cells has not yet been established. In contrast to the results obtained with [^{3}H]testosterone, [^{3}H]estradiol (0.1 μg per 100 g of body weight) in male and female rats showed nuclear estrogen concentration not only in gonadotrophs but also in acidophils and chromophobes. Sixty to 80 percent of anterior pituitary cells concentrated estrogen, (*10*), contrary to [^{3}H]testosterone, in which the labeling index of anterior pituitary amounted to about 15 percent. Approximately 60 percent of the gonadotrophs were labeled with the radioactivity after the injection of [^{3}H]testosterone.

The nuclear concentration of radioactivity in gonadotrophs in the present study suggests that such retention of radioactivity is related to the action of androgen, as it has been demonstrated in the ventral prostate and seminal vesicles (*12*). 5α-Dihydrotestosterone (DHT) is retained in prostatic cell nuclei (*12*), and the major component of the radioactivity retained in the pituitary 1 hour after the intravenous injection of 0.1 μg of [1,2-^{3}H]testosterone per 100 g of body weight, into adult male rats castrated 4 weeks before being killed, has also been identified as DHT (*6*). Kniewald and co-workers (*13*) incubated anterior pituitary tissues from normal and castrated adult male rats with [^{14}C]testosterone and found that testosterone is converted into its active metabolite DHT.

This chemical identification of the radioactive label and the autoradiographic differences in localization between [^{3}H]testosterone and [^{3}H]estradiol exclude the possibility that these results after [^{3}H]testosterone injection are due to its conversion to [^{3}H]estradiol. Leavitt *et al.* (*8*) did not find nuclear binding of [^{3}H]testosterone with anterior pituitary homogenate incubated at 23°C. This negative finding is probably attributable to the small fraction of anterior pituitary cells that concentrate androgen.

The autoradiographic evidence for nuclear accumulation of radioactivity by anterior pituitary cells after injection of [^{3}H]testosterone suggests that gonadotrophs are target cells for androgen and that the nuclear concentration of androgen may represent a major event in the direct androgen action on the anterior pituitary. Our results do not exclude the possibility that other cell types in the pituitary may be involved in androgen action. The autoradiographic observations agree with and extend the recent findings of Kamberi and McCann (*2*), Gersten and Baker (*3*), Kingsley and Bogdanove (*4*), as well as those of Debeljuk and co-workers (*14*), which also suggest that estrogen and androgen can act directly on the pituitary gland in addition to the action on the brain.

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Delta-9-Tetrahydrocannabinol: Localization in Body Fat

Abstract. *[^{14}C]Δ^9-Tetrahydrocannabinol (Δ^9THC) was injected subcutaneously in rats every day for 1 to 26 days. Concentrations of Δ^9THC and its metabolites, 11-hydroxytetrahydrocannabinol and 8,11-dihydroxytetrahydrocannabinol, were determined in various tissues. After a single injection, the concentration of Δ^9THC in fat was ten times greater than in any other tissue examined, and persisted in this tissue for 2 weeks. With repeated injection, Δ^9THC and its metabolites accumulated in fat and brain.*

Previous studies have shown that [^{14}C]Δ^9-tetrahydrocannabinol (Δ^9THC) persists in the plasma of man for several days after its intravenous administration (*1*) and that, after a single injection of [^{3}H]Δ^9THC to experimental animals, total radioactivity remained in fat (*2, 3*) and brain (*4*) for several days. A major metabolite of Δ^9THC, 11-hydroxytetrahydrocannabinol (11-hydroxy THC) (*5, 6*), is behaviorally active in animals (*5*) and humans (*7*), whereas 8,11-dihydroxytetrahydrocannabinol (8,-11-dihydroxy THC) has been demonstrated to be a nonactive metabolite (*1, 5, 8*).

Because of the lipophilic nature of Δ^9THC, its persistence in plasma might be due to sequestration in and slow release from fat. In chronic marihuana users the effects of Δ^9THC might result from accumulation of Δ^9THC or an active metabolite in brain. We now describe the selective accumulation and retention of Δ^9THC and its metabolites in fat after single and repeated subcutaneous doses of [^{14}C]Δ^9THC to rats.

Female Sprague-Dawley rats weighing 150 g were injected subcutaneously just below the scapula every other day with 14 μl of an ethanol solution (1 mg/ml, 17.5 μc/mg) of [^{14}C]Δ^9THC (*9*). Forty-four hours after 1, 3, 6, 9, or 13 doses of the THC solution, four rats were decapitated. The brain, lung, and parts of the liver and perirenal fat pads were homogenized, and the Δ^9THC, 11-hydroxy THC, and 8,11-dihydroxy THC were separated and measured by extraction into heptane of various polarities (*10*).

