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19. We thank J. Washington for technical assistance. Supported by Veterans Administration research funds and grant GB7285X from NSF. G.O.B. is an international postdoctoral fellow of NIH (FO 5 TW 1616).

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Inhibition of Normal Growth by Chronic Administration of Δ-9-Tetrahydrocannabinol

Abstract. *Body weight, food and water intake, and feces weight of 20 albino rats were recorded daily for 70 days. On days 11 to 40, 12 rats received behaviorally effective doses of Δ-9-tetrahydrocannabinol, either orally or intraperitoneally. These rats ate significantly less than placebo-dosed controls during the treatment period, and gained significantly less weight. Food intake recovered in the 30-day posttreatment period, but the former drug group still weighed less than the controls on day 70. In addition, all rats who had received intraperitoneal injections of Δ-9-tetrahydrocannabinol showed evidence of chronic diffuse nonsuppurative peritonitis.*

The isolation of marihuana's major active component, Δ-9-tetrahydrocannabinol (△-9-THC) (*1*), and its subsequent synthesis in relatively pure form (*2*) has provided the opportunity for better controlled quantitative research in the fields of physiology, biochemistry, pharmacology, and psychology (see *3*, *4*). The present study was begun as an attempt to verify under controlled conditions two incidental observations on the effects of Δ-9-THC in laboratory rats. First, rats being given daily intraperitoneal injections of Δ-9-THC very often showed precipitous losses in body weight, despite having free access to both food and water 22 hours of each day. Secondly, six of eight rats involved in a shock-avoidance study died during or shortly after the 2-week period during which they received daily intraperitoneal injections of Δ-9-THC. Postmortem examination suggested that death in all six cases had resulted from complications associated with an irritative or chemical peritonitis. We now report our observations of decreased body weight and food intake of rats given doses of Δ-9-THC daily for 30 days. Rats receiving intraperitoneal injections of the drug developed peritonitis in every case, while no rat receiving oral doses of the drug developed this condition. Both groups, however, consumed less food than controls over 30 days of drug administration and weighed considerably less even after 30 "recovery" days.

The subjects were 20 male albino rats, weighing between 250 and 300 g at the start of the study. All animals were individually housed in metal metabolism cages, with free access to bottled tap water and 45-mg animal feed pellets (P. J. Noyes Co.). The room was lighted for exactly 12 hours daily and temperature ranged between 20° and 24°C. Body weights were recorded daily, along with food and water intake and feces weight. After 10 days of this regimen, subjects were assigned randomly into four groups. Two groups of six animals subsequently received doses of Δ-9-THC each morning for the next 30 days (*5*). These two drug groups differed in both route of administration (intraperitoneal for one, gavage for the other) and dosage (4 mg/kg for the intraperitoneal groups, and 8 mg/kg for the oral group). The remaining eight rats served as placebo controls. Four received daily intraperitoneal injections of vehicle and four received daily vehicle doses via gavage.

Figure 1 shows the effect of daily Δ-9-THC injections on body weight. It is clear that both control groups, which were not significantly different from each other, continued to gain weight throughout the 30 days of placebo administration (mean gain, 53 g). The two drug groups, which also did not differ from each other, showed a notable loss in weight over the first 4 days of Δ-9-THC administration, followed by a very slow recovery up to their initial weights (mean gain, 0.7 g). Analysis of variance on net change in body weight during these 30 days revealed a significant treatment effect ($F = 9.49$; d.f. = 3,16; $P < .005$). Individual comparisons indicated that route of administration was not a significant factor in body weight changes. The drug versus placebo control comparison was significant beyond the .001 level ($F = 27.8$; d.f. = 1,16), however. After 30 additional days, during which neither drug nor placebo was administered, there was still a significant difference in body weight between the former drug and control groups ($t = 4.13$; d.f. = 17; $P < .001$; two-tailed). However, analysis of variance on weight *gain* over this 30 days posttreatment period failed to detect a significant effect of prior drug treatment.

