STUDIES ON THE PHARMACOLOGY AND ACUTE TOXICITY OF COMPOUNDS WITH MARIHUANA ACTIVITY[1]

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The various data on the toxicity of marihuana (13, 8) refer to crude hemp preparations. They are of limited interest and not necessarily correlated with the toxicity of the active principles of the drug. The first prerequisite for a systematic study of the toxic aspects of the pure principles is their isolation and identification. This was accomplished several years ago (1, 8, 14).

Whereas the specific activity of more than seventy pure substances with marihuana activity has been studied quantitatively during the past seven years (8, 5, 6), only four have become available in amounts sufficient to permit investigation of their general toxicity. Three of these compounds were the main components of hemp oil. Two of them, a natural tetrahydrocannabinol and cannabinol, possess marihuana activity, whereas the third, cannabidiol, is inert. The fourth, 1-hydroxy-3-*n*-hexyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (parahexyl), a marihuana-active congener of tetrahydrocannabinol, is a synthetic compound and was chosen for comparison with the natural substances.

SUBSTANCES STUDIED. I. *Tetrahydrocannabinol (acetate; THC).* The compound employed in this study[3] was the highly marihuana-active laevorotatory natural tetrahydrocannabinol from Oriental cannabis resin (charas) previously described (14). It was prepared from the resin after conversion into the acetyl ester and was employed only in the ester form.

II. *Cannabinol.* Two different specimens of this component of hemp oil, the feeble marihuana activity of which was only recently demonstrated (9), were employed. The one was a highly purified, repeatedly recrystallized cannabinol with an MP of 75.5–76.0°C., prepared from charas (7),[3] the other one was a less purified synthetic product (4).[4]

III. *Cannabidiol.* This was available as the pure laevorotatory substance prepared from American hemp oil (2).[4]

IV. *Parahexyl.* This compound was the racemic synthetic hexyl homolog of an isomer of tetrahydrocannabinol, as first synthesized by R. Adams (3).[5]

TECHNIC OF ADMINISTRATION. Experiments with the substances listed above were complicated by their poor water solubility and by the great tolerance of

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all species of animals to those actions with which this study was chiefly concerned.

Organic solvents had to be used. The compounds were all soluble in any proportion in ethanol and, as free alcohols, in propylene glycol. The ester, THC, was administered in dipropylene glycol. The glycol solvents were given preference because of their lower toxicity. The oral $L.D._{50}$ of propylene glycol in the strain of mice employed was determined to be 26.0 cc./kg. $\pm$ 0.70, which is in satisfactory accordance with the value of 23.9 cc./kg. reported by others (15), and that of dipropylene glycol to be 20.1 cc./kg. $\pm$ 0.65. In some instances, ethanol had to be employed, the $L.D._{50}$ of which is much smaller. In a few experiments with smaller doses, homogenized, aqueous emulsions of concentrated solutions in oil, stabilized by the aid of lecithin, were used.

For oral administration in mice, the stomach tube was employed. In dogs, the undiluted substances were fed in gelatin capsules.

LETHAL DOSE. The data relating to the mean lethal dose of the four substances are summarized in table 1. When possible, the $L.D._{50}$ was established by graphic interpolation (12). In these cases, the reliability of the result is illustrated by the standard error. In many instances the number of experiments was too small to allow statistical evaluation of the $L.D._{50}$, and only a rough estimate of the threshold lethal range was obtained.

When organic solvents were used, *i.e.*, in all experiments except the oral ones in dogs, their amounts were kept under one-half the $L.D._{50}$ whenever possible. In the higher dose experiments the amount of the solvent was sometimes greater than one-half the $L.D._{50}$.

The figures obtained should be considered as minimum values of the $L.D._{50}$. Participation of the solvent in the lethal effect cannot be completely excluded even in those experiments in which its amount was only a relatively small fraction of the $L.D._{50}$.

The time interval between administration and death was usually one or more hours or occasionally even days, with the following exceptions: (a) In all intravenous experiments in mice, death followed the injection within a few minutes. (b) Death was similarly rapid in rabbits when an intravenous dose was administered in 20 per cent and not, as usually, in 80 per cent concentration. In this case, the dose of the solvent was close to, although always less than, 0.7 cc./kg., an amount which was well tolerated when given without solute. (c) When the solvent was ethanol, the survival time was shorter and the lethal dose smaller.

