PRECLINICAL STUDIES IN ANIMALS

The following section summarizes a wide range of animal investigations designed to learn some of the implications of cannabis administration in a variety of animal species. It is included primarily for the technically sophisticated reader as a summary of the present state of marihuana preclinical investigation. It should be emphasized that such research may have no immediate relevance to human use of marihuana, and that it could be a serious error to translate these findings directly to the human case. High dose levels are frequently employed in animals to learn the limits of toxicity (not possible in human experimentation). Moreover, the methods of drug administration (and form of the drug) are often markedly different from the usual ways in which marihuana is used by people and may have different implications. Nevertheless animal work is essential to a more sophisticated understanding of the action of the drug and to developing useful clues to fruitful lines of investigation in man. Where specific findings appear to have direct relevance to human use of marihuana, an attempt has been made to interpret this in the summary or in other relevant sections of the report.

Prior to 1968, research on the effects of cannabinoids in animals was carried out with cannabis extracts prepared by the investigator himself and such preparations frequently lacked definite analysis of their active components. This has made it difficult to correlate physiological effects with chemical composition in the earlier studies. Recently, the availability of pure Delta-9 and Delta-8-THC has spurred research in the pharmacological area and so far, pharmacologic effects of the tetrahydrocannabinols seem to generally replicate, at least qualitatively, those of the cannabis extracts.

TOXICITY STUDIES

Single dose toxicity studies in rats indicate that the lethal dose in 50% of animals, i.e., the L1),0 for Delta-9-THC, is between 20-40 mg/kg by intravenous injection, and between 800-1400 mg/kg orally, depending on sex and species (66,59). Animals receiving these compounds at these very high dose levels die in respiratory arrest. Postmortem studies done after treatment with marihuana derivatives revealed pulmonary edema with hemorrhage (49).

Tolerance to cannabis action has been reported in a number of animal species (rats, dogs) and in birds (pigeons) (52). In 1968, using behavioral methods (rope climbing, operant behavior), Carlini showed that seven out of ten rats became tolerant after fifteen chronic, intraperitoneal injections of cannabis extract (20). However, there was no cross-tolerance between cannabis
extract or Delta-9-THC and LSD-25 or mescaline sulfate. This seems to indicate that tolerance to cannabis must involve a different mechanism from that of LSD or mescaline, since cross-tolerance has been established for the two latter drugs. Among the cannabinoids, cross-tolerance has been found between Delta-8- and Delta-9-THC (53), between the cannabis analogue, synhexyl and Delta-9-T1-IC and between the dimethylheptyl analogue and Delta-9-THC (9). Tolerance has been found in dogs but not in rabbits (48). Tolerance to Delta-9-THC in dogs can be blocked by prior administration of an enzyme inhibitor, such as SKF 525A. Thus, research on comparative metabolism between different species and enzyme induction studies may provide a clue to these species differences.

CENTRAL NERVOUS SYSTEM EFFECTS

Reports on the effects on the brain and the nervous system, sketchy at first, are now the subjects of various investigations. (Effects on the electroencephalogram are reported under Neurophysiological Effects.)

In animals, analgesia has been the most frequently used parameter of cannabis effects. Bicher and Mechoulam (8) have assessed this effect in mice for both Delta-8 and Delta-9-THC, by the hot plate and tail flick tests. Twenty milligrams per kilogram of either isomer, intraperitoneally, was found to be equivalent to 10 mg of morphine given subcutaneously, and the analygesic effect of the tetrahydrocannabinol lasted at least two hours. Others have reported that Delta-9-THC is a more potent analgesic agent than Delta-8-THC (41). A combination of morphine (2.1 mg/kg) and THC (1.25-5.0 mg/kg) was found to possess additive analgesic effects (19). It was also noted that drugs which decrease serotomn brain levels do not modify THC analgesia.