There was a tenfold greater concentration of Δ^9THC in fat than in the other tissues (Fig. 1A), and there was a fourfold increase over the initial concentration in fat with repeated injection. In brain Δ^9THC could not be detected at day 2, but by day 7 could be measured (0.37 ng per gram of tissue), and this concentration doubled by day 27.

The accumulation of 11-hydroxy THC, the active metabolite of Δ^9THC, shows a similar distribution (Fig. 1B) except that its concentration in fat, although higher than that for the other tissues, was less than that of Δ^9THC in fat. In brain, 11-hydroxy THC was undetectable at day 2 but by day 27 reached a concentration of 0.45 ng per gram of tissue.

The accumulation of 8,11-dihydroxy

THC (Fig. 1C) is similar except for fivefold greater accumulation in liver than in lung; 8,11-dihydroxy THC has been shown to be formed readily in vitro in liver but not in lung (*11*).

The retention of Δ^9THC and its metabolites in fat (Fig. 1D) and the other tissues was examined by injection of a single dose of [^{14}C]Δ^9THC and analyzing the tissues periodically over 14 days for Δ^9THC and metabolites. An approximate half-life of 5 days was found for Δ^9THC in fat, while 11-hydroxy THC and 8,11-dihydroxy THC persisted in smaller amounts over 14 days. In liver small amounts (0.44 ng per gram of tissue) of Δ^9THC and its metabolites were present for 14 days, while in lung similar amounts were present for 2 days only.

Estimates were made of the residual unidentified polar metabolites (*12*). After 13 doses of [^{14}C]Δ^9THC, there were negligible amounts in brain, small amounts in fat (0 to 5 ng per gram of tissue) and lung (3 to 10 ng per gram of tissue), and large amounts (30 to 60 ng per gram of tissue) in liver. The amounts of polar metabolites accumulating in liver and lung were greater than the sum of Δ^9THC, 11-hydroxy THC, and 8,11-dihydroxy THC in these tissues.

The disappearance curve for Δ^9THC in the plasma of man (*1*) and of total radioactivity in rats (*2*) shows an initial rapid decline (half-time of minutes) after intravenous administration followed by a long slow phase (half-time of days), suggesting that Δ^9THC is rapidly taken up in tissues or metabolized or both. Since the disappearance curve for total metabolites is also biphasic (*1*), and Δ^9THC is present in plasma for a week after a single tracer dose (*1*), it is probable that tissue sequestration, especially in fat, plays a dominant role in the disposition of Δ^9THC. The importance of fat localization of drugs in explaining their duration of action has been shown for drugs such as thiopental (*13*), dibenamine (*14*), and DDT (*15*). These drugs show a similar biphasic disappearance curve from plasma, a high localization in fat, and a comparable rate of accumulation in fat with repeated administration. DDT reaches maximum levels in fat of man after 1 year of the normal amounts found in food (*16*). If the period of injection of Δ^9THC had been extended over a longer time, the plateau for Δ^9THC accumulation in fat might reach a much higher value than that reported in Fig. 1A. With starvation, DDT concentrations increase in rat brain because of mobilization from fat stores (*17*). It would be of interest to study this phenomenon in those chronic marihuana users who report flashback (*18*).