EFFECTS ON CENTRAL NERVOUS SYSTEM. a. *Ataxia.* An extensive literature (11, 8) describes the numerous typical features of the motor disturbances produced by marihuana in the dog, the classic test animal for this drug. The picture of marihuana ataxia in the dog is a complex of astasia, dysbasia and dysmetria, manifested in adiadokokinesia, swaying, staggering and stumbling due to lateral, frontal and caudal pulsion. Also observed are increased muscle tension, coarse twitching, and weakness at rest characterized by dropping of the head and "slipping" of the extremities.

Other species differ from the dog in that a varying number of the components of the characteristic pattern of ataxia is lacking. Motor symptoms in cats, in

TABLE 1

Mean lethal doses of marihuana components and related substances

SPECIES	ROUTE	PARAHEXYL			TETRAHYDROCANNABINOL			CANNABINOL		CANNABIDIOL	
		No. of expts.	L.D.$_{50}$ gm./kg.	St. error	No. of expts.	L.D.$_{50}$ gm./kg.	St. error	No. of expts.	L.D.$_{50}$ gm./kg.	No. of expts.	L.D.$_{50}$ gm./kg.
Mouse	oral	72	13.5	1.85	62	>21.6		3	13.5	11	>12.7
Dog	oral	1	<0.93								
Mouse	subcut.	21	>34.0		23	>11.0					
Mouse	vein	40	0.170	0.050	22	0.175	0.024				
Mouse	vein	8	0.200*								
Rabbit	vein	15	0.143	0.026	8	0.155	0.02	1	<0.126		
Dog	vein	22	0.223	0.041	9	0.100		4	>0.254		
Guinea pig	intraperiton.	7	0.850								

* In aqueous emulsion.

which only the low-dosage range was studied, resemble those in the dog, but somewhat higher doses are required to elicit them. Yet the symptoms of swaying and stumbling are never as manifest as in the dog. This may be partly explained by the fact that the cat rarely assumes an upright posture with legs extended. In rabbits, unequivocal symptoms of ataxia occurred only rarely. In most rabbits only the less characteristic symptoms are present, "slipping" of the extremities and dropping of the head. A frequent feature in rabbits and the dominant one in guinea pigs and mice is hypomotility and motor weakness. When the animal walks, there are often a lack of direction, a tendency to zig-zag and sometimes circling movements. The doses producing motor effects in all three species of rodents are at least twenty to fifty times higher than the threshold dose for ataxia in dogs, and are higher in mice and guinea pigs than in rabbits. No motor symptoms were observed with cannabidiol.

b. *Catalepsy.* Cateleptic phenomena, a great variety of which has been described in the dog (8), were observed in all species studied.

Rabbits, like dogs, maintained an extended position when suspended prone in space by supports under the pelvis and upper thorax.

In the mouse, the cataleptic state is best manifested when the animal is placed prone upon an arrangement (brim of a beaker or two parallel wires) for supporting it only at the thighs and the jaw. After an adequate dose of parahexyl or THC, an animal of otherwise normal agility maintains this extended position for an indefinite time without sagging, until an adequate stimulus arouses it. A similar catalepsy test can be used in guinea pigs.

The cateleptic response is the most sensitive symptom of marihuana action in the mouse. However its usefulness is limited by two circumstances. First: Sub-lethal doses of propylene glycol and dipropylene glycol even in doses as low as 5 cc./kg., can induce a similar position in mice, although it can be distinguished from that seen after marihuana-active substances by sagging and by less responsiveness to stimuli. Secondly, the hypermotility occurring in certain phases of marihuana action interfered with the cataleptic response in a varying percentage of mice. This was particularly true when small doses of ethanol were employed as a solvent.