In 1965, Carlini and Carlini (23) compared the effects of cannabis extract (10 or 100 mg/kg) i.p., and strychnine on the content of RNA and DNA in the rat brain. Cannabis had no effect on RNA content of rat brain but significantly increased DNA content in a dose related manner (12% and 82%, respectively). Changes in DNA content may be involved in the short term memory deficits reported in humans.

In terms of effects on biogenic amines, cannabis resin was found to increase serotonin brain levels in mice (43) and rats (12). Norepinephrine in mice, 24 hours after i.p. injection, was found to be decreased by 5-10 mg/kg Delta-9-THC, but significantly increased by doses of 200-500 mg/kg. These changes in biogenic amines may be due to a direct central effect or the result of
AUTONOMIC AND CARDIOVASCULAR EFFECTS

Hashish resin extract had been reported (12, 13) to antagonize acetylcholine induced contractions of rat uterus and intestine in a dose related manner. In the same experiments, serotonin activity was also antagonized.

Reports of the effects of cannabis on the adrenergic system are controversial. Some (12) report that cannabis resin antagonized adrenergic effects such as the pressor response to occlusion of the carotid artery in dogs, the positive inotropic effects of epinephrine and nor-epinephrine in isolated frog heart and the action of epinephrine in the rabbit duodenum. This inhibition of the pressor response is not due to ganglionic blockade (26). Others have found that both Delta-8 and 1)elta-9-THC potentiate all parameters of norepinephrine and epinephrine and that Delta-8-THC is more potent in reversing reserpine induced blepharoptosis in mice. Cannabis resin also antagonizes the spasmogenic actions of carbachol, histamine, barium chloride and pitocm on rat and guinea pig intestines by a direct musculotropic effect (13), Dewey, et al. (26) confirmed this inhibition in isolated guinea pig ileum, remarking that the Delta-9-THC block is reversible, while Delta-8-THC is not. They found the Delta-9 isomer nearly twice as potent as Delta-8-THC in inhibiting GI propulsion in mice in vivo.

Marihuana compounds (Delta-8 and Delta-9-THC) produce a gradual prolonged fall in blood pressure (34, 27), but the synthetic analogues such as the dimethylheptyl may be more potent in this respect (24, 25). This hypotensive effect is not dependent on an intact vagus nerves not diminished by atropine, dibenamine or hexamethonium and is not due to ganglionic blockade (27). This effect can be abolished by spinal section at C-1, at least with the 1,2 dimethylheptyl derivative of TUC (40). The cardiovascular responses to direct stimulation of the hypothalamus and medullary vasomotor areas are not blocked by this compound, so it is postulated that this hypotension results from decreased central sympathetic outflow.

In the isolated, perfused rat heart Manno, et al. (50) have found that, as the dose of Delta-9-THC is increased, perfusion pressure is increased ncreased (vasoconstriction) but the force of contraction is decreased. For both of these effects, no definitive dose-response relationship could be defined.
PRECLINICAL STUDIES IN ANIMALS

EFFECT ON RESPIRATION

Cannabis usually depresses respiration rate, at least in moderate to high doses (33, 34), although stimulation has also been reported (13). As mentioned earlier, toxic doses produce breathing impairment.

HYPOTHERMIC EFFECT

Doses of Delta-9-THC greater than 1 mg/kg have been found to consistently produce hypothermia in mice, and 500 mg/kg lowered the body temperature by 5-6 degrees within 10 minutes after i.p. administration. This effect usually lasted 24 hours (43). Marked hypothermia was also observed after intercerebral administration of cannabis extract (33).

HORMONAL EFFECTS

In rats, cannabis extract (250 mg/kg, i.p.) given prior to injection of V" in rats, significantly depressed thyroidal uptake of the radio-iodine (54).

The effect of cannabis on blood sugar is not established. Miras found biphasic fluctuation of blood sugar within normal limits, but El Sourogy (28) using an extract of cannabis, found a significant increase in blood glucose, while liver glycogen was decreased and muscle glycogen remained normal, suggesting potentiation of glycogenolysis. Unfortunately, there was no mention of control animals receiving injections, so the possibility remains that a stress response was being measured.

