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BLOOD LEVELS OF ^{3}H-Δ^9-TETRAHYDROCANNABINOL AND ITS METABOLITES IN TOLERANT AND NONTOLERANT PIGEONS*

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Abstract—Pigeons were made tolerant to the behavioral effects of 1-Δ^9-trans-tetrahydrocannabinol (Δ^9-THC) by repeated intramuscular injections. The tolerant birds, as well as birds that had not received Δ^9-THC previously, were injected with ^{3}H-Δ^9-THC and blood samples were drawn over a period from 1 min to 2 weeks after injection. High levels of radioactivity appeared in the blood 1 min after injection and peak levels were reached in 30 min, after which there was a gradual decline with some radioactivity still present after 2 weeks. The levels of radioactivity in the petroleum ether-extractable fraction [mostly Δ^9-THC by thin-layer chromatography (TLC)], in the diethyl ether-extractable fraction (mostly hydroxylated metabolites by TLC), and in the residue fraction of the plasma were also determined over the 2-week period. There were no differences between tolerant and nontolerant birds in levels of radioactivity in total plasma, or in any of the plasma fractions. In other experiments, seven injections of ^{3}H-Δ^9-THC were given to pigeons over a 2-week period during which behavioral tolerance developed. Radioactivity gradually accumulated in the plasma of these birds; however, most of the radioactivity was accounted for in the residue fraction, with levels of radioactivity in the petroleum ether-extractable fraction and the diethyl ether-extractable fractions remaining at about the same levels after seven injections as after one injection. These results suggest that tolerant birds handle an injection of Δ^9-THC in much the same manner as nontolerant birds, and that levels of Δ^9-THC and its metabolites are as high as or higher in the blood of tolerant birds than they are in the blood of nontolerant birds. Thus, tolerance to Δ^9-THC in the pigeon does not appear to be metabolic in origin.

WHEN marihuana or its active constituents, the tetrahydrocannabinols, are administered repeatedly to animals a marked tolerance develops to both the behavioral[1–3] and the physiological effects.[4,5] A similar tolerance may also develop in man.[6,7]

Tolerance to Δ^9-tetrahydrocannabinol (Δ^9-THC), the major tetrahydrocannabinol[8] in marihuana, has been characterized in animals by its large magnitude, rapid development, long duration and generality across species.[9] Although these parameters of tolerance development have been described for Δ^9-THC, as yet there have been few attempts to elucidate the mechanism underlying the tolerance development.

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One mechanism that might underlie the tolerance development to Δ^9-THC is a differential distribution of the drug in tolerant and nontolerant animals. If a lower level of Δ^9-THC or its possible active metabolites[10] occurs in blood, or in brain, of animals repeatedly dosed with Δ^9-THC, the basis of the mechanism of the tolerance to Δ^9-THC might be apparent. In its least interesting form, such a drug distribution tolerance might result from poor absorption of the drug after repeated administration. The build-up of nodules after intraperitoneal administration of Δ^9-THC to rats[11] suggests that this is a possible mechanism. A more interesting form of drug distributional tolerance would be the induction of the metabolic enzymes responsible for the inactivation of Δ^9-THC, such as already has been demonstrated for the barbiturates.[12,13] Failure to demonstrate lowered levels of Δ^9-THC or its metabolites in the blood or brain of tolerant animals would suggest that the tolerance to Δ^9-THC is not drug dispositional, but depends on other mechanisms. With these considerations in mind, we administered ^{3}H-Δ^9-THC to pigeons and studied the time course of radioactivity in the plasma and in various solvent-extractable plasma fractions of tolerant and nontolerant birds.

MATERIALS AND METHODS

Subjects. The subjects were adult male White Carneaux pigeons weighing between 400 and 580 g when given free access to food and water. All birds were deprived of food until they reached 80 per cent of their free-feeding weights and were subsequently maintained at these weights during the experiments. Since some birds were used only in the behavioral studies, while other birds were used only for the analysis of blood samples, and others were used in both types of experiments, Table 1 is provided to show how the birds were utilized in these experiments.

Behavioral apparatus. The experimental chambers for pigeons, after Ferster and Skinner,[14] were sound attenuating. A translucent plastic response key, 2 cm dia., was mounted on a false wall inside each chamber. The minimum force required to operate the key was about 15 g. Opening of the key contacts defined a pecking response. The key could be transilluminated by red or blue lamps. Directly below the key was a rectangular opening through which the pigeon could be given 4-sec access to grain after a response. The chamber was illuminated by a 25-W bulb, and white noise was present at all times.

Procedure for measuring schedule-controlled behavior. The multiple fixed-ratio, fixed-interval (mult FR FI) schedule has been described in detail elsewhere,[14] as has its application to behavioral pharmacology.[15,16]

In the present experiment, in the presence of a distinctive key light (blue), the *n*th key peck produced 4-sec access to grain. In the presence of another key light (red), the first response after *n* minutes resulted in 4-sec access to grain. If a bird did not respond within 40 sec after *n* minutes had elapsed in the presence of the red light, the schedule changed to the FR component. If the bird did not make *n* responses within 40 sec in the presence of the blue light, the schedule changed to the FI component.

