consisting of 3 volumes of absolute ethanol and 7 volumes of sulphuric acid. After centrifugation, the methylene chloride phase was discarded and the reagent layer was left to stand at room temperature. Fluorescence was measured with an exciting wavelength of 470 mμ and an emitted wavelength of 530 mμ (uncorrected wavelengths) 30 min after the reagent had first been mixed with the methylene chloride solution. In each experiment a standard sample containing 0.3 μg of corticosterone and a blank sample were carried through the procedure. An Aminco Bowman spectrofluorimeter was used with the slit widths set in the 'arrangement No. 3' described in the instruction manual. No correction was made for non-specific fluorescence present in solutions derived from plasma (Silber, 1966).

In the experiments in which mice were immobilized, the tail of each mouse was secured with adhesive tape and the rest of the body was loosely held down with three wire hoops. The immobility index was measured by the ring test which is described elsewhere (Pertwee, 1972).

Finally, unless stated otherwise, differences between drug and control groups were evaluated by Student's t test ($P >$ or < 0.05) and limits of error are expressed as standard error of the means.

Results

Thermoneutrality and the effect of cannabis on the thymus gland

Preliminary experiments were carried out with weanling female mice housed in groups of six at room temperature. The mice received subcutaneous injections of cannabis extract (500 mg/kg) or Tween 80 once daily (between 11 h 00 min and 12 h 00 min) for 3 consecutive days. It was found that the cannabis lowered ($P < 0.05$) thymus gland weights by up to 50% of those of the Tween controls. The thymus weights of Tween- and saline-treated mice did not differ from each other. At a dose level of 100 mg/kg, cannabis did not significantly affect thymus weights. In these experiments cannabis had no significant effect on body weight or on the weights of liver or spleen. Table 1 summarizes these results and also shows that similar results were obtained with adult male mice that had been injected daily between 15 h 00 min and 16 h 00 minutes.

To test the possibility that the hypothermic effect of cannabis contributed towards the effect of the drug on thymus weight, experiments were carried out on mice housed (1) in separate cages at room temperature (20° C) since mice can conserve body heat by huddling together, or (2) in groups of six at room temperature or (3) in groups of six at a thermoneutral environmental temperature (30-32° C, Herrington, 1940) at which cannabis does not lower the core temperature of mice (Paton & Pertwee, 1972). Table 2 shows that as in the preliminary experiments, cannabis (500 mg/kg s.c.), which in these experiments was administered once on each of three alternate days, significantly reduced the thymus gland weights of the mice housed at room temperature below control levels. However, the cannabis pretreatment did not significantly lower the thymus weights of the mice housed at the thermoneutral temperatures of 30-32° C.

Another set of experiments (Table 3) showed that if mice were housed in groups at 30-32° C instead of in separate cages at room temperature, the acute lethality of cannabis extract was also affected significantly ($P < 0.05$). In these

Table 2 Effect of three injections of cannabis (CAN; 500 mg/kg s.c. at 11 h 00 min to 12 h 00 min) on the weights (means with s.e. mean) of thymus gland, spleen and liver of batches of 6 mice housed separately at room temperature or in groups either at room temperature or at 30-32° C

Experimental conditions	*Drug* Day 1, 3 and 5	*Body weight (g)* Day 6	*Organ weights (mg/g body weight)* Thymus Day 6	Spleen Day 6	Liver Day 6
Isolated (20° C)	CAN	21.8 ± 1.8	1.2 ± 0.3*	4.1 ± 0.7	59.4 ± 1.5
	Tween 80	24.9 ± 0.8	2.4 ± 0.2	5.2 ± 0.4	59.7 ± 2.2
Grouped (20° C)	CAN	25.6 ± 0.6	1.7 ± 0.2*	4.7 ± 0.3	61.5 ± 2.6
	Tween 80	25.8 ± 0.5	2.7 ± 0.2	6.4 ± 0.8	59.2 ± 2.3
Grouped (30-32° C)	CAN	24.8 ± 0.5	2.2 ± 0.2	5.2 ± 0.4	59.6 ± 1.3
	Tween 80	26.1 ± 0.3	2.4 ± 0.1	5.8 ± 0.4	56.6 ± 1.6

