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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References and Notes

1. Y. Gaoni and R. Mechoulam, *J. Am. Chem. Soc.* **86**, 1646 (1964).
2. The Δ-9-tetrahydrocannabinol used in this study was obtained from Dr. J. A. Scigliano of the Center for Studies of Narcotic and Drug Abuse of NIMH. The material was from batch No. SSC-61516 of synthesized Δ-9-THC and was assayed to be 95 percent pure.
3. F. Lipparini, A. DeCarolis, V. A. Longo, *Physiol. Behav.* **4**, 527 (1969); D. Holtzman, R. A. Lovell, J. H. Jaffe, D. X. Freedman, *Science* **163**, 1464 (1969); J. C. Garriott, L. J. King, R. B. Forney, F. W. Hughes, *Life Sci.* **6**, 2119 (1967).
4. A. T. Weil, N. E. Zinberg, J. M. Nelson, *Science* **162**, 1234 (1968).
5. The Δ-9-THC was received dissolved in ethanol in a concentration of 0.20 g/ml and was subsequently diluted with propylene glycol so that each dose was approximately 0.2 ml in volume. Concentrations used, therefore, were 6.6 mg/ml for the intraperitoneal rats, and 13.2 for the oral ones. Placebos consisted of propylene glycol and ethanol in amounts equal to those in the corresponding drug solution. Exact dose volume for each animal was determined from his weight on the first day of drug administration and was held constant in spite of changes in the animals weight.
6. H. Barry, J. L. Perhach, R. K. Kubena, *Pharmacologist* **12**, 258 (1970).
7. C. L. Scheckel, E. Boff, P. Dahler, T. Smart, *Science* **160**, 1467 (1968); D. E. McMillan, L. S. Harris, J. M. Frankenheim, J. S. Kennedy, *ibid.* **169**, 501 (1970); R. K. Siegel, *Psychopharmacologia* **15**, 1 (1969); E. S. Boyd, E. D. Hutchinson, L. C. Gardner, D. A. Merritt, *Arch. Int. Pharmacodyn.* **144**, 533 (1963).
8. E. A. Carlini, *Pharmacology* **1**, 135 (1968); D. E. McMillan, L. S. Harris, J. M. Frankenheim, J. S. Kennedy, *Science* **169**, 501 (1970); M. B. Black, J. H. Woods, E. F. Domino, *Pharmacologist* **12**, 258 (1970).
9. D. E. Smith and C. Mehl, in *The New Social Drug*, D. Smith, Ed. (Prentiss-Hall, Englewood Cliffs, N.J., 1970), p. 63.
10. D. F. Caldwell, S. A. Meyers, E. F. Domino, P. E. Merriam, *Percept. Motor Skills* **29**, 755 (1970).

11. We thank Mr. Doug Tang of the Statistics Department, Division of Biometrics, Walter Reed Army Institute of Research, for advice and assistance in the analysis of the data; also, Dr. Paul Hildebrandt, Chief, Department of Veterinary Pathology, Division of Veterinary Medicine, Walter Reed Army Institute of Research, for his painstaking pathology reports. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

27 July 1971

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.

not affected (*13*). In its strongly reduced, bivalent condition vanadium has been found in a porphyrin complex of the feathers of some South African birds (*14*).

The experiments reported here were carried out with the trace element–controlled environment system created in our laboratory to study unrecognized trace element–deficiency diseases (*15*). Trace element contamination is kept at a minimum by ultraclean, trace element sterile isolators, and by amino acid diets composed of highly purified individual ingredients (*16*). The system permits definitive experiments on unidentified trace element requirements, provided that sensitive analytical methods are available to determine the elements in question. By this technique we recently demonstrated that tin is essential for the growing rat (*17*).

Animals on the basal amino acid ration inside the controlled isolator show a variety of deficiency symptoms. They grow poorly, lose hair, develop seborrhea-like conditions, lose tonicity, and show other changes. The amino acid diet supplies all known essential elements at adequate, balanced amounts (*18*). It is deficient in more than one additional trace element, which has been shown by chemical fractionation of yeast ash. Besides tin, vanadium is one of these missing elements.

In tests carried out from 1965 to 1969 with less advanced techniques, positive but nonsignificant effects of 10 to 12 percent were seen occasionally with supplements of 5 to 25 μg of vanadium per 100 g of ration, added to the diet as vanadyl chloride or sodium orthovanadate. Neutron activation analysis revealed that the organic components of the diet (amino acids, sucrose, fat, and vitamins) were very low in vanadium, but the inorganic salts were a source of vanadium contamination (*19*). Significant effects of vanadium were demonstrated upon subsequent improvements in the isolator (*16*), weaning procedure, and composition of the salt mixture. Inbred, littermate, male, weanling Fischer 344 rats were used (*20*). Animals inside the isolator were housed in individual plastic cages. They did not come in contact with metal, glass, rubber, dust, or caretaking personnel. Each experiment consisted of four groups of eight animals. As an additional control to each group in the isolator, six rats were maintained in metal cages under conventional conditions on each of the test diets. The animals were weighed and evaluated as to their appearance at 3- to 4-day intervals. Growth rates and standard errors of the means were computed by covariance analysis of the weight gains. The experiments were terminated after 26 to 28 days, at which time the rats were autopsied.