For two of the pigeons used to determine the time course of the behavioral effect of 10 mg/kg of Δ^9-THC (Table 1), the schedule was a mult FR 50 response FI 2-min schedule; for the other two pigeons in this part of the experiment (Table 1), the schedule was a mult FR 30 response FI 5-min schedule of food presentation. The

TABLE 1. ASSIGNMENT OF BIRDS TO BEHAVIORAL AND BIOCHEMICAL EXPERIMENTS

Treatment	Behavioral measures	Blood samples	*n*
One injection 10 mg/kg Δ^9-THC	Mult FR 30 FI 5 schedule to determine onset and duration of effect	None taken	2
One injection 10 mg/kg Δ^9-THC	Mult FR 50 FI 2 schedule to determine onset and duration of effect	None taken	2
One injection 10 mg/kg ^{3}H-Δ^9-THC (5 μCi)	Visual observation of posture and feeding to determine onset and duration of effect	Blood samples at irregular intervals for pilot studies	14
Six Triton-X injections, then 10 mg/kg ^{3}H-Δ^9-THC (5 μCi)	FR 30 schedule to show lack of effect of drug vehicle and duration of effect	Blood samples from 1 min to 14 days after ^{3}H injection to nontolerant birds	4
Six Δ^9-THC injections, then 10 mg/kg ^{3}H-Δ^9-THC (5 μCi)	FR 30 schedule to show development of tolerance to Δ^9-THC	Blood samples from 1 min to 14 days after ^{3}H injection to tolerant birds	4
Seven injections ^{3}H-Δ^9-THC (5 μCi)	FR 30 schedule to show development of tolerance to ^{3}H-Δ^9-THC	Blood samples 2·5 hr after each injection	4

pigeons under the mult FR 50 FI 2 schedule were drug naïve, and the pigeons under the mult FR 30 FI 5 schedule had received drugs previously, but not Δ^9-THC, and no drugs had been given to them for several months.

Twelve other pigeons (Table 1) were conditioned to key peck for food under a FR 30 schedule in the presence of a blue light. These birds had not received drugs previously.

Procedure for gross observation of pigeon behavior. The 14 pigeons (Table 1) used for preliminary determinations of plasma radioactivity were observed visually at 30-min intervals for 2 hr after receiving 10 mg/kg of ^{3}H-Δ^9-THC. Subsequent observations were made at 6 and 24 hr after injection, and then daily until the end of the experiment. Food was not available to these pigeons until 6 hr after injection, after which 20 g was placed in the food cup daily.

Materials. Unlabeled Δ^9-THC was suspended in a 5% Triton X-100 solution. The concentration of Δ^9-THC was 10 mg/cc, so that each pigeon could be injected at a volume of 1 cc/kg. On days when labeled Δ^9-THC was given, 0·01 cc (or 0·1 mg) of the unlabeled Δ^9-THC was replaced by 0·2 cc (also 0·1 mg) of tritium-labeled Δ^9-THC suspended in a Triton X-100 solution. The dose of tritium administered was 5·0 μCi in 0·2 cc.

Analysis of the blood samples. Approximately 1 cc of blood was drawn into a heparinized syringe from the cephalic vein of the pigeon. The blood was placed in a glass tube and centrifuged at 3000 rev/min for 10 min in a Sorvall RC 2-B refrigerated centrifuge. The plasma was removed and placed in a 10-ml beaker from which a 0·1-ml aliquot was taken and placed in a scintillation vial containing 15 ml of scintillation fluid for counting in a scintillation counter. The scintillation fluid was

a mixture of 1 liter of purified Triton X-100, 8 g PPO,* 200 mg dimethyl POPOP* and 2 l. of toluene.

The volume of the remainder of the sample was measured and then was washed four times with 2 ml of petroleum ether to extract the Δ^9-THC.[17] The petroleum ether fraction (organic phase) was decanted, pooled and evaporated to dryness at room temperature. The dried petroleum ether fraction was redissolved in 0·2 ml of petroleum ether, 0·1 ml of which was placed in scintillation fluid for counting. The aqueous phase was then washed three times with 2 ml of diethyl ether to extract the hydroxylated metabolites of Δ^9-THC.[17] After separation, the diethyl ether fraction (organic phase) was taken to dryness at room temperature and redissolved in 0·2 ml diethyl ether, 0·1 ml of which was placed in scintillation fluid for tritium counting. For most of the samples, an aliquot of 0·1 ml of the total residue (aqueous phase) remaining after the diethyl ether washings was placed in scintillation fluid for tritium counting. All samples were corrected for quenching by internal standardization. Of the total radioactivity in plasma, an average of 82 per cent (standard error of 4·3 per cent) could be recovered from the plasma fractions.

To determine if the radioactivity was associated with the plasma and not the red blood cells, after the plasma was removed from the centrifuge tube, the red blood cells were placed on filter paper and weighed. After drying, the red blood cells and the filter paper were burned in a Packard Automatic X-100 Oxidizer and the tritiated water was collected and placed in scintillation fluid for counting. As a further control, plasma samples were counted from four pigeons which had not received Δ^9-THC.