Barry, et al, (6) have found activation of pituitary-adrenal function in rats following 4-16 mg/kg i.p., Delta-9-THC, probably resulting from a central nervous mechanism for hypersecretion of corticotropin, since corticosterone levels sometimes triple following THC but do not after hypophysectomy, pentobarbital or morphine. Inhibition of antidiuretic hormone was also indicated in view of the doubled urine output; these two effects also occur following alcohol intoxication.
ANTIBIOTIC ACTIVITY

Cannabis preparations have long been known to possess antibiotic activity against gram positive bacteria in vitro or in topical administration; recently, this activity has been narrowed down to the cannabidiol fraction of the plant (60).

INTERACTION WITH OTHER DRUGS: BIOCHEMICAL STUDIES

So far, few authors have reported on the interaction of cannabis with other drugs. The other interaction which has been well reported is the interaction with barbiturates as those compounds were used to determine the effects of THC on the central nervous system. Natural (extracted), synthetic THC and synhexyl (a synthetic analogue) have been shown to potentiate hexobarbital and barbital sleeping time (35, 46). However, the mechanism of this potentiation, possibly through enzyme induction, is still debated. The results of Truitt showing decreased sleeping time in mice when the animals were pre-treated twice daily for three days with Delta-8-THC (3-30 mg/kg., i.p.) then given 65 mg/kg pentobarbital, seem to support the enzyme induction theory (NIMII contract PH-43-68-1338), but others question it.

Potentiation of amphetamine has been noticed after administration of cannabis compounds both acutely (one hour post injection) and chronically (three days after) (35). Delta-9-THC, 16 mg/kg, enhanced the stimulant effect of amphetamine, 4 mg/kg, but was found to protect some subjects from a toxic methamphetamine dose.

In an in vitro study (26), Delta-8 and Delta-9-THC have been found to cause some inhibition of the metabolism of aminopyrine and ethyl-morphine in rat liver homogenates. This was not found in vivo.

NEUROPHYSIOLOGICAL EFFECTS

Cannabis has long been suspected of having tranquilizing properties. In evaluating this potential, Salustiano, et al (61) used chlorpromazine as a standard of comparison for cannabis extract. Cannabis extract was found to be twice as active as chlorpromazine in decreasing
isolation-induced aggressive behavior in mice, but was much less efficient in protecting the mice from d-amphetamine toxicity.

Sampaio (62) has observed that extract of cannabis, THC and synbexyl abolishes the linguomandibular reflex in the dog even after atropine administration. Chlorpromazine produces the same effects in a comparable dose range and the effect is abolished by administration of strychnine. In search of the mechanism of this action, the same group (47) found that THC depresses the presynaptic potential in the trigeminal nerve, while the tibialis nerve was unaffected, suggesting a specific central depressant action. Others, using the synthetic analogue dimethylheptyl and neurophysiologic methods (16) have also observed this depression in cats and localized the effect to the forebrain area, as facilitation of the linguomandibular reflex resulting from forebrain stimulation was also depressed. They also found that dimethylheptyl occasionally depressed the monosynaptic myotatic reflex and depressed lower motor neurons, thus resembling the effects of thiopental, only more inconsistently. Boyd and Meritt have also observed that 0.2 mg/kg dimethylheptyl is equivalent to 2 mg/kg thiopental in raising the threshold for both EEG and behavioral arousal by action on the ascending reticular formation (17).

In animals, the cannabinoids produce definite changes in the electroencephalograms (EEG) after acute and chronic administration. However, dosage levels used in animal studies are usually higher than those administered in humans.