Significant changes in food intake were also observed during the course of the study, and the data suggest that the effects on body weight reported above were due predominantly, if not entirely, to these changes, for the four groups of rats were essentially indistinguishable in terms of water consumed and feces weight. The animals in the two groups receiving Δ-9-THC consumed an average of only 522 g of food, while controls averaged 602 g ($F = 22.64$; d.f. = 1,16; $P < .001$). The two drug groups did not differ significantly from each other on this measure, nor did the controls. In order to assess our hypothesis that this difference in food intake was the primary cause of the observed differences in body weight, an analysis of covariance was performed, with food intake as the covariate or predictor variable. With total food intake controlled for in this way, the significant effect upon body weight disappears ($F = 1.95$; d.f. = 3,16). This close parallel between food intake and body weight is substantiated further by the data from the 30-day posttreatment period. No significant differences between groups was seen in food intake during this period ($F = 2.80$; d.f. = 3,15), just as there were no differences in weight gain. There was still a striking difference in the

body weights of former drug and control rats however, and there was also still a significant difference in total food intake over the entire experiment ($t = 3.36$; d.f. = 17; $P < .001$, two-tailed).

At the end of the experiment all animals were killed, and a thorough necropsy was performed. All of the rats given intraperitoneal injections of Δ-9-THC showed evidence of having suffered from chronic diffuse chemical peritonitis. With the exception of one rat that had a large localized abscess of the mesenteric lymph nodes, from which *Pseudomonas aeruginosa* was isolated, no organisms were cultured. Rats are very resistant to infectious peritonitis, and when present it is usually manifested by focal areas of necrosis and acute inflammatory cells. Although the peritonitis seen in these rats was in the healing stage, the diffuse distribution of the chronic inflammatory cells and mild fibroplasia of serosal surfaces is in accord with changes expected from an irritative inoculum rather than a septic injection. The presence of this peritonitis is not surprising, since many other phenolic compounds are highly caustic as well as Δ-9-THC; what is of concern is that intraperitoneal injection has been the predominant route of administration in animal studies of Δ-9-THC. No such damage was found in any animals from the control or oral Δ-9-THC groups. What is puzzling is that only one rat died during the course of this experiment, and from causes unrelated to peritonitis. Yet it was the abnormally high death rate in a shock-avoidance study which led to the study reported here. Apparently the shock-avoidance procedure was an important factor in the earlier study. Preliminary results from experiments currently under way indicate that all three factors, avoidance, peritonitis, and Δ-9-THC, must act in conjunction to produce a high death rate.

We feel that several aspects of these data deserve close attention from those engaged in or planning research with Δ-9-THC. First, despite the fact that it has frequently been used in animal studies, intraperitoneal injection is clearly not the administration route of choice. Severe irritation of the peritoneum may be presumed to have behavioral consequences in itself, perhaps obscuring or distorting those produced by the central nervous system effects. This suspicion has been supported by our difficulties in replicating, with oral administration, certain behavioral effects reliably seen after intraperitoneal injections of Δ-9-THC in the rat. In addition, peritonitis might also play an important role in certain endocrine responses to intraperitoneal injections of Δ-9-THC (*6*).