Thin-layer chromatography. From selected samples (1 min, 1 day, 1 and 2 weeks from tolerant and nontolerant birds), a 0·1-ml aliquot of the petroleum ether extract, the diethyl ether extract, and the residue were saved for analysis by thin-layer chromatography (TLC).[18] The two solvent extracts were evaporated to dryness and then brought to a volume of 0·6 ml with the respective solvents. The total solvent was streaked on an Eastman Kodak 6060 Silica gel G TLC plate. Each plate was developed in a hexane–acetone (3:1) solvent system. After development, each plate was visualized with an aqueous solution of diazo blue B salt. Zones corresponding to the migration of standards of Δ^9-THC and the 8,11-dihydroxy and 11-hydroxy metabolites of Δ^9-THC were scraped from each plate into scintillation fluid and counted to determine the percentage of total radioactivity in the zones corresponding to each reference standard. The plasma residue remaining after the ether extractions was analyzed on TLC plates in a similar manner.

RESULTS

Time course of the behavioral effects of Δ^9-THC. The onset and duration of action of 10 mg/kg of Δ^9-THC in birds that had not received Δ^9-THC previously are shown both for the schedule-controlled behavior and for the visual observations of behavior in Fig. 1. All of the behavioral measures show much the same onset and duration of effect of 10 mg/kg of Δ^9-THC. For the first 30 min after injection, there is little if any effect. From 30 to 60 min after the injection, rates of key pecking begin to fall and the previously reported characteristic slumped posture[9] seen in pigeons after Δ^9-THC can be observed. By 2 hr after 10 mg/kg of Δ^9-THC, none of the birds are

* PPO = 2,5-diphenyloxazole; POPOP = 1,4-*bis*-2-(4-methyl 5-phenyloxazolyl) benzene.

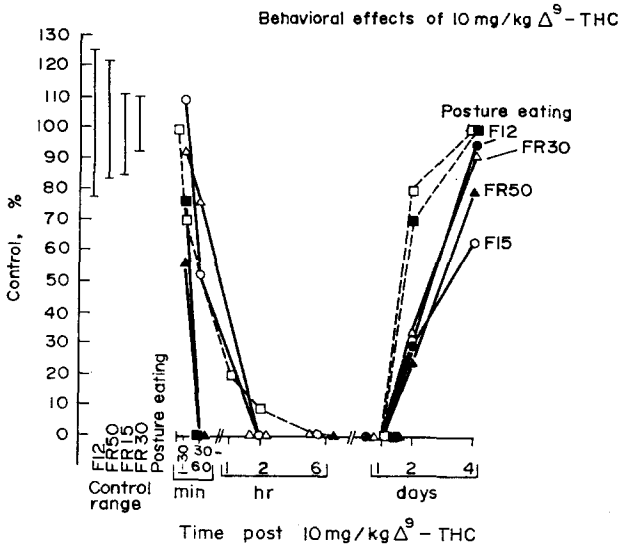

FIG. 1. Effects of Δ^9-THC on posture, eating and schedule-controlled behavior. Abscissa, time; ordinate, rate of key pecking as a per cent of the control rate, or percentage of birds eating and showing normal posture. The brackets at Control Range show the range around the control mean from at least four non-injection sessions. The number of animals contributing to each point in the figure can be found in Table 1.

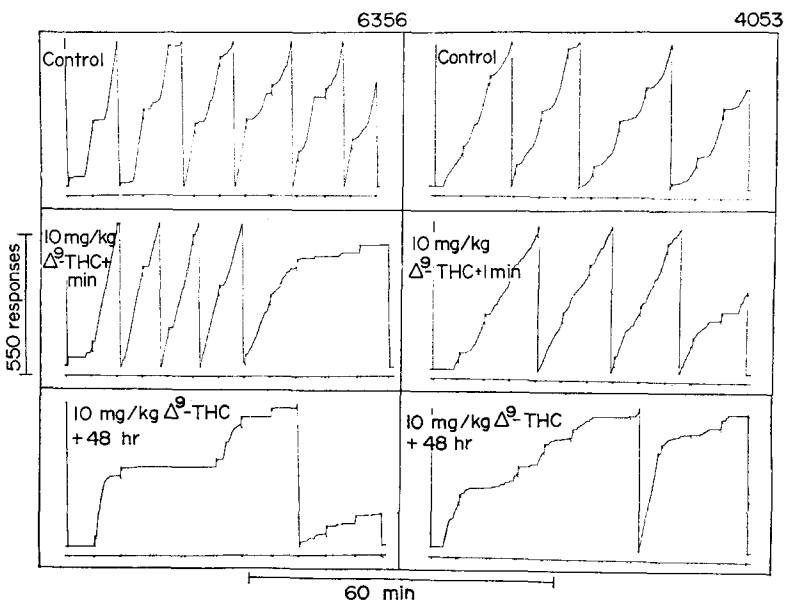

FIG. 2. Cumulative response records showing the onset and duration of the effects of 10 mg/kg of Δ^9-THC on behavior under the mult FR 30 FI 5 schedule of food presentation. Abscissa, time; ordinate, cumulative number of responses. The FR and FI components are alternated. The components change after food delivery (short diagonal lines on the cumulative record and on the horizontal line), or after 40 sec without food in the FR component (diagonal lines on the horizontal line), or after 40 sec without food after 5 min have elapsed in the FI component (diagonal lines on the horizontal line).

key pecking and postural signs are obvious in almost all of the pigeons. Essentially the same behavioral signs are observable at 6 and 24 hr after injection, during which time pigeons do not eat freely available grain.