* Shown by Student's t test to have fallen significantly ($P < 0.05$) below values found in mice that had received Tween 80 200 mg/kg under the same experimental conditions.

experiments mice isolated at room temperature or grouped at 30-32°C each received a subcutaneous injection of cannabis extract at a dose level of 2.5, 5.0 or 7.5 g/kg. All the deaths noted in the subsequent 7 day period occurred within 48 h of injection. Analysis of the results by the method of Litchfield & Wilcoxon (1949) showed that the LD_{50}'s for cannabis extract (g/kg) together with the 95% confidence limits are 3.3 (2.5 and 4.3) for isolated mice at room temperature and 5.7 (4.6 and 6.9) for mice grouped at 30-32°C. The LD_{50} for cannabis extract in mice grouped at room temperature lay between 5 and 7.5 g/kg.

Thermoneutrality and the effect of Δ^1-tetrahydrocannabinol on plasma levels of corticosterone

Dose levels of both 10 and 20 mg/kg of Δ^1-THC elevated corticosterone plasma concentrations significantly above control levels when administered intraperitoneally to mice housed in separate cages at room temperature (Table 4). In contrast, although Δ^1-THC 5 mg/kg raised plasma corticosterone levels, the increase was not statistically significant. Mean plasma levels of corticosterone 60 and 120 min after injection of Δ^1-THC 10 mg/kg were respectively 31.3 ± 4.3 and 24.2 ± 1.9 μg/100 ml. The mean level was 34.5 ± 4 μg/100 ml 60 min after injection of Δ^1-THC 20 mg/kg, and 13.8 ± 3.4 μg/100 ml at the same time after the control treatment (Tween 80; 20 mg/kg i.p.). The corticosterone level after injection of Tween 80 was higher than the level in a group of untreated mice (six mice grouped at room temperature) which was only 7.2 ± 1.9 μg/100 ml. However this difference was not statistically significant.

The housing of mice in groups of six at thermoneutral environmental temperatures, a procedure that had abolished the effect of cannabis on the thymus gland, did not alter the effect of Δ^1-THC on corticosterone plasma levels. Thus 60 min after injection of Tween 80, or of 2, 5, 10 or 20 mg/kg of Δ^1-THC, levels of corticosterone were about the same in the plasma of mice housed separately at room temperature as they were in the plasma of mice housed in groups at 30-32°C (Table 4). Similarly, the effect of cannabis extract on corticosterone was approximately equal under both sets of conditions. Sixty and 120 min after the subcutaneous injection of 500 mg/kg of cannabis extract, corticosterone plasma levels were respectively 38.2 ± 5.1 and 35.1 ± 4 μg/100 ml at room temperature and 46.5 ± 5.6 and 29.2 ± 5.0 at 30-32°C.

Table 3 The lethality of cannabis extract in isolated or grouped mice at ambient temperatures of 20° or 30-32°C

	Proportion of deaths in each batch (%)		
Dose (g/kg s.c.)	*Isolated (20°C)*	*Grouped (20°C)*	*Grouped (30-32°C)*
7.5	100 (10)*	100 (20)	90 (20)
5.0	90 (10)	30 (20)	30 (10)
2.5	20 (10)	0 (20)	0 (10)

* Numbers in parentheses denote number of mice in each batch of animals.

Table 4 Effect of Δ^1-THC on plasma corticosterone levels (means with s.e. mean) of batches of 6 or 8 mice housed separately at room temperature or in groups at 30-32°C

		Plasma corticosterone levels (μg/100 ml)	
Drug	*Dose (mg/kg i.p.)*	*Isolated (20°C)*	*Grouped (30-32°C)*
Δ^1-THC	2	14.0 ± 2.5 (4)**	15.4 ± 8.4 (3)
	5	24.9 ± 4.0 (3)	21.3 ± 0.4 (3)
	10	31.3 ± 4.3 (3)*	28.9 ± 8.2 (3)
	20	34.5 ± 4.0 (4)*	38.6 ± 4.1 (3)*
Tween 80	40	13.8 ± 3.4 (4)	13.6 ± 4.9 (3)

* Shown by Student's *t* test to have risen significantly ($P < 0.05$) above levels found in mice that had received Tween 80 under the same experimental conditions.

