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# STEROIDOGENIC AND LIPOLYTIC EFFECTS OF CANNABINOLS IN THE RAT AND THE RABBIT

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**Abstract**—$\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC), $\Delta^8$-tetrahydrocannabinol ($\Delta^8$-THC) and dimethylheptyl-$\Delta^{6a-10a}$-tetrahydrocannabinol (dimethylheptylpyran, DMHP) deplete the adrenals of cholesterol ester and ascorbic acid and increase the plasma concentrations of corticosterone and unesterified fatty acids in intact rats. These effects are similar to those evoked by adrenocorticotropic hormone (ACTH) and are abolished after hypophysectomy. In rabbits, however, the cannabinols increase neither unesterified fatty acids nor plasma cortisol levels, although responsiveness to exogenous ACTH was well established. It is suggested that the apparent discrepancy between the results obtained in the two species might be due (a) to the greater handling stress in the rabbit, and (b) to the fact that cortisol exerts a stronger inhibitory effect than corticosterone on the hypothalamic feed-back mechanism regulating the secretion of ACTH.

Besides its effect on the central nervous system of laboratory animals (e.g. Domino *et al.* [1]), $\Delta^9$-THC has been shown to deplete the adrenals of ascorbic acid in intact rats [2] and to increase plasma corticosterone concentrations in intact but not in hypophysectomized or pentobarbitone- or morphine-treated rats [3]. These effects strongly suggest that, like other centrally active agents [4], the cannabinols stimulate the release of ACTH. In our studies on the mode of action of ACTH (unpublished), the adrenal effect of the hormone is estimated by reference to the increase in the concentration of plasma corticosterone and to the depletion of cholesterol esters as well as ascorbic acid. Lipolysis affords an additional parameter for determining extraadrenal effects of ACTH.

In the experiments reported below, the effects of $\Delta^9$-THC, $\Delta^8$-THC and DMHP on the adrenal content of cholesterol esters and ascorbic acid and on the plasma concentrations of corticosterone and unesterified fatty acids were investigated in rats. They were compared with their effects on the plasma concentration of cortisol and unesterified fatty acids in rabbits.

## MATERIALS AND METHODS

*Chemicals.* The 1-*trans* forms of $\Delta^9$-THC and $\Delta^8$-THC and the racemate of DMHP (formulae Fig. 1) were synthesized in our chemistry laboratories by Dr. W. Bencze. Corticotropin-(1–24)-tetracosapeptide (tetracosactide, Synacthen®, Ciba) [5] was used as an ACTH standard.

*Animals.* The experiments were carried out on male albino rats of the Sprague–Dawley strain (Tierfarm Sisseln, Switzerland) weighing about 200 g. Hypophysectomy was performed by the parapharyngeal route under hexobarbitone sodium (Evipan®, Bayer, Germany) anaesthesia, 24 hr before the experiment. Bilateral adrenalectomy was carried out under ether anaesthesia, 4 days before the experiment. The rats were given 1% saline solution.

Rabbits of no particular breed weighing 1·5–2 kg were used.

*Experimental procedure.* Until the start of the experiments, the rats were kept five to a cage under controlled lighting conditions, and were fed a normal laboratory diet with water *ad lib.* The cannabinols, suspended in saline solution containing 6% Tween 80, were injected intraperitoneally at a dose of 30 mg/kg. The controls received the vehicle only. The rats were killed by a blow on the neck, the utmost care being taken to avoid handling stress, and subsequently exsanguinated. The adrenal glands were removed immediately, separated from adhering fat and kept frozen on dry ice until the determinations. Blood was collected in heparinized tubes and centrifuged to obtain the plasma.

The rabbits first received 10 μg/kg ACTH by subcutaneous injection, then 8 days later an intraperitoneal injection of 30 mg/kg of the cannabinols and after a further 6–8 days a second subcutaneous injection of 10 μg/kg ACTH. Blood was drawn from the veins of the ears into heparinized tubes and spun to separate the plasma. Each of the cannabinols was given to a different group of rabbits.

*Determinations.* The adrenal content of cholesterol was estimated by the method of Schoenheimer and Sperry [6], the quantity of esterified cholesterol being calculated by subtraction of the free from the total cholesterol, which were determined separately. Since the cholesterol content was measured in pooled adrenals from 7 to 10 rats, no standard error can be shown. Plasma corticosterone (rats) and cortisol (rabbits) were determined fluorometrically by the Mattingly [7] technique.