Bose (14) found that 15-30 mg/kg, i.p., of cannabis extract initially increased frequency in the rabbit's frontal cortex indicating stimulation while the parietal area was depressed; one hour after administration, both areas were depressed. Recovery was characterized by appearance of sharp waves and gradually increasing voltage suggesting increased excitability of neurons. Lipparini, et al (48) showed that, in animals with chronically implanted electrodes, 0.5-1 mg/kg IV Delta-8 or Delta-9-THC will abolish theta waves in the rabbit hippocampus, flatten the EEG and give rise to traces of high voltage spike and waves. However, increasing the dose to 10 mg/kg did not produce grand mal EEG tracings but only increased stupor. Racemic Delta-8-THC (less than 6 mg/kg) produced no EEG or behavioral changes or corneal anesthesia.

Bicher and Meadulam (8) also found changed cortical activity as evidenced by strong beta rhythm in the electrocorticogram (ECoG) in rabbits following Delta-8 or Delta-9-THC (8 mg/kg IV) treatment. The cortical arousal threshold was lowered and the length of ECoG morphine action, which produces a decrease in frequency in EEG and an elevated threshold of arousal response, can be differentiated from cannabis.
Similar effects were also reported by Boyd and Merritt (17) for the dimethylheptyl synthetic derivative. Studies with cats (6 mg/kg of Delta-9-THC i.p.) produced only moderate synchronization of EEG, which was easily interrupted by external stimuli.

In doses greater than 5 mg/kg, IV, Lipparinit et al. (48) showed corneal arreflexia, marked motor deficit, synchronization of EEG and insensitivity to external stimuli after 1-cannabinol. This is surprising as cannabidiol had previously been reported as being physiologically and pharmacologically inactive. Its effects, however, differ from those of Delta-8 and Delta-9-TIIC in that spike and wave EEG pattern and diminution of voltage are not seen. These authors suggest that flattening of EEG tracings, disappearance of hippocampal theta waves, and spike and wave configuration of the EEG could replace conical arreflexia as a specific bioassay of THC activity. They also suggest that synthetic derivatives of Delta-8-TIC such as the methyl anedimethyl, which are 5-10 times more potent than Delta-8-TIC and show the same spectrum of activity in rabbits and cats as Delta-8 and Delta-9-THC, will have cannabis activity in man.

Barratt, et al. (4) have noticed, in preliminary experiments, EEG changes in cats treated chronically (i.p. 16 mg/kg/day) or by inhalation with a marihuana extract. After 10-12 days, slow waves with spiking appeared in baseline recordings. Treatment was continued for a total of 23 days and the abnormal baseline persisted 22 days following end of drug administration. At lower doses (2 mg/kg) abnormal EEG tracings did not appear until the 25th day. Behaviorally, these cats eventually became less playful and more withdrawn; normal behavior returned 3 days following the end of treatment. Seizures of any nature were not apparent. Chronic, high dose administration of Delta-9-THC has been found to reduce paradoxical sleep in rats.

Fenimore, et al. (29), using autoradiography methods with tritium labeled Delta-9-THC, showed distribution of Delta-9-THC in various cortical and subcortical structures of the monkey brain. Relatively high accumulations were found in the lateral geniculate nuclei at a time when the animal would appear to be hallucinating. Similarly high concentrations were discovered in the amygdala' hippocampus, inferior and superior colliculi at the time of behavioral effects and marked amounts in the cerebellum corresponded well with the monkey's motor incoordination. It thus appears that behavioral effects may be related to increasing concentration of cannabinoids in specific brain areas.

**BEHAVIORAL EFFECTS**
The effects of cannabis are behaviorally both dose and species related but setting can also be a factor. Barry and Kubena (5) have demonstrated that rats show increase and/or decrease of spontaneous activity following Delta-9-THC, given intraperitoneally. They found that low doses (4 mg/kg) produced initial excitation followed by depression; the excitation could be exacerbated by using laboratory naïve and nonacclimated rats and could be abolished by a higher dose (16 mg/kg). Rats' behavior with Delta-8 or Delta-9-THC has also been studied by Grtrunfeld and Edery (39). Following a 20 mg/kg i.e. injection with these compounds, rats have been observed to be ataxic, cateleptoid and flaccid. This dose disrupts learned behavior but reactions to unconditioned stimuli remain intact. Vieira, et al. (68) have suppressed a conditioned avoidance response in mice and rats with 125 mg/kg, i.p. extract. Mice show a similar response accompanied by partial ptosis and piloerection. Irwin (45) found mydriasis in racemic Delta-8-THC treated mice and miosis after Delta-9-THC in the same species. The minimal oral dose for behavioral effects with Delta8-THC in mice and cats was low (0.1 mg/kg) and peak effect was 2 hr. post-administration. Mice also exhibit decreased performance in the rotating rod test when given 10 mg Delta-9-THC, i.p.; no effect, however, could be elicited following subcutaneous injection.

