Table 1. Volatile substances in the mandibular glands of males of five *Camponotus* species (++ denotes major component, + denotes minor component, − denotes not detected).

Species	Methyl 6-methyl salicylate	2,4-Dimethyl-2-hexenoic acid	Methyl anthranilate
C. nearcticus	++	+	+
C. rasilis	−	+	++
C. subbarbatus	++		−
C. noveboracensis	++	−	−
C. pennsylvanicus	++	−	−

tense M^+ ion, and the like) and lost CH_3OH and $COOCH_3$, suggesting it to be an aminobenzoic acid methyl ester. This structure was confirmed by the formation of an *N*-acetate (M^+ 193) that lost both the elements of ketene and C_2H_2O plus CH_3OH. The methyl ester of *p*-aminobenzoic acid gives a loss of CH_3O, rather than CH_3OH, and the retention times of both the para and the meta isomers are incorrect for this third peak. However, the ortho isomer, methyl anthranilate, has an identical retention time and mass spectrum to those of the natural product.

The extract of *C. nearcticus* male heads also contained several long chain fatty acids having α-methyl branching and unsaturation. Of the other species investigated, males of *C. rasilis* produce both the 2,4-dimethyl-2-hexenoic acid and methyl anthranilate, whereas males of *C. pennsylvanicus* and *C. noveboracensis* apparently produce only methyl 6-methyl salicylate as a major component. The mandibular glands of *C. subbarbatus* (*6*) males yield methyl 6-methyl salicylate in addition to several other components, one of which may be either geranic or nerolic acid. These results are summarized in Table 1. Excision of the mandibular glands from the males established that all compounds were present in these exocrine structures. None of these substances could be detected in the heads of either alate females or workers of any of these species.

As the mandibular gland secretion of males of *C. ligniperda* was shown to cause an identical response in females of *C. herculeanus* during swarming (*1*), this exudate cannot be regarded as species-specific or as a species isolating mechanism. Both of these European *Camponotus* species belong in the subgenus *Camponotus*, as do the two North American species, *C. pennsylvanicus* and *C. noveboracensis*, and this investigation has established that these latter two species contain the same major volatile compound, methyl 6-methyl salicylate, in their mandibular glands. The three other species studied, *C. nearcticus*, *C. rasilis*, and *C. subbarbatus*, belong in the subgenus *Myrmentoma*, and all have considerably more complex secretions. In spite of the fact that we have analyzed only five species in a genus with more than 600 species (*7*), it appears that some species have the same major volatile substance, whereas others may have a blend distinctive of the species.

In *C. herculeanus*, it is the male mandibular gland secretion which stimulates the females and induces them to fly off. In contrast, this same secretion of another formicine, *Lasius niger*, elicits an indifferent response from females of its species and, instead, excites the males themselves and causes them to fly off from the nest (*1*). This gross difference in their behavior may not be surprising as *Camponotus* and *Lasius* are not closely related formicine genera. While heads of males of *L. alienus*, *L. neoniger* and *Acanthomyops claviger* contain an indole, possibly skatole, which does not occur in the heads of workers, both workers and males of *L. alienus* and *A. claviger* contain appreciable quantities of volatile substances (*8*). Our investigation of these five *Camponotus* species has shown that it is only the males that produce detectable quantities of volatile substances. In at least the five *Camponotus* species studied these compounds are therefore truly caste-specific. The identification of these novel and caste-specific compounds may provide the means for comprehending the function and significance of these exocrine products in the mandibular glands of many male *Camponotus* species.