Plasma unesterified fatty acids (FFA) were determined colorimetrically according to Duncombe [8]. Blood glucose concentrations were measured by the glucose-oxidase method.

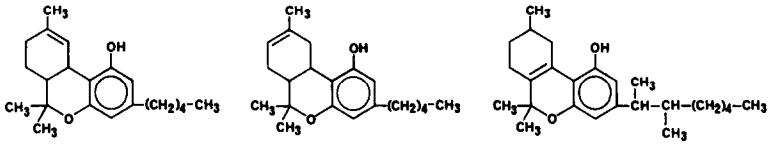


Fig. 1. Structures of the cannabinols tested.

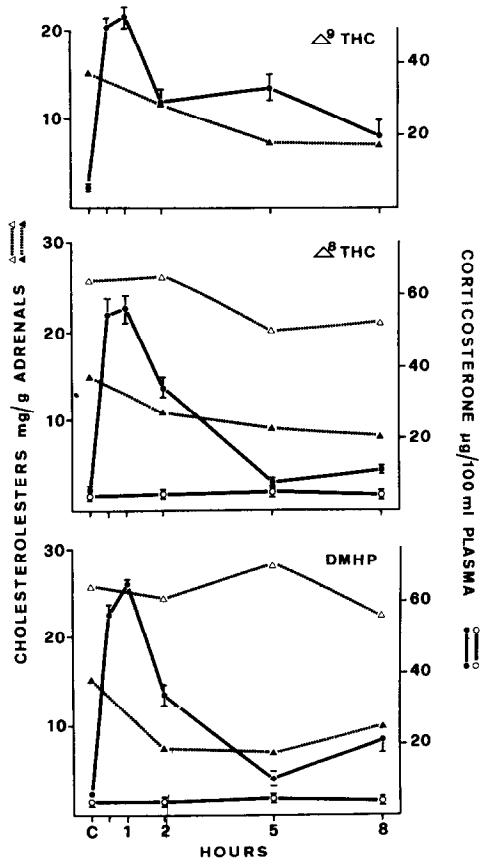


Fig. 2. Time-course of the effects of the three cannabinols (30 mg/kg, i.p.) on adrenal cholesterol-ester content (dotted lines, left ordinate) and plasma corticosterone concentration (solid lines, right ordinate). Closed symbols represent intact rats, open symbols indicate rats hypophysectomized 24 hr previously. C on the abscissa denotes basal values without treatment. Each point represents the mean of 12–40 animals ± S.E.M. Saline-injected controls exhibited corticosterone levels of 3·8, 3·4, 5·2 and 12·4 µg/100 ml plasma after 1, 2, 5 and 8 hr, respectively.

*Statistics.* Standard errors of the mean and the statistical significance of the results according to Student's *t*-test were calculated on a Hewlett-Packard 9100A calculator.

## RESULTS

*Adrenal effects.* The effects of $\Delta^9$-THC, $\Delta^8$-THC and DMHP in rats given an intraperitoneal dose of 30 mg/kg were compared over a period of 8 hr (Fig. 2), which, in our experience, is a suitable length of time for estimating the action of ACTH on the adrenal cholesterol–ester content. All three cannabinols lowered the concentration of cholesterol esters. Maximum depletion was reached between 2 and 5 hr after administration, and from then until at least the 8th hr the cholesterol-ester values remained steady at about half the basal level. The concomitant rise in the plasma concentration of corticosterone induced by all three cannabinols was identical. The time-curves are superimposable for the first 2 hr. Maximum values were reached in ½ hr and persisted for a further ½ hr, after which there was a rapid decline, between 1 and 2 hr after the injection. Five hr after administration, $\Delta^9$-THC still exhibited a considerable degree of activity, similar to that noted after 2 hr. $\Delta^8$-THC and DMHP, however, no longer exerted any corticotropic effect at that time. The small increase between 5 and 8 hr after the injection reflects the diurnal variation in the corticosterone concentration, as is apparent from the values for the saline-treated control rats shown in Table 1.