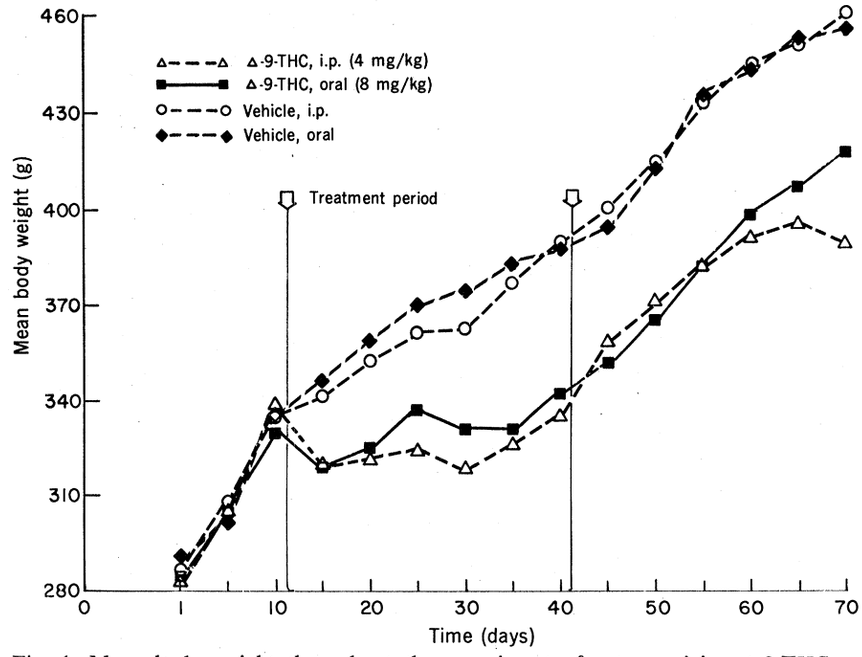

Fig. 1. Mean body weight throughout the experiment of rats receiving Δ-9-THC or placebo by one of two routes (intraperitoneal or oral) daily during the interval marked "treatment period."

A second caveat for Δ-9-THC investigators stems directly from the decrease in food intake shown by our rats. By far the most common finding in work on Δ-9-THC and food-motivated behavior in animals has been dramatic slowing or cessation of such behavior (*7*). The depression in food intake observed in our animals suggests that Δ-9-THC may reduce the effectiveness of food reinforcers. This possibility should not be overlooked in attempts to account for decreases in food-motivated behavior following Δ-9-THC administration. It should also be noted that this problem is not obviated by avoiding the intraperitoneal route of administration. Our oral Δ-9-THC rats, all free of notable pathology, showed declines in weight and food intake very similar to those of the rats given Δ-9-THC intraperitoneally, at least during the period of drug administration. An extension of this experiment, encompassing a much wider dose range, revealed that the effects reported here are by no means specific to the particular dose levels chosen. We repeated our observation of body weight and food and water intake on three additional groups of six rats given Δ-9-THC orally for 30 days. Doses employed were 0.5 mg/kg, 2.0 mg/kg, and 32 mg/kg. Figure 2 summarizes the weight changes seen in these animals and combines these data with those of the oral placebo and 8 mg/kg oral groups reported in detail here. It is clear that even at 0.5 mg/kg the drug has a detectable effect on normal weight gain. At the higher dose levels, far more typical of those employed in most reported behavioral studies of Δ-9-THC in animals, the effect is striking. Experiments employing the drug at these doses should therefore be approached cautiously.

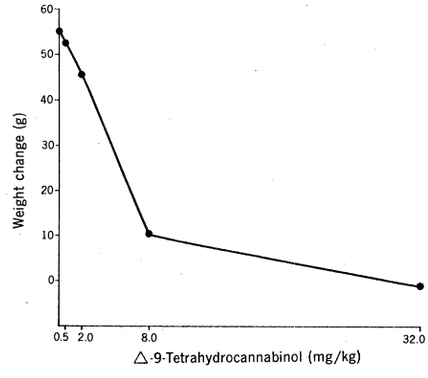

Fig. 2. Mean change in body weight of rats during 30 days of daily oral administration of Δ-9-THC or placebo.