Table 2. Effect of different vanadium salts on growth of rats in an "ultraclean" environment.

Dose (μg/100 g of diet)	Rats (No.)	Average increase (%)	*P*
Sodium metavanadate, $NaVO_3 \cdot 4H_2O$			
5	8	8	
5	8	18	
Sodium pyrovanadate, $Na_4V_2O_7$			
5	8	−8	
10	8	9	
Vanadyl sulfate, $VOSO_4$			
1	8	4	
5	7	27	< .05
Vanadyl acetate, $VO(CH_3COO^-)_2$			
5	7	16	
10	8	21	< .05
25	8	18	< .10

The diet, based on an optimal mixture of L-amino acids, was identical to that described (*17*, *21*), except for the salt mixture which was simplified to eliminate potential trace contaminants (*22*). The revised salt mixture, contained in the diet at a 3.5 percent level, and the trace element supplement are shown in (*23*). All diets contained 100 μg of tin per 100 g of ration, given as stannic sulfate, $Sn(SO_4)_2 \cdot 2H_2O$. Vanadium compounds to be assayed were added to the diet as freshly prepared aqueous solutions. All diets were freshly prepared and replaced at weekly intervals.

Significant growth effects of vanadium were obtained regularly under the described conditions. Most experiments were carried out with sodium orthovanadate, Na_3VO_4, which appeared to give the best results. To obtain a dose response curve, a series of five consecutive tests with dose levels varying from 1 to 50 μg of vanadium per 100 g of diet (0.01 to 0.50 ppm) (Table 1). A 21 percent increase in growth, obtained by the 1 μg supplement, was not statistically valid; but 5 and 10 μg produced significant, nearly optimum responses. The maximum effect of over 40 percent was obtained with the 25 and 50 μg dose levels. None of the other deficiency symptoms seen inside the isolators were significantly affected by vanadium supplementation. Animals kept on identical diets in (vanadium-containing) stainless steel cages under conventional conditions had higher basal growth rates and showed no effect of vanadium supplementation to their diet.

Experiments with other vanadium compounds (Table 2) showed that, in analogy to findings with selenium (*24*), chromium (*25*), and tin (*17*), different vanadium compounds vary in potency. Differences in activity were observed between the various salts derived from vanadium pentoxide, V_2O_5. These are formally analogous to phosphate salts derived from P_2O_5, but the vanadates have little in common chemically or structurally with the phosphates (*26*). Sodium metavanadate at 5 μg per 100 g of ration produced only marginal responses. It may be effective if given in higher amounts since it can be slowly converted to the orthovanadate. Sodium pyrovanadate at 5 and 10 μg levels was clearly inactive. The pyrovanadate ion, $V_2O_7^{4-}$, the most stable hydration product of V_2O_5, is sluggish in oxidation-reduction reactions and in establishing equilibria with the other ions derived from V_2O_5. The results indicate that it is poorly utilized.

Two derivatives of tetravalent vanadium, the steadiest oxidation state of the element, were effective at 5, 10, and 25 μg per 100 g of ration, even though the observed increases in growth rates did not appear as great as those seen with sodium orthovanadate. Both compounds tested, vanadyl sulfate and vanadyl acetate, are derivatives of oxovanadium, VO^{2+}, considered one of the most stable biatomic ions in existence; it can persist unchanged in a large variety of chemical reactions. Whether the two lower oxidation states of vanadium can satisfy the vanadium requirement of the rat is not known. However, derivatives of bivalent and trivalent vanadium have been found in nature (*14*, *27*). These oxidation states have strong reducing activities, but within the organism they may be stabilized by complex formation with organic ligands which would change the oxidation potential.

The amounts of vanadium needed to elicit the growth response in rats are physiological, that is, they are within the range of concentrations found naturally in foods, feeds, and tissues of higher animals (*4*). It appears as if the physiologically essential levels of vanadium lie at or below 0.1 ppm of dry matter: Rats require in their diets approximately as much vanadium to grow as is needed in the media for *Aspergillus niger* and *Scenedesmus obliquus* to support growth; the effective concentration in these media was in

the order of 10^{-8}, equivalent to 0.1 ppm of dry weight in the medium. The amount of vanadium required by the rat is also quite close to that calculated as the daily vanadium intake for man. A rat weighing 75 g needs 1 to 2 μg of vanadium per day (25 μg per 100 g of diet) for an optimal growth response, while a human with an average weight of 75 kg has been estimated to consume 2 mg of vanadium daily. This calculation is based on dietary surveys and "a well-balanced diet." The total amount in man's body is estimated to be 17 to 43 mg (*4*).