Ten µg/kg of ACTH provoked a maximum elevation of corticosterone of the same magnitude as that elicited by the cannabinols. The effect of ACTH followed a similar course to that of the cannabinols until one hour after administration, but subsided more quickly thereafter (Table 1). The control values after 30 min were elevated owing to the stress of the injection. In rats hypophysectomized 24 hr previously, the elevated basal content of cholesterol esters was hardly influenced by $\Delta^8$-THC and DMHP; $\Delta^9$-THC was not tested (Fig. 2).

Table 1. Time-course of the effect of ACTH in intact rats

| Treatment | Dose (µg/kg i.v.) | Concns of corticosterone at various times after injection (µg/100 ml plasma) 0 hr | ½ hr | 1 hr | 2 hr | 5 hr | 8 hr | N |
|---|---|---|---|---|---|---|---|---|
| Saline | — | 4·1 ± 0·2 | 25·1 ± 2·3 | 9·2 ± 1·0 | 10·7 ± 1·3 | 7·1 ± 0·9 | 13·8 ± 3·0 | 24 |
| ACTH | 10 | — | 59·1 ± 1·6 | 57·1 ± 2·4 | 10·0 ± 0·9 | — | — | 12 |

Results are mean ± S.E.M.

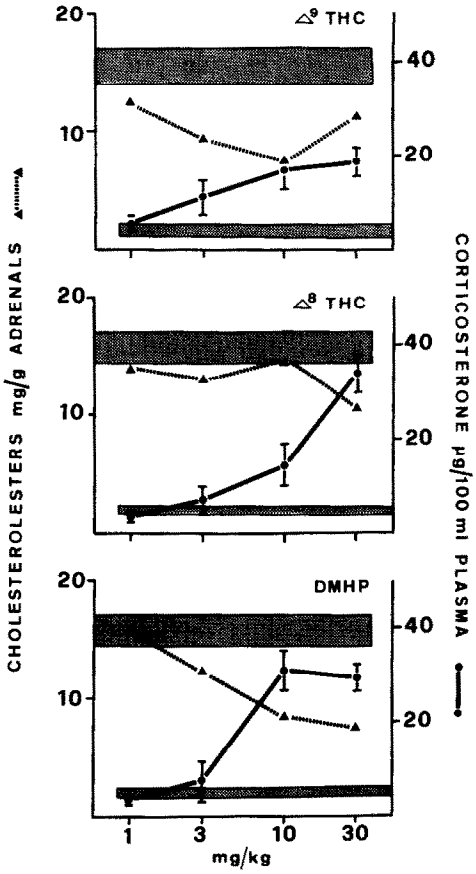


Fig. 3. Dose–response relation of the three cannabinols for adrenal cholesterol-ester content (dotted line, left ordinate) and plasma corticosterone concentration (solid line, right ordinate) in intact rats. The doses indicated on the abscissa in mg per kg body wt were administered intraperitoneally 2 hr before sacrifice. The shaded areas represent basal values for adrenal cholesterol esters (upper) and for plasma corticosterone (lower) concentrations. Each point represents the mean of 10 animals ± S.E.M.; both parameters were measured in the same rats.

The dose–response relations of $\Delta^9$-THC, $\Delta^8$-THC and DMHP are shown in Fig. 3. All three cannabinols elicited a dose-dependent increase in plasma corticosterone concentrations. The slopes of the dose–response curves, however, were quite different. $\Delta^9$-THC gave a rather flat, almost linear curve, while the effect of $\Delta^8$-THC increased markedly at the 30 mg/kg dose-level. DMHP exhibited an effect similar to that of $\Delta^8$-THC, but the maximum response was elicited by a dose three times smaller. The dose–response curve for DMHP showed a plateau between 10 and 30 mg/kg.

Judging from the extent to which the adrenal cholesterol-ester content was reduced, $\Delta^9$-THC was about 3 times more potent than DMHP and about 10 times more potent than $\Delta^8$-THC at threshold doses.

The ascorbic-acid content of the rat adrenals was decreased to a similar extent by the cannabinols and by 10 µg/kg ACTH, as is shown in Fig. 4. The effect was rapid in onset and sustained.