By 48 hr after the injection of 10 mg/kg of Δ^9-THC, some signs of recovery are readily apparent. Most of the birds have started to eat and postural signs have disappeared. At this time, key pecking begins to occur again, although the rates are well below control levels and the pattern of responding is abnormal. Four days after the injection, the gross appearance of the pigeons is normal and rates of key pecking either have returned to control levels or are very close to it.

Figure 2 shows the cumulative response record of the key pecking of two different birds. Note that the pattern of responding begins to appear abnormal about 25 min after injection. This was the earliest that we observed disruption of behavior after intramuscular injections of 10 mg/kg of Δ^9-THC. The pattern of responding under the mult FR 30 FI 5 schedule was not disrupted for bird 6356 until about 45 min after the injection.

Tolerance to the effect of Δ^9-THC on behavior. Eight pigeons (Table 1) were injected three times weekly (Monday, Wednesday and Friday) with either labeled or unlabeled Δ^9-THC and the rate of responding under the FR 30 schedule was determined 2 hr after each injection. Four other pigeons were treated similarly, except that they received vehicle injections of 5% Triton X-100 rather than Δ^9-THC. After six of these injections, all 12 birds received labeled Δ^9-THC (10 mg/kg and 5 μCi) as a part of the seventh injection. Figure 3 shows that an initial injection of 10 mg/kg of either labeled or unlabeled Δ^9-THC completely eliminated responding, while injections of the control vehicle were without effect. Tolerance developed rapidly in both groups to the repeated administration of 10 mg/kg of Δ^9-THC, so that after four injections

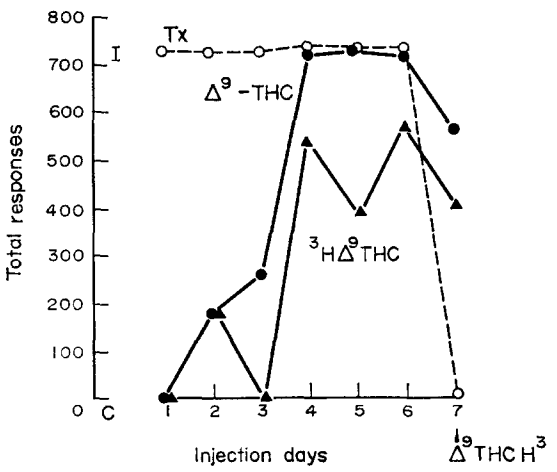

Fig. 3. Development of tolerance to the rate-decreasing effects of Δ^9-THC on behavior under the FR 30 schedule. Abscissa: injection days, with birds being injected three times weekly (behavior on the days between injections not shown). Ordinate: number of key peck responses during a 30-min session. The brackets at C show the range of control performances during at least four non-injection sessions. Each point is the mean of single observations in each of four birds.

of Δ^9-THC the birds were pecking the key almost as much as during their premedication period. However, the first Δ^9-THC injection given to the birds that were receiving repeated injections of Triton X-100 completely eliminated responding.

A similar development of tolerance to 10 mg/kg of Δ^9-THC could be demonstrated by visual observation of the birds used to determine levels of Δ^9-THC in the blood (Table 1). The initial injection of Δ^9-THC produced postural changes in all birds, and none of these birds ate grain 6 hr after the injection. However, after the third and subsequent injections, the posture of all birds appeared normal and the birds ate their daily grain ration as soon as it was offered.

In summary, 10 mg/kg of Δ^9-THC produced marked effects on both schedule-controlled behavior and gross behavior. The onset of these effects is slow, taking from 20 min or more to develop after an intramuscular injection. The effects last for 3 or 4 days. However, if this dose is administered repeatedly, these behavioral effects almost disappear after four or five injections and approach control levels prior to the seventh injection. Thus, the seven injections given over 2·5 weeks (three/week) are ample for a marked tolerance to develop to the effects of 10 mg/kg of Δ^9-THC.

Tritium in red blood cells and in plasma of untreated birds. The red blood cells of a few birds that had received ^{3}H-Δ^9-THC (5 μCi) were dried and oxidized. The labeled material was collected as tritiated water and placed in scintillation fluid for counting. All these samples counted near background level, indicating that little, if any, radioactivity was associated with the red blood cells after ^{3}H-Δ^9-THC.

A 0·1-ml aliquot of plasma from birds that had not received any Δ^9-THC was placed in scintillation fluid and counted. All these samples also counted at background level, suggesting that the plasma of untreated birds did not contain a substance which might incorrectly be interpreted as tritium.