With cannabinol, a cataleptic response in the mouse was obtained only after a lethal oral dose of 15.4 gm./kg. In the case of cannabidiol the response was absent even after 12.7 gm./kg., the highest dose tested in mice.

c. *Corneal areflexia.* Suppression of the lid reflex of the rabbit is a systemic effect observed after all isomeric tetrahydrocannabinols and numerous congeners. Parahexyl as well as THC were effective even in small doses, but corneal areflexia was not obtained with any dosage of cannabinol and cannabidiol, nor in any species other than the rabbit. A xerotic condition of the cornea was seen in dogs after several days of motor depression produced by near-lethal doses of parahexyl, but the wink reflex was not abolished.

Even in the rabbit, corneal areflexia is by no means a consistent response nor does it parallel other effects of marihuana. When referred to ataxia activity,

the areflexia activity of parahexyl is much greater than that of THC, as was recently reported in detail (10).

d. *Central excitant action.* Whereas states of central excitement and hyperactivity after both parahexyl and THC were rare in dogs and guinea pigs, they were often exhibited by rabbits, and were frequent in mice.

Rabbits, after doses of 100 mg./kg. and up, often showed markedly increased locomotor activity somewhat resembling that produced by apomorphine. Attacks of running, particularly when elicited by a stimulus of touch or pain, would begin with one or several jumps, and precede or interrupt the phases of hypomotility and catalepsy.

In the mouse, motor excitation can be observed with great individual variations and occurs even after very small doses. Attacks of running, jumping and dancing could be elicited by slight stimuli. Simultaneously, the animals were often aggressive and pugnacious. As in the rabbit, excitement and hyperexcitability may prevent cataleptic responses and terminate the cataleptic state.

e. *Hypnotic action.* Typical anesthesia or narcosis was never observed in any species after any dose of the four substances. At the height of the effect of large doses, dogs and sometimes other animals to a lesser extent may exhibit a picture of apathy superficially resembling that produced by a hypnotic. But a characteristic difference from the effects of anesthetics or hypnotics is the ease with which the marihuana animals can be aroused.

Gastro-intestinal Symptoms. Salivation, retching, nausea and vomiting occur frequently in dogs after oral and intravenous administration of all the marihuana-active substances in doses which produce pronounced ataxia. Such symptoms are unrelated to dosage and are absent in cats.

Diarrhea was observed only in mice. In this species it was a regular symptom in the highest range of oral THC and parahexyl doses when the animals survived more than 15 hours. The only dog succumbing after oral administration of parahexyl developed a profuse bloody diarrhea and had extensive hyperemic areas in the intestinal tract.

Respiratory Symptoms. All dogs succumbing to an intravenous dose of parahexyl exhibited signs of pulmonary edema (11). The weight of the lungs was between two and five times greater than normal.

The respiratory rate was observed in experiments in dogs. An increase did not occur in animals restrained for blood pressure recording, but did occur in unrestrained animals, more frequently after small than after large doses. The increase was transitory, and occurred within 30 minutes after the injection, *i.e.*, before the ataxia was completely developed. The original rate was exceeded by a maximum of between 30 and 100 per cent.

Subsequent to the initial stimulation, the respiration was usually decreased. A decrease was also observed in animals tied to the board for blood pressure recording. The greatest respiratory depression, with no preceding stimulation, was observed in the dog receiving the largest dose of parahexyl (926 mg./kg, by mouth).

There appeared to be no consistent difference in the respiratory symptoms produced by THC and parahexyl.

CIRCULATORY SYMPTOMS. a. *Blood pressure.* No influence upon the blood pressure of dogs was observed in a wide range of dosage of either THC (four experiments) or parahexyl (eight experiments).

b. *Pulse rate.* Increase in pulse rate in man has been repeatedly described as a characteristic action of cannabis preparations, and has also been observed occasionally in the non-anesthetized dog after THC (8). It was observed, however, only in a few out of the 28 experiments in dogs reported here. The only significant rise in pulse rate in an unrestrained animal was seen in one instance after a very large intravenous dose of parahexyl, 326 mg./kg. The increase in this case coincided with the development of labored respiration, and both phenomena may be attributed to a rapidly developing pulmonary edema.

A moderate decrease of the pulse rate was a much more consistent sign. It was always present in unrestrained animals, and was seen in about half of the restrained animals. The decrease appeared not to be related to the size of the dose or to the degree of motor depression. The pulse rate began to decrease soon after the drug was injected and remained slow for hours. The maximum effect usually occurred in the early phase of this period, but in some instances the decrease progressed steadily as long as the animal was observed. The decrease was abolished by sectioning the vagus nerves or by administering atropine.