In rabbits, the plasma cortisol concentrations (Fig. 5) remained unchanged over a period of 5 hr after

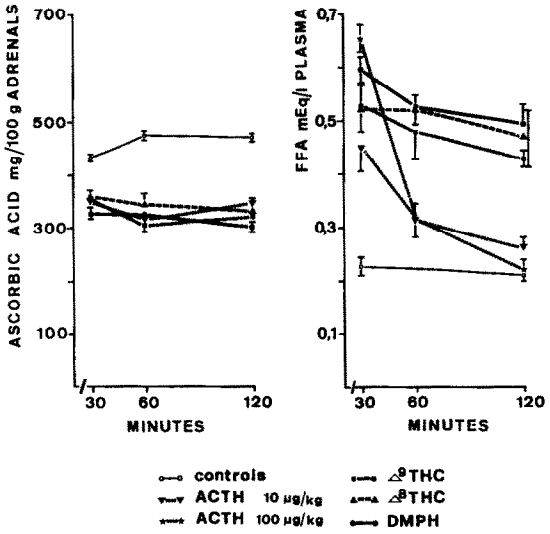


Fig. 4. Effect of cannabinols (30 mg/kg i.p.) and ACTH (i.v.) on adrenal content of ascorbic acid (left panel) and on plasma free fatty acids in intact rats. Controls were treated with the vehicle only. Each plot in the left graph is the mean of 6 rats and in the right graph of 14–21 rats ± S.E.M. Basal values in untreated rats ranged from 450 to 520 mg/100 g adrenal tissue for ascrobic acid and from 200 to 250 µEq/l. plasma for fatty acids.

the injection of all three cannabinols at a dose of 30 mg/kg. ACTH (10 µg/kg), however, elicited a marked rise in the cortisol concentration, which reached its maximum 1 hr after administration and persisted at that level for more than 2 hr. The high initial values reflect handling stress. The blood was drawn from conscious animals, which were held in narrow boxes. The basal values (n = 22 from 12 rabbits) ranged from 14·9 to 41·9 µg cortisol per 100 ml plasma, the mean being 28·0 ± 1·7 µg/ml.

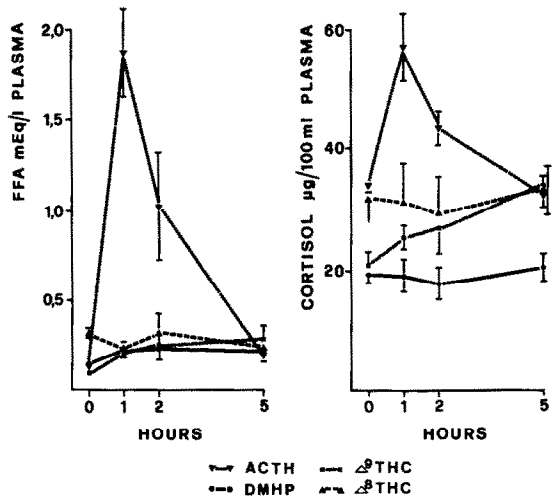


Fig. 5. Effect of cannabinols (30 mg/kg i.p.) and ACTH (10 µg/kg s.c.) on plasma concentrations of free fatty acids (left panel) and cortisol (right panel) in intact rabbits. Each curve represents the mean of 4–6 rabbits ± S.E.M. Both FFA and cortisol were determined in the blood of the same rabbits.

Table 2. Time-course of the effect of $\Delta^8$-THC in adrenalectomized and sham-operated rats

| Treatment | Dose (mg/kg i.p.) | Corticosterone (μg/ml plasma) 0 hr | ½ hr | 1 hr | 2 hr | Unesterified fatty acids (μEq/l. plasma) 0 hr | ½ hr | 1 hr | 2 hr | N |
|---|---|---|---|---|---|---|---|---|---|---|
| Adrenalectomized | | | | | | | | | | |
| Controls | | 2·9 ± 0·3 | 2·7 ± 0·5 | 2·5 ± 0·3 | 1·8 ± 0·4 | 114 ± 19 | 269 ± 8 | 121 ± 10 | 152 ± 14 | 7 |
| $\Delta^8$-THC | 30 | | 2·5 ± 0·3 NS | 1·8 ± 0·2 NS | 2·5 ± 0·2 NS | | 268 ± 7 NS | 255 ± 38‡ | 197 ± 15* | 7 |
| Sham-operated | | | | | | | | | | |
| Controls | | 4·1 ± 0·2 | 18·9 ± 3·9 | 4·6 ± 1·9 | 3·8 ± 0·7 | 116 ± 12 | 227 ± 29 | 103 ± 7 | 94 ± 5 | 7 |
| $\Delta^8$-THC | 30 | | 42·0 ± 1·2‡ | 42·9 ± 1·3‡ | 22·1 ± 4·5† | | 367 ± 39* | 259 ± 22‡ | 215 ± 26‡ | 7 |