Time course of radioactivity in tolerant and nontolerant birds. Figure 4 shows the number of dis/min in 0·1 ml plasma at various times after the injection of ^{3}H-Δ^9-THC

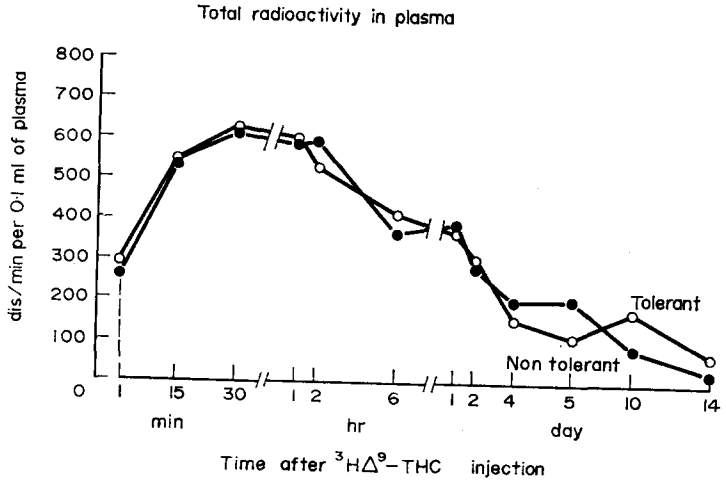

FIG. 4. Levels of total radioactivity in plasma. Abscissa, time; ordinate, disintegrations per minute per 0·1 ml of plasma. Each point is the mean of single observations in each of four birds. Samples were drawn repeatedly from each bird.

(5 μCi in 10 mg/kg). Radioactivity in plasma was surprisingly high in both tolerant and nontolerant birds 1 min after intramuscular injection, indicating a rapid absorption. The approximately 300 dis/min/0·1 ml seen 1 min after injection represents almost half the peak level. Radioactivity in the plasma approached a peak level within 15 min and it remained at a high level (about 550–600 dis/min/0·1 ml) for more than 2 hr after the injection. Six hours after the injection, total radioactivity in the plasma had begun to decline and the levels of radioactivity slowly decreased over a 2-week period. Approximately half of the peak level of radioactivity was still present 2 days after injection of ^{3}H-Δ^9-THC with about 10 per cent of the maximum still present 2 weeks after injection, at which time the experiment was terminated.

There is a striking similarity in the curves describing the time course of radioactivity in blood for tolerant and nontolerant birds that have received the same dose of ^{3}H-Δ^9-THC. The almost identical time course curves were obtained, despite the fact that the behavior of the two groups of birds differed markedly. Two hours after 10 mg/kg of Δ^9-THC, nontolerant birds did not peck the key to obtain food and they showed marked postural disturbances (Figs. 1, 2 and 3). These effects lasted for several days. In contrast, the tolerant birds key pecked at near normal rates and no postural changes were observed.

Thin-layer chromatography of plasma fractions. The TLC plates were spotted with standards and with 0·1-ml aliquots from the petroleum ether fraction, the diethyl ether fraction, and the residue fraction, using samples taken 1 hr after injection of ^{3}H-Δ^9-THC to both tolerant and nontolerant birds. Figure 5 shows the mean radioactivity from the scraping of different zones of the TLC plates spotted with samples from tolerant and nontolerant birds.

The TLC plate spotted with the petroleum ether fraction shows that by far the area of greatest activity was zone 5, which is the zone into which the Δ^9-THC standard migrated. The TLC plate spotted with the diethyl ether fraction had high levels of radioactivity in the combined zones 2, 3 and 4, which are the zones associated with the 8,11-dihydroxy and 11-hydroxy metabolite migrations. There was also a high level of radioactivity in zone 5 of the TLC plates spotted with the diethyl ether fraction, suggesting the presence of some unmetabolized Δ^9-THC. In the TLC plates spotted with the residue fraction, most of the radioactivity did not migrate from the origin (zone 1), although there was some radioactivity in zones 2, 3 and 4, which is associated with the hydroxylated metabolite standards. Thus, these solvent fractionations are not clean and clearcut in their separation of the parent compound and the metabolites. In addition, other metabolites which may have similar migration patterns could be contained in the zones counted. The non-migrating polar material remains unidentified.

Time course of radioactivity in the plasma fractions of tolerant and nontolerant birds. Figure 6 shows the time course for the levels of radioactivity in the petroleum ether-soluble fraction (mostly Δ^9-THC by TLC) of the plasma. Within 1 min, radioactivity in this fraction is fairly high and by 15 min it approaches peak levels. However, within an hour after injection, there is a suggestion that the radioactivity in the petroleum ether fraction is beginning to drop and by 2 hr the trend is quite apparent. There is almost no radioactivity in the petroleum ether fraction 24 hr after the injection of ^{3}H-Δ^9-THC, although the animals are still markedly affected.

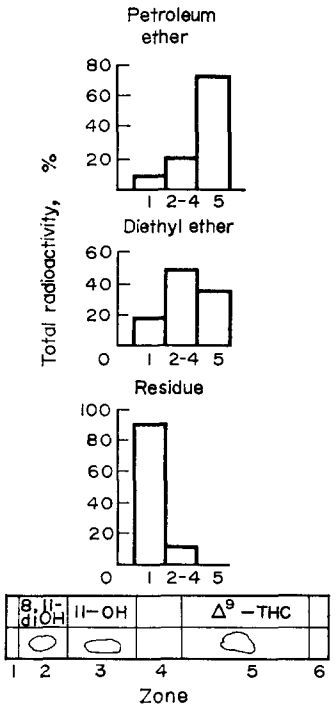

FIG. 5. Thin-layer chromatographic analysis of content of plasma fractions. Abscissa, TLC zone; ordinate, per cent of total radioactivity on the TLC plate accounted for by scrapings from each zone. All data are averaged across tolerant and nontolerant birds 1 hr after injection of ^{3}H-Δ^{9}-THC. At the bottom of the figure are tracings from the visualization of the TLC standards showing their migration on the plate into the different zones.