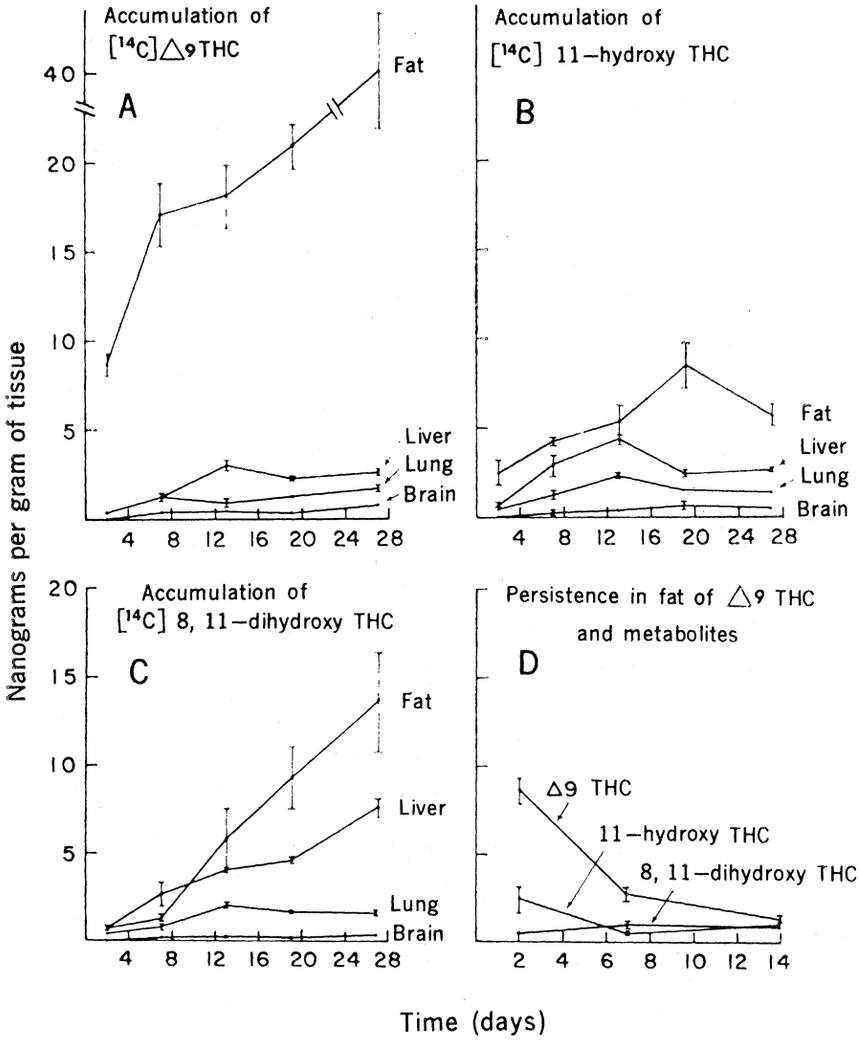

Fig. 1. The distribution of Δ^9THC, 11-hydroxy THC, and 8,11-dihydroxy THC in rat tissues after repeated subcutaneous doses of [^{14}C]Δ^9THC. (A to C) The [^{14}C]Δ^9THC was given every other day for the stated number of days. (D) A single dose of [^{14}C]Δ^9THC was given, and tissues were examined at the times indicated. Results are expressed as mean ± standard error of the mean for four animals at each time point.

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9. A Hamilton microsyringe was used. Examination of the injection site after 13 injections revealed no gross pathological changes, and an ethanol extract of the tissues at the site revealed 500 to 1000 count/min.
10. The tissues were homogenized in three volumes of KH_2PO_4–Na_2HPO_4 buffer (0.05*M*, *p*H 7.0) with a Polytron homogenizer. Four volumes of heptane were added, and the mixture was agitated with a Vortex mixer for 1 minute, then shaken in a mechanical shaker for 30 minutes. After centrifugation at 1500*g* for 10 minutes, the organic extracts were dried with a gentle stream of nitrogen at room temperature to a volume of 0 to 4 ml, to which 1 ml of ethanol and 10 ml of phosphor

were added for determination of radioactivity in a Packard scintillation counter. The samples were counted for 20 to 50 minutes to obtain statistical significance, and correction was made for quenching by channel ratio. The organic extracts used for chromatography were dried completely, then 50 to 100 μl of ethanol was added for application to Eastman silica gel sheets. This heptane extraction was followed sequentially by identical procedures with two other solvent mixtures: heptane and 1.5 percent isoamyl alcohol, and heptane and 3 percent isoamyl alcohol. The heptane extracted 90 percent of the Δ^9THC, 10 percent of the 11-hydroxy THC, and none of the 8,11-dihydroxy THC, whereas the heptane containing 1.5 percent isoamyl alcohol extracted the remaining Δ^9THC, 60 percent of the total 11-hydroxy THC, and 20 percent of the 8,11-dihydroxy THC. The final extraction with heptane and 3 percent isoamyl alcohol recovered the remaining 11-hydroxy THC, and 60 percent of the total 8,11-dihydroxy THC. The amounts of each of the three compounds were determined by simultaneous equations. The precision and specificity of the method was confirmed three times on three thin-layer chromatography systems (hexane : acetone, 3 : 1; chloroform : ethanol, 19 : 1; chloroform : acetone, 9 : 1). The partitions in all four tissues were the same. The standard error of the mean for these procedures was 1.1 percent ($N = 16$).