Controls were injected i.p. with NaCl–Tween (1:1) solution.
NS, not significant.
* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

*Extra-adrenal effects.* The lipolytic effects of $\Delta^9$-THC, $\Delta^8$-THC and DMHP (30 mg/kg, i.p.) were compared with that of 10 and 100 μg/kg ACTH administered intravenously to intact rats (Fig. 4). The three cannabinols exhibited identical lipolytic activity. All three cannabinols and ACTH elicited maximum effects within 30 min of administration, those of the cannabinols corresponding to that of ACTH in a dose between 10 and 100 μg/kg. Whereas the effects of both doses of ACTH subsided within little more than 60 min, the effects of the cannabinols were much more persistent: 2 hr after the administration of all three cannabinols plasma FFA concentrations were still significantly elevated.

In adrenalectomized rats, an intraperitoneal challenge of 30 mg/kg $\Delta^8$-THC administered 4 days postoperatively elicited an increase in plasma unesterified fatty acids. The values measured 1 and 2 hr after administration were significant (Table 2). In sham-operated rats, the effect of the challenge on fatty acids was slightly more pronounced and the corticosterone content increased to higher values (Table 2) than in intact animals (Fig. 2).

In rabbits, plasma FFA concentrations (Fig. 5) showed no change over a period of 5 hr after the injection of 30 mg/kg of all three cannabinols. Ten μg/kg ACTH, however, provoked a very marked rise in the FFA concentration, reaching a maximum after one hour and lasting more than 2 hr. This effect was similar to the influence of ACTH on plasma cortisol levels. ACTH was administered before and after the injection of the cannabinols, at intervals of 1 week. Both administrations of ACTH yielded similar responses.

*Blood glucose.* In two experiments in which the effects of the three cannabinols on the adrenal gland were estimated, blood glucose concentrations were also determined. No change was observed up to 8 hr after administration (Table 3).

Table 3. Effects of cannabinols (30 mg/kg, i.p.) on blood glucose concentration in intact rats

| Treatment | Blood glucose (mg/100 ml) 2 hr | 5 hr | 8 hr |
|---|---|---|---|
| Vehicle | 97 ± 3 | 105 ± 2 | 101 ± 1 |
| $\Delta^9$-THC | 99 ± 2 | 110 ± 3 | — |
| $\Delta^8$-THC | 99 ± 2 | 100 ± 3 | 96 ± 3 |
| DMHP | 93 ± 2 | 101 ± 2 | 97 ± 2 |

Each value represents the mean of six determinations ± S.E.M.

## DISCUSSION

Our results show that the three cannabinols tested elicit marked adrenal and extra-adrenal effects in intact rats. So far, several adrenal effects of $\Delta^9$-THC have been observed in this species. Dewey *et al.* [2] reported depletion of ascorbic acid and Kubena *et al.* [3] noted an increase in the plasma corticosterone concentration, which was abolished after hypophysectomy.

Our findings confirm these observations and enlarge upon them in as much as further parameters and additional cannabinols were investigated. In general, both $\Delta^8$-THC and DMHP produced quantitatively similar effects to those of $\Delta^9$-THC. DMHP tended to be more potent in its activity than the other two cannabinols, as is evidenced by its effects on plasma corticosterone concentrations (Figs. 2 and 3). This greater potency is in keeping with its effects on catecholamine-accumulation in certain regions of the brain [9] or with the inhibition of the conditioned-avoidance response it evokes [9]. Its lipolytic activity, however, was similar to that of $\Delta^9$-THC and $\Delta^8$-THC (Fig. 4).