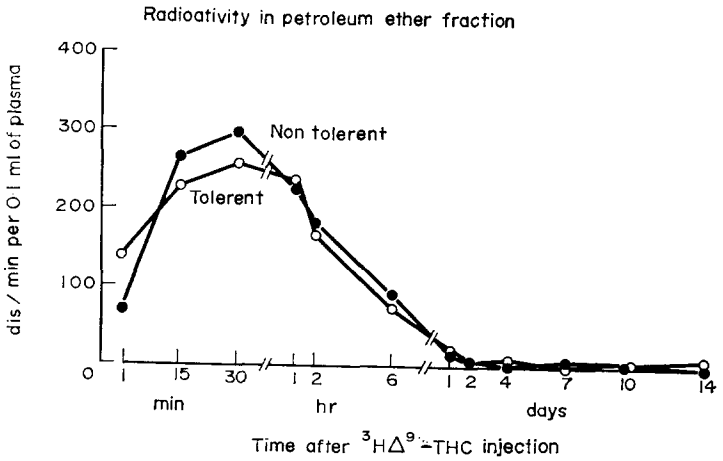

FIG. 6. Radioactivity in the petroleum ether-extractable fraction of the plasma. Abscissa, time; ordinate, dis./min per 0·1 ml of plasma. Each point is the mean of a single observation in each of four birds.

As was the case with the total radioactivity in total plasma, there is no difference between tolerant and nontolerant birds in the time course of radioactivity in the petroleum ether-soluble fraction of plasma. It appears that radioactivity attributable to Δ^9-THC rapidly appears in the blood of both tolerant and nontolerant birds, and that the levels of radioactivity in the petroleum ether fraction decline at the same rate in these two groups of birds over the 2-week period.

Figure 7 shows that the increase in the levels of radioactivity in the diethyl ether fraction (mostly 11-hydroxy and 8,11-dihydroxy Δ^9-THC by TLC) is much slower than the increase in levels in the petroleum ether fraction. This is especially true for the nontolerant birds; however, by 15–30 min, the radioactivity in the diethyl ether fraction reaches a similar maximum level. Unlike the level of radioactivity in the petroleum ether fraction, which began to fall rapidly a few hours after injection, radioactivity in the diethyl ether fraction decreased much more slowly, so that more than half the maximum levels were still present on the day after injection.

There were few differences, if any, in the time curves for radioactivity in the diethyl ether fraction of the plasma of tolerant and nontolerant birds. During the first 30 min after injection, there was a tendency for slightly higher levels of radioactivity to occur in the diethyl ether fraction of tolerant birds than from nontolerant birds; however, these differences were small. Thus, it appears that the radioactivity attributable to the hydroxylated metabolites does not differ in tolerant and nontolerant birds.

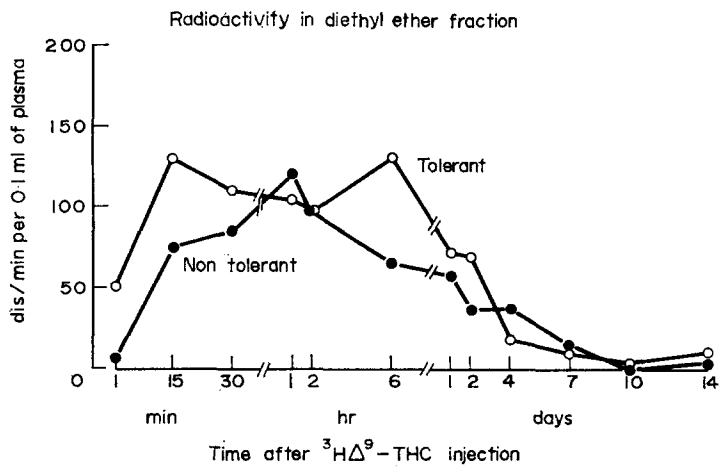

FIG. 7. Radioactivity in the diethyl ether-extractable fraction of the plasma. Abscissa, time; ordinate, dis./min per 0·1 ml of plasma. Each point is the mean of a single observation in each of four birds.

Figure 8 shows the time course for levels of radioactivity in the residue after the petroleum ether and diethyl ether fractions were removed. The levels of radioactivity in the residue rose much more slowly than they did in the solvent-extractable fractions, not reaching a peak until 24 hr after the injection. Thereafter, the levels of radioactivity began to decline slowly, but about one-third to one-half the peak levels of radioactivity were still present 14 days after the injection. Again, as with the other two

fractions, the curves describing the time course of radioactivity in the residue fraction were similar for tolerant and nontolerant birds.

To summarize the pattern of radioactivity in the plasma fractions, 1 min after injection of ^{3}H-Δ^9-THC, the great bulk of radioactivity in the plasma is accounted for by radioactivity in the petroleum ether fraction (mostly Δ^9-THC), with relatively small contributions being made by the diethyl ether (mostly hydroxylated metabolites of Δ^9-THC) or residue fractions. Subsequently, the contribution of the petroleum ether fraction declines steadily. Although part of the decline in the contribution of the petroleum ether fraction is accounted for by a rise in the contribution of the diethyl ether fraction at the early times, most of the decline can be attributed to the increasingly large contribution of the residue fraction. As early as 6 hr after injection, the residue fraction accounts for more radioactivity than either of the ether fractions, and by 24 hr after injection, the residue fraction is accounting for most of the radioactivity.