Pulse irregularities, if present before administration of the drug, occasionally improved after the drug was given, but in dogs with normal sinus rhythm the injection appeared sometimes to produce cardiac irregularities.

DISCUSSION AND CONCLUSIONS. A major conclusion of these studies is that under all conditions the lethal dose of the pure marihuana principles is extremely high compared both with the small doses required for the specific pharmacodynamic effects and with the lethal doses reported for crude hemp preparations. Parahexyl, THC and cannabinol, three congeners of very different marihuana activity, as well as cannabidiol, the main relatively inactive component of hemp oil, can be given to albino mice without persistent harm in larger doses than sodium chloride. The ratio between the oral lethal dose of THC in the mouse and the intravenous threshold dose producing ataxia in the dog is more than 200,000. The ratio between the oral $L.D._{50}$ in the mouse and the threshold dose for the psychic action in man is more than 40,000 for THC, and more than 4,500 for parahexyl.

Various findings indicate that the cause of death is not correlated with the mechanism of the specific marihuana action. THC is about six times more effective than parahexyl in producing ataxia in dogs, but has by mouth a greater, and by vein the same $L.D._{50}$. The enormous species differences in specific marihuana activity were not paralleled by differences in lethal toxicity. In the mouse, which is refractory to marihuana action, the intravenous $L.D._{50}$ is the same as in the dog which is highly susceptible to marihuana-induced ataxia. In the dog, the difference between the loci of the lethal action and the specific

central nervous effect was particularly evident. In this species, death was due to acute intestinal effects after oral, and acute pulmonary alterations after intravenous administration.

The extremely poor water-solubility of the cannabinol and cannabidiol drugs appears not to be the main factor responsible for the low toxicity. Whereas the lethal dose in the mouse may be about 100 times higher by mouth than by vein, the ataxia dose in the dog is only ten to twenty times higher by vein than by mouth, and the lethal dose less than four times higher. In addition to solubility, the sensitivity of different sites of action in different species appears to be a factor of major importance for the nature and the degree of toxicity.

The species differences in sensitivity are such that cataleptic effects were the only manifestation of marihuana action common to all the species of animals studied. Certain actions upon the circulation and respiration, which in the past have ben considered to parallel marihuana activity, particularly an increase in pulse rate, were not found to occur with any regularity, nor were they found to be correlated with dosage or specific action. The only regular circulatory change in dogs was a moderate decrease in pulse rate.

An important result of the study of pure hemp substances is the refutation of the general belief in their hypnotic action. Neither the two highly marihuana-active substances nor the two major components of hemp possessing negligible marihuana activity manifested any hypnotic or sedative activity in any dose or species of animals. The actions of marihuana-active compounds upon consciousness, psychic functions and sensori-motor reactions are much more selective. It does not appear justified to align these substances with the hypnotics or anesthetics.

SUMMARY

1. The acute toxicity of the highly marihuana-active natural charas tetrahydrocannabinol (acetate), its somewhat less active synthetic homolog, parahexyl, the feebly active hemp component cannabinol, and the inactive hemp component cannabidiol was studied in mice, rabbits, guinea pigs, cats and dogs.

2. The $L.D._{50}$ of all four substances was very high as compared with the dose causing specific symptoms, namely, over 10.0 gm./kg. in mice by mouth and still higher subcutaneously, and between 100 and 230 mg./kg. by vein in mice, rabbits and dogs.

3. The ataxia activity varies widely in different species of animals. Corneal areflexia is elicited only in rabbits. The only sign of marihuana action common to all species of animals is a cataleptic effect. The only consistent circulatory effect in dogs was a moderate decrease in pulse rate. Excessive oral doses in mice produce diarrhea.

4. Hypnotic action is completely absent in all four substances in all species.

5. Lethal effects and specific pharmacologic activity are not correlated. The four substances vary greatly in ataxia potency, but little in lethal dose. Dogs die from pulmonary edema after intravenous administration. One dog succumbed from intestinal hemorrhage following parahexyl given by mouth.

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