The effects on social behavior in animals were studied by Carlini, et al (22). They found that chronic administration of cannabis extract, 10 mg/kg, i.p., could evoke fighting behavior in rats only with starvation as part of the regimen. On the other hand, a single dose of cannabis extract (10 mg/kg) has been shown by Santos (63) to decrease aggression in mice by 80% while motor activity remained unchanged; this response was demonstrated for fighter and non-fighter mice and the effect lasted nearly 7 hours. This decrease in aggressiveness has been compared by Garattini (33) to the effects of chlordiazepoxide. Siegel and Poole (65) have confirmed this effect and also noticed less group aggregation and temporary disruption of social hierarchies in a mice community. Synhexyl (15 mg/kg, i.p.) in operant behavior tests, has been shown to increase curiosity in the rat by Abel and Schiff (2) and also to decrease food, but not water consumption. They also showed disruption of the suppressive effect in a conditioned emotional response situation (1).

Carlini and Kramer (21) observed improved maze performance by rats given 10 mg/kg, i.p., cannabis extract prior to testing. However, post-trial administration produced no effect or disruption of activity, thus distinguishing cannabis from other CNS stimulants (strychnine or picrotoxin) which improve maze performance when given pre- or post-trial. Higher doses of cannabis were explored but, at these doses, motor activity was impaired.

Boyd, et al (15) also studied the effects of synthetic tetrahydrocannabinols (DMHP) in the rat in various operant behavior tests using positive food reinforcers at various dose levels. These compounds were found to depress all measures of behavior except on a mixed schedule, where they appeared to increase the ability of the animal to judge elapsed time; general performance
PRECLINICAL STUDIES IN ANIMALS

on fixed ratio schedules was found more sensitive to these drugs than on fixed intervals. The overall effects were similar to that of pentobarbital. Sheckel, et al (64) report that monkeys receiving racemic Delta-9-THC (32 or 64 mg/kg, i.p.) exhibit initial excitation, including fine hand tremors, pamelike states, hallucinatory activity and unusual limb positions. These signs lasted three hours and were followed by depression; nine subjects died after the high dose treatment. This study also revealed that racemic Delta-9-THC (4 or 8 m/kg) reduced response rate by 50% in a continuance avoidance schedule, whereas 16-64 mg/kg increased responding 200%. Effects of the Delta-8 were different from those of the Delta-9. Delta-8-THC increased lever responding in the lower doses (2, 4 and 8 mg/kg) but the higher doses did not cause the depression or death seen with Delta-9. The monkeys also seemed to lack ability or motivation to perform complex tasks. Francois’ et al (31) have confirmed this behavioral spectrum in monkeys and also report consistent vomiting after 8 mg/kg i.p. of Marihuana Extract Distillate (MED). The social dominance hierarchy was not changed by the drug, but expressions of dominance were changed, that is, the monkeys were less aggressive.

The general behavior of dogs is not unlike that of other animals previously studied, but excessive salivation, retching, vomiting and overt ataxia seem specific for dogs. This typical ataxia has since been used for a bioassay of cannabis action as well as the corneal arreflexia M rabbits (36). In pigeons Frankenheim, et al. (32) found that both Delta-9- (0.3-3.0 mg/kg) and Delta -8-THC (3-10 mg/kg) given intramuscularly caused a dose dependent decrease in the rate of key pecking in a multiple operant behavior schedule. The Delta-9 isomer was found to be more than twice as potent as Delta-8—TIIC and tolerance was found after seven days of chronic administration.