Effects of repeated injections of ^{3}H-Δ^9-THC. The preceding series of experiments provide information as to how a dose of radiolabeled Δ^9-THC is handled by birds tolerant to Δ^9-THC, compared to birds that had not received Δ^9-THC previously; however, the experiments provide no information about the possible accumulation of Δ^9-THC when it is given repeatedly. Therefore, we administered ^{3}H-Δ^9-THC (10 mg/kg, 5 μCi) seven times over a 2·5-week period and measured the levels of radioactivity in the plasma and in the plasma fractions 2·5 hr after each injection. These data are shown in Fig. 9.

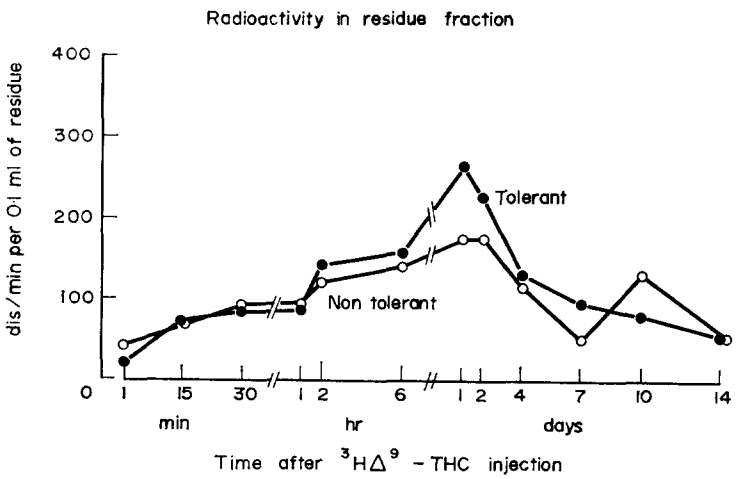

FIG. 8. Levels of radioactivity in plasma after the solvent extractions (residue). Abscissa, time; ordinate, dis./min per 0·1 ml of residue. Each point is the mean of single observations in each of four birds.

Figure 9 shows that there is a gradual accumulation of total radioactivity with the repeated injections of ^{3}H-Δ^9-THC. The initial value of 500 dis/min is very close to that obtained for both tolerant and nontolerant birds in the previous experiments. However, total radioactivity accumulated until there was more than three times as

much radioactivity (1820 dis/min/0·1 ml) in the plasma of these birds after the seventh injection of ^{3}H-Δ^9-THC than was seen when only the seventh injection of Δ^9-THC contained tritium. Most of the accumulated radioactivity could be accounted

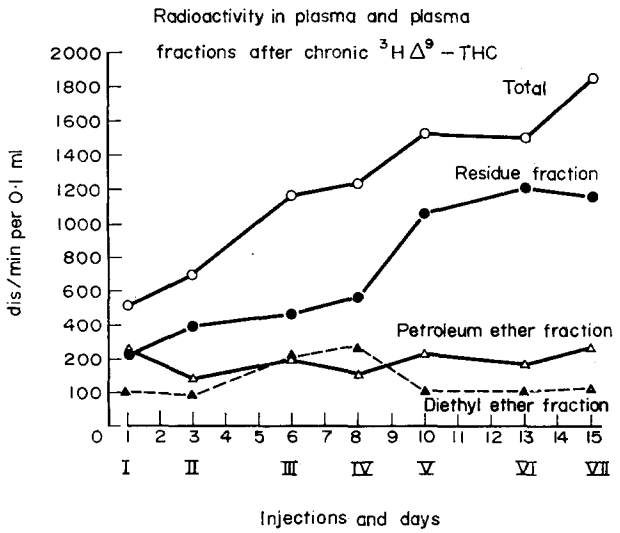

FIG. 9. Radioactivity in plasma and in the plasma fractions after seven injections of ^{3}H-Δ^9-THC given over a 15-day period. Abscissa, days (Arabic numerals) and injections (Roman numerals); ordinate, dis./min per 0·1 ml. Radioactivity was measured only on injection days, 2·5 hr after injection. Each point is a mean of single observations in each of four birds.

for in the residue fraction, where radioactivity increased steadily. Radioactivity levels in the petroleum ether and diethyl ether fractions, attributable to Δ^9-THC and its hydroxylated metabolites, never got very much higher than they were after the initial injection. Thus, radioactivity in total plasma and radioactivity in the residue fraction were much higher after seven injections than after a single injection, while the levels of radioactivity in the petroleum ether fraction and in the diethyl ether fraction were about the same after the seventh injection as they were after the first injection.

Figures 4, 6, 7 and 8 showed the time course of total radioactivity in plasma and the time course of radioactivity in the plasma fractions for nontolerant birds. By looking at these curves, it can be determined how much radioactivity is present at a given time after a single injection. By summing values at various times after a single injection, it would be possible to predict the accumulation of radioactivity after multiple injections. Our seven injections of ^{3}H-Δ^9-THC were administered over a period of 14 days. By extrapolating from the curves of Figs. 4, 6, 7 and 8 and summing the various values obtained after a single ^{3}H-Δ^9-THC injection, values which should be obtained after seven injections of ^{3}H-Δ^9-THC can be predicted. The predicted values are close to the observed values, except for the diethyl ether fraction, where the observed values are somewhat lower than the predicted values.