As all three cannabinols increased plasma corticosterone concentrations, it is evident that the adrenal cholesterol-ester content decreased concomitantly. Since both these effects and also the depletion of adrenal ascorbic acid are known consequences of ACTH, it is reasonable to assume that the cannabinols stimulate the release of ACTH. Our data do not permit any definitive conclusions to be drawn as to whether this stimulation takes place in the pituitary or the hypothalamus. The findings of Maître *et al.* [9] appear to suggest involvement of the hypothalamus, since it was this region of the brain that was found to be most affected by the cannabinols. With regard to extra-adrenal activity of the cannabinols, to the best of our knowledge no results have yet been published concerning their effects on plasma FFA concentrations in experimental animals; in man, however, no change was found [10, 11].

In our experiments, all three cannabinols elicited a sustained elevation of plasma FFA to between 2 and 3 times the basal values (Fig. 4). The effect deviated considerably from that of a single injection

of ACTH, implying that the cannabinols give rise to a prolonged stimulation of ACTH release. Since corticosterone is known to elicit lipolysis through inhibition of re-esterification of the hydrolyzed fatty acids before they leave adipose tissue [12], an experiment with adrenalectomized rats was performed (Table 2). The results showed that lipolysis was indeed a direct effect of endogenous ACTH released by cannabinols rather than a result of elevated corticoid levels.

In rabbits Paton *et al.* [13] found no significant effect on resting blood glucose levels or glucose tolerance after treatment with 50 mg/kg $\Delta^9$-THC daily over a period of 60 days. This is consistent with our results, which revealed no change within 8 hr of the administration of any of the three cannabinols (Table 2). In man much attention has been focused on blood glucose, since cannabinols are known to elicit a sensation of hunger some hours after ingestion [14]; in rats similar observations have been made [15]. Hollister [10] found no change in glucose levels, and Podolsky [16] reported similar results with regard to fasting hypoglycaemia, but the glucose tolerance curve reached higher maximum levels that persisted for the same length of time after marihuana had been smoked for 7 days as those observed during a similar period of abstinence in the same subjects. Sprague *et al.* [17] reported that $\Delta^9$-THC depleted liver glycogen by up to 75% in monkeys, rabbits and rats, without causing any change in blood glucose concentrations. Assuming that no change in the turnover of blood glucose had occurred, the authors suggested that the glucose made available from the glycogen stores was utilized to cover the energy expended in metabolizing the cannabinol in the liver.

Although it seems likely that the cannabinols act by stimulating the release of ACTH from the pituitary, they elicit longer-lasting effects than the intravenous injection of high doses of ACTH. This is particularly clearly evident from their effect on plasma FFA concentrations in the rat. The different route of administration, the difference in solubility between the cannabinols and the ACTH peptide and the considerably shorter half-life of the latter may account for this disparity.

In our experiment on rabbits, the cannabinols showed little or no lipolytic and steroidogenic activity (Fig. 5). This is in contrast to our findings in rats, but in comparing the two species it must be borne in mind that there are profound endocrine differences between them. The principal glucocorticoid secreted by the rat is corticosterone, whereas in the rabbit it is cortisol.

It has been reported [18, 19] that cortisol is a more potent inhibitor of the hypothalamus than corticosterone, acting preferentially on the so-called fast feedback and blocking the immediate excretion of ACTH.

Stress induced by handling is much less pronounced in rats than in rabbits. In the rat the stressful effects of an injection subside within $\frac{1}{2}$ hr and do not interfere with later measurements. In rabbits, repeated handling or confinement in a restraint box, as is necessary when blood samples have to be withdrawn hourly in the conscious state, causes prolonged stress and elicits a high level of endogenous ACTH and consequently a high cortisol level (cf. control values). Thus the stores in both the pituitary and the adrenal gland are more exhausted in the rabbit than in the rat, and as a result the drug effect becomes marginal.

In our view the strong feed-back inhibition of cortisol and the influence of handling stress are more plausible explanations for the unresponsiveness of the rabbits than decreased absorption of the drugs from the peritoneum. Impaired absorption is unlikely since all the rabbits showed marked central effects after treatment with all three cannabinols, and they responded normally to an injection of ACTH.

It is interesting to note that no increase in plasma cortisol concentrations was observed in habitual users of cannabis. When drawing comparisons between the results of these animal experiments and the effects of the substances in man, however, it must be borne in mind that the animals were treated with a single dose whereas in man the use of the drugs is generally chronic.

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