TERATOLOGY

One of the pertinent questions regarding marihuana use in the population concerns the effects of repeated usage during pregnancy.

The experimental evidence reported so far has been contradictory. Once more, results seem to vary with species, mode of administration and doses used.

Miras (54) found that rats impregnated after being fed a diet containing 0.2% marihuana extract for several months showed a reduced fertility but the offspring produced were normal. In
another study, pregnant mice injected intraperitoneally (i.p.) with 16 mg/kg of Cannabis resin on
day six of gestation produced offspring which were stunted but not malformed. The same dose
given on days 1-6 caused complete fetal resorption (57). In a second experiment using rats, the
injection of 4.2 mg/kg of cannabis extract on days 1-6 resulted in a high frequency of
malformed progeny (58). Congenital malformations and abnormal fluid accumulations were also
observed in fetal hamsters and rabbits after prenatal administration of large, multiple doses of
marihuana resin (100-500 mg/kg), the teratogenicity being influenced by plant origin and
seasonal variations (37, 38).

However, Borgen, et al. (10) administering the pure Delta-9-THC subcutaneously, to female rats
in doses of 0.01 to 200 mg/kg as a solution in olive oil from day 1-20 of gestation did not find
congenital abnormalities or stunting of offspring. However, average litter size was, however,
reduced by doses of 100 to 200 mg/kg. At doses of 10 mg/kg and above, maternal weight gain
during pregnancy was diminished and length of gestation was increased by 1-2 days. Doses
above 25 mg/kg caused a marked postnatal mortality of pups ap- parently due to inadequate
maternal lactation. Females sacri- ficed on day 21 after 100 and 200 mg/kg dosages showed
increases in the size of the adrenals, thyroid, and heart, while the mass of liver was reduced.
Thus in contrast to published re- search with marihuana extracts, Delta-9-THC does not appear
to be teratogenic in rats in doses up to 200 mg/kg given throughout gestation. The major effects
noted were on the female rather than the progeny, and these appeared only with higher
dosages.

This lack of teratogenic effect cannot be the result of a lack of penetration of Delta-9-THC
through the placental barrier as IdampaanHeikkila,, et al. (44) found that 15 minutes post i.p.
administration, Delta-9-THC-H' crossed the placenta and peak concentration was achieved 30
minutes after the administration in the hamster. Fetuses from animals injected early in
pregnancy contained nearly three times more radioactivity than fetuses from animals injected at
a later time in pregnancy; this difference was even more apparent after subcutaneous
administration. The placenta was shown to contain more radioactive label than the fetus by
either route and the fetus contained more label than maternal plasma or brain.

A few investigators have studied the cytogenic effects of marihuana and so far no observable
chromosomal changes have been found (55, 51).

METABOLISM

With the availability of Tritium and Carbon-14 radioactive. labeled Delta-8 and Delta-9-THCs
last year, major advances in the study of the metabolism of these compounds took place. These
studies showed that the cannabinoids disappear rapidly from the blood and metabolism occurs mostly through the liver of the species studied: mice, rats and rabbits. So far, metabolism is mainly an hydroxylation process (3, 7, 18, 30, 56, 67, 69) and the 11-hydroxy metabolites of Delta-8 and Delta-9-THC have been reported to have the same pharmacological profiles as the parent compounds (67, 69). Distribution studies after intravenous administration and inhalation have shown relatively high concentration of radioactivity in the lungs (3, 42). Excretion is mostly through the feces. Even after single dose administration, radioactivity can be found in the feces for days after administration. So far, only two metabolites have been characterized (the mono and dihydroxy (leriatives) but a significant number of uncharacterized metabolites have been reported by the various researchers (3, 69). Preliminary experiments indicate that the primary metabolite may vary with the species, which would explain species differences in terms of response to cannabis effects.

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