DISCUSSION

Our experiments strongly suggest that the marked tolerance that develops to

Δ^9-THC in pigeons does not result from a differential absorption of the drug into the bloodstream, nor does it appear to result from a differential metabolism of the drug by tolerant birds.

Significant amounts of radioactivity appeared in the plasma of both tolerant and nontolerant birds within a minute after intramuscular injection of ^{3}H-Δ^9-THC, suggesting that the Δ^9-THC was rapidly absorbed. The time course of radioactivity in total plasma of tolerant and nontolerant birds was almost identical, despite the marked behavioral differences in tolerant and nontolerant birds after Δ^9-THC. These data would seem to eliminate any explanation of Δ^9-THC tolerance based on poor absorption of the drug due to tissue damage, or encapsulation of the drug, during repeated administration.

It is possible that the time course of the metabolites of Δ^9-THC in plasma might differ in tolerant and nontolerant birds, even though the time course of radioactivity in total plasma was the same for both groups. For this reason, we measured radioactivity in the different plasma fractions and attempted to identify the source of the radioactivity in the fractions by TLC. Again, the time course of radioactivity in the petroleum ether- and diethyl ether-extractable fractions, as well as in the plasma residue, was similar for tolerant and nontolerant birds. These data suggest that tolerant birds are handling a dose of Δ^9-THC in the same manner as birds that have never received the drug previously. Further, the experiments where ^{3}H-Δ^9-THC was administered repeatedly showed that the final levels of radioactivity in total plasma, in either of the organic solvent-extractable fractions, and radioactivity in the residue all were as high as or higher than the corresponding levels of radioactivity after a single injection of ^{3}H-Δ^9-THC to nontolerant birds. Thus, Δ^9-THC and its metabolites appear to be present in the plasma of tolerant birds in concentrations at least as high as, if not higher than, the concentrations of Δ^9-THC and its metabolites in the plasma of nontolerant birds. Taken together, all these findings suggest that the tolerance to Δ^9-THC in pigeons does not result from factors such as poor absorption, increased rates of metabolism or more rapid elimination of the drug from the body. Although our experiments make tolerance to Δ^9-THC seem unlikely to be based on drug distributional factors, these factors cannot be eliminated completely, since the picture in the blood may not reflect accurately events occurring in the brain. In addition, the separation and identification techniques employed in this study do not eliminate the presence of as yet unidentified additional metabolites which may be involved in the phenomenon of tolerance.

If tolerance to Δ^9-THC is not metabolic or drug distributional, the question of the mechanism underlying the phenomenon remains. The tremendous magnitude of the tolerance[19] and the fact that tolerance appears to occur to lethal doses[20] seem to eliminate the possibility that the tolerance is a learned tolerance of the type suggested by Dews.[21] By a process of elimination, a pharmacodynamic tolerance, perhaps resembling that which develops to morphine,[22,23] seems most likely.

Our failure to find a more rapid metabolism of Δ^9-THC upon repeated injection to pigeons apparently is in contradiction to recent findings in man where it has recently been suggested that chronic marihuana users may metabolize Δ^9-THC more rapidly than non-users.[24] Whether this represents a species difference or some other factor is not apparent. Obviously, the dose levels of unlabeled Δ^9-THC were much higher in our experiments than those that are used by humans and it is much

easier to control the previous drug experiences of laboratory animals than it is to control these experiences in man.

There have been several reports that the 11-hydroxy metabolite of Δ^9-THC is responsible for the biological activity.[10,25] Our data bear indirectly on this problem. Levels of total radioactivity in drug-naïve birds were quite high 1 min after injection of ^{3}H-Δ^9-THC and maximal within 15 min, yet behavioral effects never appeared before 25 min after injection, and usually these effects occurred even later. Such data are consistent with the idea that Δ^9-THC is converted to an active metabolite. Actually, there was no correlation between the behavioral effects of Δ^9-THC and total radioactivity, or between the behavioral effects and the radioactivity in any of the plasma fractions. This is not surprising, since the duration of action depends primarily on the dose of unlabeled Δ^9-THC.[26,27] However, it should again be pointed out that our fractionation and identification procedures are not definitive.

If Δ^9-THC is converted to an active metabolite, then the tolerance that occurs with repeated injections of Δ^9-THC is actually tolerance to the active metabolite. Our data lend some support to this notion. Figure 6 shows that very little radioactivity was present in the petroleum ether fraction 24 hr after ^{3}H-Δ^9-THC injection, yet tolerance develops with injections spaced 48–72 hr apart (or even with injections 1 week apart[28]). Since this fraction contains mostly Δ^9-THC, it may not be necessary for significant blood levels of Δ^9-THC to be maintained between injections for tolerance to develop. Unfortunately, our TLC analysis showed that there was some Δ^9-THC in the diethyl ether fraction, which showed a longer duration of radioactivity. Until we can achieve a better separation of Δ^9-THC from its hydroxylated metabolites and secure a more definitive identification of the metabolites, this point will remain unclear.

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