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31 August 1972

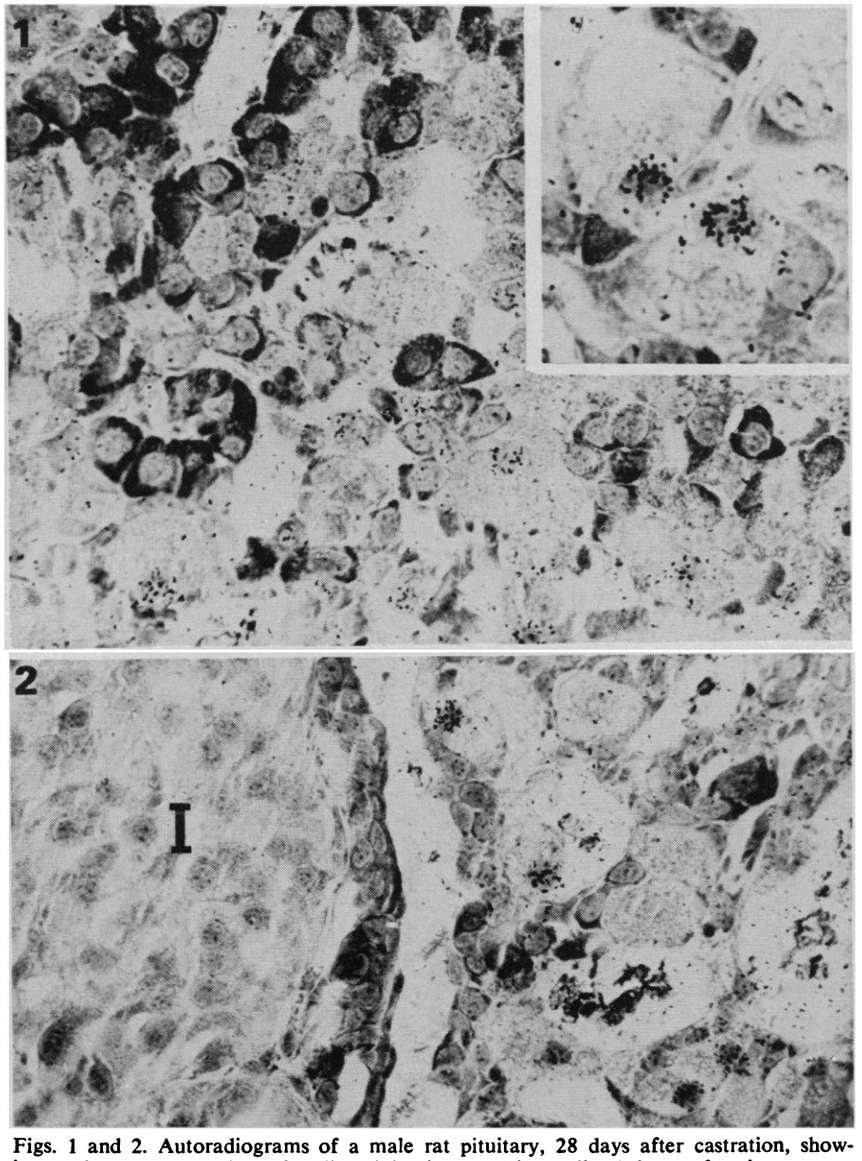
Pituitary Gonadotrophs: Nuclear Concentration of Radioactivity after Injection of [^{3}H]Testosterone

Abstract. *Gonadotrophs and castration cells in the male rat pituitary showed nuclear concentration of radioactivity 1 hour after [1,2,6,7-^{3}H]testosterone injection. Thyrotrophs and acidophils did not retain radioactivity; also the cells of the intermediate and posterior lobes did not accumulate radioactivity. The autoradiographic results suggest a direct and selective action of androgen on gonadotrophs, which contrasts with the action of estradiol which was shown earlier to bind not only to basophils but to acidophils and chromophobes as well.*

It is well established that androgen feedback control of gonadotrophin secretion is mediated through the hypothalamus (*1*). However, it is still debated whether androgen feedback exists at the pituitary level (*1*), since intrapituitary implants of testosterone have yielded controversial results. More recent studies suggest that both estrogen and androgen can act directly on the pituitary (*2–4*). While the pituitary has been shown to selectively concentrate radioactivity after the injection of labeled testosterone (*5–7*), incubation of anterior pituitary homogenate did not result in a nuclear binding of [^{3}H]-testosterone or a radioactive metabolite of it (*8*). By use of the dry-mount auto-

radiographic technique (*9*), estrogen and glucocorticoid target cells in the brain and pituitary have been demonstrated (*10*). With use of the same technique, androgen-concentrating neurons in the brain have also been described (*11*). This report deals with the autoradiographic localization of radioactivity in the male rat pituitary after the injection of [^{3}H]testosterone.