** Numbers in parentheses denote number of samples of plasma analysed; each sample was derived from the blood of two mice 60 min after injection.

Tolerance to the effect of cannabis on plasma levels of corticosterone

A dose level of 50 mg/kg (s.c.) of cannabis extract was effective in producing a significant degree of tolerance to the effect of a challenging dose of Δ^1-THC (1.0 mg/kg i.v.) on immobility index when the cannabis was administered to a group of six mice at the thermoneutral temperatures of 30-32°C once on each of three alternate days. Thus, 15 min after injection of Δ^1-THC the mean immobility index rose to 62 ± 3 in Tween pretreated mice but only to 42 ± 4 in the cannabis pretreated animals. The same cannabis pretreatment also produced tolerance to the effect of a challenging dose of Δ^1-THC (10 mg/kg i.p.) on corticosterone plasma levels. Thus, as shown in Table 5, Δ^1-THC raised corticosterone levels to 47 ± 7.3 μg/100 ml in the plasma of mice pretreated with Tween 80 but had no detectable effect on corticosterone levels in mice pretreated with cannabis.

However, the cannabis pretreatment did not significantly affect the response of mice to immobilization; restraint for 2 h at room temperature elevated levels of corticosterone to 51.6 ± 2.3 μg/100 ml in mice pretreated with cannabis and to 57.8 ± 3.9 μg/100 ml in Tween pretreated animals. It is noteworthy that the increases in corticosterone levels that resulted from immobilization were significantly ($P < 0.05$) greater in mice that had been pretreated with Tween 80 than in mice that had received no pretreatment at all.

When Δ^1-THC was substituted for cannabis extract in the above pretreatment schedule, tolerance to the effect of Δ^1-THC on corticosterone levels could still be produced. Thus injections of Δ^1-THC (10 or 30 mg/kg s.c.) made on 3 alternate days abolished the effect of the challenging injection of Δ^1-THC (10 mg/kg i.p.) on corticosterone plasma levels. Pretreatment over 3 alternate days with two injections of Tween 80 followed by a single injection of Δ^1-THC (10 mg/kg s.c.) was also effective in producing tolerance to the challenging injection of Δ^1-THC. In this case, although a rise in corticosterone level was induced by the challenging injection, it was significantly less than one induced in mice that had been pretreated on 3 alternate days with Tween 80 (200 mg/kg s.c.). By the same criterion, no significant tolerance to the challenging injection of Δ^1-THC could be detected after pretreatment on 3 alternate days with Δ^1-THC at a dose level of 2 mg/kg (s.c.).

Finally, it was found (Table 5) that pretreatments with cannabis extract or with Δ^1-THC were equally effective in producing tolerance to the effect of a challenging injection of Δ^1-THC (10 mg/kg i.p.) on corticosterone plasma

Table 5 Effect of pretreatment with cannabis extract or Δ^1-THC on increases in mouse plasma corticosterone levels caused by immobilization for 2 h or produced 60 min after an injection of Δ^1-THC (10 mg/kg i.p.)

		Pretreatment			*Corticosterone levels*
Experiment	*Experimental conditions*	*Drug*	*Dose (mg/kg s.c.)*	*Treatment*	*(μg/100 ml)*
		Days 1, 3 and 5		*Day 6*	
1	Isolated (20°C)	CAN	500	Δ^1-THC	12.4 ± 3.0*
		Tween 80	200	Δ^1-THC	43.9 ± 4.9
2	Grouped (30-32°C)	CAN	500	Δ^1-THC	15.9 ± 3.2*
		Tween 80	200	Δ^1-THC	47.0 ± 7.3
3		CAN	500	Tween 80	19.2 ± 3.6
		Tween 80	200	Tween 80	20.3 ± 3.0
4		CAN	500	Immobilization	51.6 ± 2.3
		Tween 80	200	Immobilization	57.8 ± 3.9
5		None	—	Immobilization	28.7 ± 6.1*
		None	—	None	7.2 ± 1.9
6		Δ^1-THC	2	Δ^1-THC	28.9 ± 6.3
		Δ^1-THC	10	Δ^1-THC	12.6 ± 2.7*
		Δ^1-THC	30	Δ^1-THC	12.0 ± 2.0*
		**Tween + Δ^1-THC	20 + 10	Δ^1-THC	22.6 ± 2.4*
		Tween 80	200	Δ^1-THC	47.0 ± 7.3