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12. Two 0.5-ml fractions were taken from each homogenate and from the final residue after the three organic extractions. To each fraction 1.5 ml of NCS solubilizer (Nuclear-Chicago) was added, and the tissue was digested for 1 to 2 days (until a clear solution was obtained). Two drops of a 1 percent solution of $SnCl_2$ were added (to reduce chemifluorescence) plus 1 ml of ethanol and 10 ml of phosphor. There was, however, considerable variation between duplicates.
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Isolation of RNA Virus from Papillary Tumors of the Human Renal Pelvis

Abstract. *Virus particles were detected by electron microscopy in three papillary cancers of the human renal pelvis. Similar particles were seen in cells from all three tumors in primary culture. An RNA virus was isolated from these tumors. The serum of tumor patients contains neutralizing antibodies against virus isolated from two of the tumors. Initial studies suggest that this agent is not a known human RNA virus.*

There is evidence that viruses may play some role in the causation of certain human tumors, such as cancer of the cervix and breast, Burkitt's lymphoma, and sarcomas (*1*). We report here studies of a virus found in papillary transitional cell cancers of the human renal pelvis.

Papillary transitional cell carcinomas of the renal pelvis were removed from three adult males. The tumors (T_1, T_2, and T_3) were minced finely, and were then placed in flasks (area, 25 cm^2) containing RPMI-1640 medium with 20 percent fetal calf serum, penicillin (400 unit/ml), and streptomycin (100 μg/ml). In addition, minced tumors were suspended in RPMI-1640 medium and then passed through a 0.22-μm filter. Throughout the procedures all specimens were handled separately. The filtrates were then placed on cell cultures of human fibroblasts, testicle, and kidney, as well as on cultures of specific pathogen-free (SPAFAS) chicken embryo fibroblasts (CEF). Normal kidney from each tumor patient was subjected to trypsin digestion and was then propagated in cell culture with the same outgrowth medium. Culture medium from these cells was negative for bacteria and mycoplasma. All work was carried out in a laboratory where there had been no previous studies on RNA viruses of animals.

The original tumor specimens, contiguous and grossly normal kidney, and other control (skin and muscle) and tumor tissue culture cells were prepared for electron microscopy by being fixed in 3 percent glutaraldehyde, osmium tetroxide, and uranyl acetate, and then being embedded in Epon. Fine-structure studies of the original tumors showed two types of virus particles. One type was present mainly in extracellular membrane-bound vesicles; the particles had a diameter of 85 to 120 nm, and resembled C-type virus (Fig. 1). The other particles had a diameter of 60 to 90 nm, and resembled the A-type virus described in simian mammary tumors (*2*), human fibrosarcomas, and breast cancer (*3*). We observed virus particles in the cytoplasm, but not in the nucleus. The tumor cells in culture often showed several large membrane-bound vesicles containing virus particles of varying size and morphology (Fig. 2). The tubular epithelium of normal kidney from two patients had occasional A-type virus particles. Budding of the virus particles from the cell membrane was not observed; thus, it is not known how this virus matures. Others who have observed similar virus-like particles in human tumors either failed to demonstrate budding (*3*), or have seen it only after special treatment of the cells in culture (*4*).

The primary tumor cells in culture grew slowly. The cells were small and appeared to be epithelial; cells in several flasks showed foci of spontaneous degeneration characterized by round, refractile cells that detached from the surface of the flasks. Replicate cultures were chronically infected with infectious virus found in supernatant fluids. Normal kidney cells in culture grew well, and spontaneous cytopathology did not occur. Within 7 days after being planted, virus was isolated from the medium of tumor cells in culture by inoculating filtered culture medium from tumor cells onto CEF cultures. Human tumor fluids, when assayed on CEF cultures, had titers of 100 to 10,000 $TCID_{50}$ per milliliter (*5*). The culture fluids from normal kidney cells had no demonstrable virus when assayed on CEF cultures. Primary cells from the three tumors have been maintained in culture for over 6 months and continue to produce infective virus.

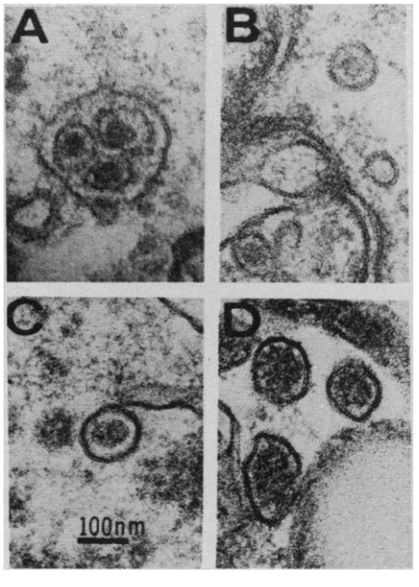

Fig. 1. Virus particles in the original tumor specimens. (A) Extracellular particles in T_1 (× 70,000). (B) Intracellular particles in T_1 (× 70,000). (C and D) Intracellular particles in cells of T_2 (× 70,000).