The chemistry of the natural occurring derivatives of vanadium is unknown. Much of the vanadium must be present in low molecular form since it tends to occur in fats and oils. The properties of vanadium make it likely that its biological function is related to oxidation-reduction mechanisms. Effects of vanadium have been observed in many systems. Notably, it catalyzes the in vitro oxidation of phospholipids by mammalian liver protein fractions (*28*). It is highly active as a catalyst in the nonenzymatic oxidation of catecholamines, dihydroxyphenylalanine, and 5-hydroxyindoles (*29*). At high dose levels it inhibits cholesterol synthesis (*30*) and lowers the amounts of phospholipid and cholesterol in the blood (*31*). It has also been reported to inhibit the development of caries through stimulation of the mineralization of teeth (*32*). Whether any of these phenomena are related to the normal function of vanadium in intermediary metabolism remains to be established. In a review on vanadium in man (*4*), Schroeder *et al.* stated in 1963: "No other trace metal has so long had so many supposed biological activities without having been proven to be essential." The above data show that vanadium is indeed essential for the growing rat.

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References and Notes

1. Earlier work was surveyed by D. Bertrand, *Bull. Amer. Museum Natur. Hist.* **94**, 407 (1950).
2. A. Nason, in *Trace Elements*, C. A. Lamb, O. G. Bentley, J. M. Beattie, Eds. (Academic Press, New York, 1958), pp. 269–296.
3. T. G. Faulkner Hudson, *Vanadium Toxicology and Biological Significance* (Elsevier, New York, 1964).
4. H. A. Schroeder, J. J. Balassa, I. H. Tipton, *J. Chron. Dis.* **16**, 1047 (1963).
5. K. Schwarz and D. B. Milne, *Fed. Proc.* **30**, 462 (1971).
6. V. M. Goldschmidt, in *Geochemistry*, A. Muir, Ed. (Clarendon, Oxford, 1958); M. Ishibashi, *Bull. Inst. Chem. Res. Kyoto Univ.* **24**, 68 (1951); see also R. Söremark *J. Nutr.* **92**, 183 (1967).
7. M. Henze, *Z. Physiol. Chem.* **72**, 494 (1911); see also (*1*).
8. Many ascidians (sea squirts belonging to the family of tunicates), some holothurians (sea cucumbers), and one mollusk (*Pleurobranchus plumula*) accumulate up to 1900 ppm (*1*). In ascidians, vanadium is located primarily in blood, where it can reach a concentration of 4 percent of the dry weight. The element is contained in green blood cells, vanadocytes, in the form of a sulfate-chromoprotein complex, vanadochrome. It is in its trivalent condition. The function of vanadium in these species is thought to be related to reduction of CO_2 or sulfate rather than to O_2 transport. Vanadium is accumulated from the soil by the fly mushroom, *Amanita muscaria*, which can contain up to 180 ppm (dry matter) of the element. No other *Amanita* species, fungus, or plant has been found to have this capacity (*1*).
9. D. Bertrand, *Ann. Inst. Pasteur* **68**, 226 (1942).
10. D. I. Arnon and G. Wessel, *Nature* **172**, 1039 (1953); D. I. Arnon, in *Trace Elements*, C. A. Lamb, O. G. Bentley, J. M. Beattie, Eds. (Academic Press, New York, 1958), pp. 1–32.
11. I. Roitman, L. R. Travassos, H. P. Azevedo, A. Cury, *Sabouraudia* **7**, 15 (1969).
12. C. K. Horner, D. Burck, F. Allison, M. S. Sherman, *J. Agr. Res.* **65**, 173 (1942); H. Takahashi and A. Nason, *Biochim. Biophys. Acta* **23**, 433 (1957).
13. L. L. Hopkins, Jr., and H. E. Mohr, in *Newer Trace Elements in Nutrition*, W. Mertz, Ed. (Dekker, New York, in press); *Fed. Proc.* **30**, 462 (1971).
14. It is bound to uroporphyrin III, replacing copper in turacin. Vanadium-porphyrin complexes are also found in large quantity in fossil fuels such as petroleum, asphalt, shale, and possibly coal. These materials frequently contain high levels of vanadium.
15. J. C. Smith and K. Schwarz, *J. Nutr.* **93**, 182 (1967).
16. K. Schwarz, in *Trace Element Metabolism in Animals*, C. F. Mills, Ed. (Livingstone, Edinburgh, 1970), pp. 25–38.
17. K. Schwarz, D. B. Milne, E. Vinyard, *Biochem. Biophys. Res. Commun*, **40**, 22 (1970); K. Schwarz, in *Newer Trace Elements in Nutrition*, W. Mertz, Ed. (Dekker, New York, in press).
18. However, all diets used in these studies were deficient in factor G, a vitamin-like unidentified dietary agent which is necessary for optimum growth of rats on amino acid diets [K. Schwarz, *J. Nutr.* **100**, 1489 (1970)].
19. The basic diet without the salt mixture contained 0.001 ppm of vanadium. An accurate estimate of the amount contributed by the salts was difficult because of matrix effects.
20. Simonsen Laboratories, Gilroy, California. To prevent intake of trace elements from the mothers' feed, chow pellets were removed from the nursing mother on the 17th day postpartum. The young were separated from the mother and shipped to the laboratory on the 19th day. They were kept in plastic cages on the basal amino acid diet for 1 day before initiation of the experiment in the isolator.
21. K. Schwarz, in *Human Ecology in Space Flight*, D. Calloway, Ed. (New York Acad. of Sciences, New York, 1968), vol. 3, pp. 54–69.
22. Growth rates supported inside the isolator by unsupplemented diets containing this salt mixture were slightly lower than those reported (*17*), apparently because of reduced trace element contamination.
23. Composition of salt mixture (percent): $CaHPO_4$, 61.0; K_2HPO_4, 6.73; monohydrate of potassium hydrogen glutamate, 12.6; monohydrate of sodium hydrogen glutamate, 12.15; $MgSO_4$, 6.87; $MnSO_4 \cdot H_2O$, 0.37; $ZnCO_3$, 0.195; and KI, 0.0153. A trace supplement of the following composition was added at a level of 0.1 percent to the diet (percent): $FeSO_4 \cdot 7H_2O$, 12.45; $CuSO_4 \cdot 5H_2O$, 2.36; $MnSO_4 \cdot H_2O$, 6.16; $CrCl_3 \cdot 6H_2O$, 2.56; $CoSO_4 \cdot 7H_2O$, 2.385; MoO_3, 0.304; and lactose, 73 781. Selenium was added as sodium selenite (15. μg of Se per 100 g of diet, dissolved in 1 ml of water).
24. K. Schwarz, *Fed. Proc.* **20**, 666 (1961).
25. ——— and W. Mertz, *Arch. Biochem. Biophys.* **85**, 292 (1959).
26. R. J. H. Clark, *The Chemistry of Titanium and Vanadium* (Elsevier, Amsterdam, 1968), p. 16 and pp. 214–219; see also I. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry* (Interscience, New York, 1966).
27. M. Henze, *Z. Physiol. Chem.* **213**, 125 (1932).
28. F. Bernheim and M. L. C. Bernheim, *J. Biol. Chem.* **128**, 79 (1939).
29. G. M. Martin, E. P. Benditt, N. Eriksen, *Nature* **186**, 884 (1960).
30. D. L. Azarnoff and G. L. Curran, *J. Amer. Chem. Soc.* **79**, 2968 (1957).
31. J. T. Mountain, F. R. Stockell, Jr., H. E. Stokinger, *Proc. Soc. Exp. Biol.* **92**, 582 (1956).
32. C. F. Geyer, *J. Dent. Res.* **32**, 590 (1953).