* Shown by Student's *t* test to differ significantly ($P < 0.05$) from the value measured either in untreated mice (experiment 5) or in mice pretreated only with Tween 80 (experiments 1, 2 and 6).

** Tween 80 (20 mg/kg) on days 1 and 3 followed by Δ^1-THC (10 mg/kg) on day 5.

levels when the experiment was carried out on mice housed in separate cages at room temperature as when the experiment was performed under conditions of thermoneutrality. Under both sets of conditions, pretreatment with cannabis (500 mg/kg s.c.) on 3 alternate days completely abolished the response to Δ^1-THC.

Discussion

The results confirm an earlier observation (Kubena *et al.*, 1971) that Δ^1-THC can raise corticosterone plasma levels and also show that the effect is not altered by thermoneutral conditions. However, unlike the work of Kubena *et al.* (1971), the experiments described in this work showed that pretreatment with cannabis or Δ^1-THC can readily produce tolerance to the effect of Δ^1-THC on corticosterone plasma levels. Thus three injections of cannabis or Δ^1-THC made over 5 days abolished the rise in corticosterone levels produced by an intraperitoneal dose of Δ^1-THC 10 mg/kg in mice naive to cannabis. Further, a single injection of Δ^1-THC preceded by two injections of the vehicle, Tween 80, also produced significant tolerance to the effect of a further injection of Δ^1-THC on corticosterone levels in plasma. The pretreatment with three injections of cannabis was one which successfully produced tolerance to the effect of Δ^1-THC on the immobility index, a parameter that measures the 'cataleptic' effect of the drug (Pertwee, 1972).

The possibility that mice tolerant to the effect of Δ^1-THC on corticosterone plasma levels no longer respond to any other stimuli that normally cause the release of corticosterone was ruled out by experiments in which corticosterone levels were raised by the immobilization of mice. The immobilization was equally effective in elevating corticosterone plasma levels in mice that had received a treatment known to produce tolerance to the effect of Δ^1-THC on corticosterone levels as in untreated animals.

The failure of the experiments of Kubena *et al.* (1971) to detect the development of tolerance to the effect of Δ^1-THC on corticosterone levels in rat plasma, must reflect the differences that existed either in experimental procedure or in the species used. A possible cause of the discrepancy emerges when the sensitivity of rats and mice to the effect of Δ^1-THC on corticosterone plasma levels is compared. The dose of Δ^1-THC (8 mg/kg i.p.) given to rats to test for the development of tolerance to the effect of the drug on corticosterone levels is a 'supramaximal' dose in this species, a maximum increase in corticosterone levels being produced by a dose of 4 mg/kg. On the other hand, in mice which appeared to be 5 to 8 times less sensitive than rats to Δ^1-THC, the challenging dose of Δ^1-THC (10 mg/kg i.p.) was 'sub-maximal'. Changes in effect of a given dose will be detected earlier when the effects of submaximal dose levels of a drug are compared, than when supramaximal dose levels are used, and may be missed altogether with the latter. It may therefore be that the effect of pretreatment with Δ^1-THC on its own capacity to elevate corticosterone plasma levels was sufficient to be detected by the use of a submaximal dose in mice but not great enough to be detected by a supramaximal dose in rats.