Six adult male Sprague-Dawley rats were used. Four were castrated and killed 4 days later, and two killed after 28 days. Those castrated for 28 days had developed castration cells in the pituitary. [1,2,6,7-^{3}H]Testosterone (specific activity, 91 c/mmole) was dissolved in 10 percent ethanol in isotonic saline, and 0.5 μg of the [^{3}H]testosterone per 100 g of body weight was injected intravenously. The animals were decapitated after 1 hour. The pituitaries were removed and frozen in −180°C propane. Frozen sections (2 and 4 μm) were cut in a Wide Range Cryostat (Harris, Cambridge, Mass.) and freeze-dried with a Cryopump (Thermovac Industries, Copiague, N.Y.). The freeze-dried, unfixed, and unembedded sections were dry-mounted on slides coated with desiccated photographic emulsion (Kodak NTB-3). After autoradiographic exposure for 3 to 6 months at −15°C, the slides were developed, fixed, and stained with Gomori's trichrome and modified aldehyde fuchsin–Masson's trichrome to differentiate cell types in the anterior pituitary. Gomori's trichrome stain differentiates basophils from acidophils, whereas aldehyde fuchsin–Masson's trichrome stain differentiates gonadotrophs, thyrotrophs, and acidophils. We also tried to stain the autoradiograms with the periodic acid–Schiff and orange G method to differentiate cell types in the pituitary, but complete fading of silver grains resulted. The dry-mount autoradiographic procedure has been described (*9*). Autoradiograms of diaphragm, prepared in the same way, served as controls. Anterior pituitary tissue from an untreated castrated male rat was also processed for autoradiograms as a control against positive chemographic artifacts.



Figs. 1 and 2. Autoradiograms of a male rat pituitary, 28 days after castration, showing nuclear concentration of radioactivity in castration cells, 1 hour after intravenous injection of 0.5 μg per 100 g of body weight of [1,2,6,7-^{3}H]testosterone (specific activity, 91 c/mmole). Exposure time, 90 days. Stained with aldehyde fuchsin and Masson's trichrome. Fig. 1 (top). Anterior pituitary, periphery (× 560). Thyrotrophs and acidophils are dark staining cells and do not show concentration of radioactivity. Inset shows two radioactively labeled castration cells, one of which is a "signet ring" cell with the "nebenkern" (× 1200). Fig. 2 (bottom). Intermediate and anterior lobe. Cells of the intermediate lobe (*I*) do not concentrate radioactivity, while gonadotrophs (castration cells) in the anterior lobe show nuclear concentration of silver grains (× 560).

In the pituitary a small number of anterior lobe cells were observed to concentrate radioactivity 1 hour after [^{3}H]testosterone injection (Figs. 1 and 2). The cells of the intermediate (Fig. 2) and posterior lobes did not retain radioactivity. In the lumen of sinusoidal capillaries, radioactivity was retained; however, in the muscle of the diaphragm, used as a control, no selective accumulation of radioactivity was observed. Autoradiograms of pituitary sections revealed that radioactivity accumulated in certain cell nuclei, although some silver grains could be seen over the cytoplasm. When Gomori's trichrome stain was used, the labeled cells were identified as basophils. The cells that showed nuclear concentration of radioactivity were the castration cells (Figs. 1 and 2) or gonadotrophs, as judged by differential staining with aldehyde fuchsin and Masson's trichrome stain. Thyrotrophs, acidophils, and chromophobes did not concentrate and retain radioactivity. Figure 1 shows a typical "signet ring" cell with nuclear labeling of radioactivity.

The autoradiographic results clearly demonstrate that labeling of anterior pituitary cells is confined to gonadotrophs, although some of the gonadotrophs do not show a nuclear concentration of radioactivity. Whether the labeled gonadotrophs are follicle-stimulating hormone or luteinizing hormone

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