33. Dr. J. Cecil Smith and Mrs. Elizabeth Vinyard participated in the earlier phase of this work. The technical assistance of David Evans, Maureen Conley, and George El-Bogdadi is acknowledged. Supported by PHS grant 08669 from the National Institute of Arthritis and Metabolic Diseases.

7 June 1971; revised 23 July 1971

Social Behavior of Monkeys Selectively Depleted of Monoamines

Abstract. *Initiated social interactions of* Macaca speciosa *are decreased during the period of treatment with alpha-methyl-*p*-tyrosine, an inhibitor of catecholamine synthesis. The treated animals maintained stable body weights and appeared to be healthy. Similar depletion of indoleamines with* p*-chlorophenylalanine does not change these same observed behaviors in spite of weight loss, hair loss, ataxia, and debilitation in some of the animals.*

The monoamines serotonin, dopamine, and norepinephrine have been implicated in such activities as the regulation of appetite, temperature, sleep, alertness, motor functions, and mood (*1*). In addition, complex disturbances in behavior have been related to an excess or deficiency of one or more of these substances, as suggested, for example, by the recent successful treatment of Parkinson's disease by the dopamine precursor dihydroxyphenylalanine (*2*). Selective experimental depletion of the indoleamines or the catecholamines has only recently allowed study of specific monoamine functions with the use of inhibitors of enzymes required for synthesis. Parachlorophenylalanine (PCPA) effectively decreases synthesis of serotonin (*3*), and alpha-methyl-*p*-tyrosine (AMPT) specifically decreases both dopamine and norepinephrine by inhib-