Chronic activation of the adrenal cortex is known to diminish thymus gland weight and this effect has been exploited to provide an indirect method for detecting rises in plasma levels of corticosterone produced by repeated injections of cannabis. The experiments showed that cannabis extract does indeed cause significant involution of the thymus glands of mice at room temperature. This supports results of experiments in which rats were treated chronically with Δ^1-THC (Ling, Thomas, Usher & Singhal, 1973). It was found, however, that in a thermoneutral environment, this no longer occurred, suggesting that the hypothermic effect of Δ^1-THC and cannabis might be stimulating or facilitating corticosterone release. But it was also found that the effect of these drugs on plasma corticosterone was the same whether the animals were at room temperature or in thermoneutral surroundings. Tolerance to Δ^1-THC was also equally readily produced under the two conditions. These results cast doubt on the reliability of thymus gland weight as an index of corticosterone release.

Finally, of possible relevance to the finding that the effect of cannabis on the thymus gland depends on environmental temperature, is the observation that the acute lethality of cannabis is lower in mice housed in groups at thermoneutral environmental temperatures than in mice housed in separate cages at room temperature.

This work was supported by the Medical Research Council. I am indebted to Dr E.W. Gill for the extraction of Δ^1-tetrahydrocannabinol from the crude resin.

References

DEWEY, W.L., PENG, T-C. & HARRIS, L.S. (1970). The effect of 1-trans-Δ^9-tetrahydrocannabinol on the hypothalamo-hypophyseal-adrenal axis of rats. *Eur. J. Pharm.*, 12, 382-384.

DOUGHERTY, T.F. (1952). Effect of hormones on lymphatic tissue. *Physiol. Rev.*, **32**, 379-401.

DREW, W.G. & SLAGEL, D.E. (1973). Δ^9-THC: selective impairment of corticosterone uptake by limbic structures of the rat. *Neuropharmac.* **12**, 902-914.

GUILLEMIN, R., CLAYTON, G.W., SMITH, J.D. & LIPSCOMB, H.S. (1958). Measurement of free corticosteroids in rat plasma: physiological validation of a method. *Endocrinology.* **63**, 349-358.

HALBERG, F., PETERSON, R.E. & SILBER, R.H. (1959). Phase relations of 24-hour periodicities in blood corticosterone, mitoses in cortical adrenal parenchyma and total body activity. *Endocrinology*, **64**, 222-230.

HERRINGTON, L.P. (1940). The heat regulation of small laboratory animals at various environmental temperatures. *Am. J. Physiol.*, **129**, 123-139.

HOLTZMAN, D., LOVELL, R.A., JAFFE, J.H. & FREEDMAN, D.X. (1969). 1-Δ^9-tetrahydrocannabinol: neurochemical and behavioural effects in the mouse. *Science, N.Y.*, **163**, 1464-1467.

KUBENA, R.K., PERHACH, J.L. & BARRY, H. (1971). Corticosterone elevation mediated centrally by Δ^1-tetrahydrocannabinol in rats. *Eur. J. Pharmac.*, **14**, 89-92.

LING, G.M., THOMAS, J.A., USHER, D.R. & SINGHAL, R.L. (1973). Effects of chronically administered Δ^1-tetrahydrocannabinol on adrenal and gonadal activity of male rats. *Int. J. Clin. Pharm. Ther. Tox.*, **7**, 1-5.

LITCHFIELD, J.T. & WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.*, **96**, 99-113.

PATON, W.D.M. & PERTWEE, R.G. (1972). Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. *Br. J. Pharmac.*, **44**, 250-261.

PATON, W.D.M. & PERTWEE, R.G. (1973). In: *Marijuana: chemistry, pharmacology, metabolism*, ed. Mechoulam, R., Chapter 5. New York: Academic Press.

PERTWEE, R.G. (1972). The ring test: a quantitative method for assessing the 'cataleptic' effect of cannabis in mice. *Br. J. Pharmac.*, **46**, 753-763.

SILBER, R.H. (1966). In: *Methods of Biochemical Analysis*, ed. Glick, D., **14**, 63-78. New York: Wiley.

SOFIA, R.D., NALEPA, S.D., HARAKAL, J.J. & VASSAR, H.B. (1973). Anti-edema and analgesic properties of Δ^9-tetrahydrocannabinol. *J. Pharmac. exp. Ther.*, **186**, 646-655.

(Received January 3, 1974).