The persistence of this hypophagia throughout the entire 30 days of drug administration may reduce somewhat the discrepancy between animal data

on tolerance to Δ-9-THC and the observations of "reverse tolerance" in human marihuana users. The behavioral effects of Δ-9-THC (or cannabis extract) in animals have most often been reported to subside after two to ten daily doses (*8*), but among human marihuana users, experienced users tend to require less, not more, marihuana to report effects than do naive users (*4*, *9*). Our data suggest that the tolerance typically shown by animals is behavioral rather than pharmacological. That is, the physiological reactions to the drug may change very little with repeated doses, but the animal is eventually able to perform despite these actions. It is possible that something similar occurred over the last few days of drug administration in the present study, since all drug groups showed substantial weight gains during this period. However, subnormal weight gains were seen for at least 3 weeks, far beyond the periods required for recovery of rope climbing or bar pressing in other experiments (*4*, *9*). The pronounced tolerance to Δ-9-THC in animals might then be analogous to the reported ability of experienced human marihuana smokers to perform adequately on some laboratory tests while reporting a normal "high" (*10*).

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Growth Effects of Vanadium in the Rat

Abstract. *Vanadium is necessary for growing rats raised inside trace element–controlled, all plastic isolators on a highly purified amino acid diet. Addition of vanadium to the diet enhances growth by over 40 percent. A nearly optimum effect is obtained with 10 micrograms of vanadium per 100 grams of diet in (0.1 part per million), supplied in the form of sodium orthovanadate, as seen from series of tests with levels ranging from 1 to 5 micrograms per 100 grams of diet. Different vanadium compounds show different potencies: sodium orthovanadate was effective, metavanadate less active, and pyrovanadate without activity. Tetravalent vanadium, supplied as vanadyl sulfate or acetate, was utilized but produced smaller responses. The amounts of vanadium required are those normally found in tissues and nutrients.*

Biological functions of vanadium have attracted much attention in the past (*1–4*). It has been suspected to play a physiological role in higher animals, but proof of its essentiality has been lacking. We have found that vanadium exerts a pronounced growth promoting effect in rats which are kept in a trace element–controlled environment and fed a highly purified amino acid diet (*5*). The element is present in tissues of higher animals, including man, at average levels of 0.1 ppm, and in plants at a mean level of 1 ppm of dry matter. Even though the vanadium content of seawater is remarkably low (0.0003 to 0.003 ppm) (*6*), most marine invertebrates contain 1 to 3 ppm (dry weight), and some accumulate extraordinarily large amounts in blood and tissues (*7*, *8*).

Thus far, an essential requirement for vanadium appears to have been clearly demonstrated only in two microorganisms: the mold *Aspergillus niger* (*9*), and the green alga *Scenedesmus obliquus* where it may be concerned with photosynthesis (*10*). However, a need for vanadium has also been demonstrated for a thermophilic yeast, *Candida slooffii*, when grown at high enough temperatures (*11*). Growth effects were also seen in higher plants, but they have been related to the capacity of vanadium to substitute for molybdenum as a catalyst of nitrogen fixation in *Azotobacter* and other bacteria (*2*, *12*). That it might be essential for animals could be inferred from the finding that it enhances the development of wing and tail feathers in chicks raised under carefully controlled conditions on a casein-based diet; however, the growth rates of these chicks were

Table 1. Growth response of rats to varying levels of vanadium supplements (sodium orthovanadate, Na_3VO_4). Pooled results of five successive experiments are shown for each dose. Weight gains (grams) are given as means ± standard errors of the means. The total increase is given as the percentage gain over a 21- to 28-day period.

Dose (μg/100 g of diet	Unsupplemented controls		Supplemented animals		Total increase (%)	*P*
	Rats (No.)*	Average daily weight gain	Rats (No.)	Average daily weight gain		
1	7	1.05 ± 0.08	7	1.27 ± 0.10	21	†
5	16	1.04 ± 0.08	16	1.38 ± 0.08	33	< .01
10	6	1.02 ± 0.14	7	1.38 ± 0.07	35	< .05
25	14	0.87 ± 0.10	14	1.21 ± 0.09	41	< .02
50	6	1.02 ± 0.14	7	1.49 ± 0.12	46	< .02

* A total of 37 rats served as controls in five successive experiments. † Not significant.