except as a decomposition product of some pesticides[26]. We observed both *ortho* and *para* isomers in mouse urine[27], but only the *para* compound appears to demonstrate a strong correlation to estrogen treatment as performed in this experiment for all strains tested.

While probably not possessing specific pheromonal activity by themselves, the compounds reported here, combined in specific ratios and in the presence of other unique volatiles recently discovered by us in mouse urine, may confer any of the releaser pheromonal effects associated with estrous urine. The physiological and behavioral properties of these compounds are currently being investigated[27].

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Respective roles of circulating T_4 and T_3 in control of TSH secretion in severely iodide-deficient rats[1,2]

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Summary. After a 6-month iodide deficiency, Wistar male rats were submitted to a normal iodine diet (20 and 50 µg of ^{127}I daily). Plasma T_3, T_4 and TSH were determined by RIA from 0 to 140 days of iodide refeeding. A highly significant correlation was found between plasma TSH and T_4 concentrations, but not between plasma TSH and T_3 levels. These data suggest that an increase in plasma T_3 alone, up to the normal value, is not able to inhibit TSH secretion. It is only when a certain plasma T_4 concentration is also reached, resulting in further T_3 formation through deiodination, that TSH secretion is inhibited.

In the thyrotrope cells, thyroid hormones modulate the thyrotropin (TSH) secretion[5-7]. However, the respective roles of circulating thyroxine (T_4) and triiodothyronine (T_3) levels in this feedback mechanism are still controversal. The particular experimental conditions of iodide refeeding in severely iodide-deficient rats allow us to study this process. Indeed, during the first days of iodide refeeding, only T_3 is newly synthesized; T_4 appears later. The present results show that in severely iodide-deficient rats under conditions of iodide refeeding, when a normal level of plasma T_3 is reached it is not able by itself to inhibit TSH secretion. The role of T_3 should not be neglected, but TSH secretion probably starts to decrease only when the T_4 concentration also increases to reach a crucial level, which after T_4 deiodination will provide a further T_3 concentration in the thyrotrope cells.

Materials and methods. Wistar male rats received a low iodide diet during 6 months (LID Remington and distilled water). During the last 2 months of LID, propylthiouracil (PTU) was added. At the end of this treatment, i.e., on day 0, a normal iodide diet was reestablished with 2 doses of iodide in drinking water: 0.9 and 2.27 µg ^{127}I/ml, i.e., approximately 20 or 50 µg ^{127}I/day/rat, and the PTU treatment was stopped. The rats were sacrificed on days 0, 1, 2, 4, 8, 12, 16, 30, 45, 80 and 140 of normal iodide diet. Two control groups received a Remington diet without PTU, and drinking water containing 0.9 or 2.27 µg ^{127}I/ml during 8 months. After sacrifice, blood was collected and centrifuged, and plasma was frozen at −20 °C. Plasma T_3, T_4 and TSH concentrations were determined by radioimmunoassay as previously described[8]. The results were analyzed by Student's t-test.

Results. For the first 4 days of iodide refeeding (50 µg daily) (fig. 1), T_3 and T_4 concentrations remained as low as on day 0. Between days 4 and 8, plasma T_4 started to increase slightly but not significantly, whereas T_3 rapidly reached its control value. From days 8 to 12, plasma T_3 remained at its normal level, whereas T_4 was higher than on

day 0 and reached the control value on day 30. Plasma TSH concentrations remained very high during the first 8 days of iodide refeeding, 10 times the control value and similar to day 0. Thereafter, they decreased abruptly, being significantly different from day 0 at day 12, and attaining their control value on day 30. Similar results were observed in rats which received 20 µg of iodide daily, but with a slight delay, normal plasma TSH ant T_4 values being reached on day 45.

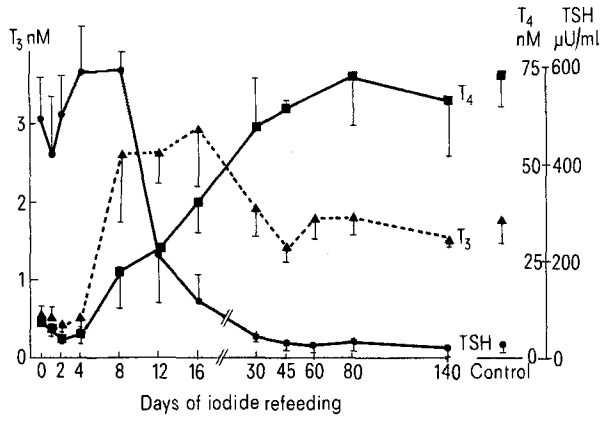

Figure 1. Evolution of plasma T_3 (▲), T_4 (■), TSH (*) concentrations during iodide refeeding (50 µg daily) in previously iodide-deficient rats. Day 0 is the last day of 6 months of iodide deficiency. Each point represents the mean ± SD of determinations performed in groups of 9 animals, except for days 45 and 140 when groups had 6 animals.

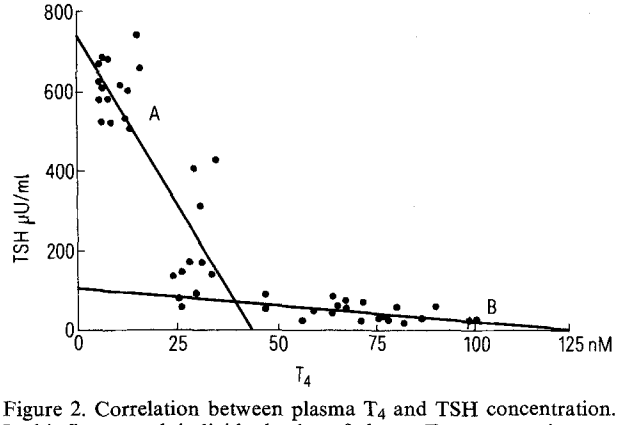

Figure 2. Correlation between plasma T_4 and TSH concentration. In this figure, each individual value of plasma T_4 concentration was plotted against plasma TSH concentration. The values were taken from fig. 1 and pooled with those obtained in group 20 (20 µg iodide daily). Curves A and B display linear regression ($y = -17.4x + 737$, $r = 0.81$, $p < 0.001$, 26 values; $y = -0.82x + 102$, $r = 0.566$, $p < 0.01$, 20 values). For T_3, equations were respectively: $y = -142.7x + 652$, $r = 0.603$, $p < 0.01$; $y = 32.1x + -13$, $r = 0.47$, $p < 0.05$.

Versus day 0	TSH	T_4	T_3
Day 0–4	NS	NS	NS
8	NS	NS	$p < 0.05$
12	$p < 0.02$	$p < 0.001$	$p < 0.001$
16–140	$p < 0.001$	$p < 0.001$	$p < 0.001$
Versus control	**TSH**	**T_4**	**T_3**
Day 0–4	$p < 0.001$	$p < 0.001$	$p < 0.001$
8	$p < 0.001$	$p < 0.001$	NS
12	$p < 0.001$	$p < 0.001$	NS
16	$p < 0.001$	$p < 0.005$	$p < 0.02$
30–140	NS	NS	NS

Plasma T_4 concentrations were plotted against plasma TSH concentrations for both iodide intakes. A highly significant correlation ($r = 0.81$) was obtained (fig. 2) while a less significant correlation was found between plasma TSH and T_3 ($r = 0.60$).

Discussion. For the first 8 days of iodide refeeding, TSH secretion remains at the same high level as on day 0, but 2 phases can be distinguished during this latent period of inhibition of TSH secretion. From day 0 to day 4, T_3 and T_4 neosecretion being not yet established, the negative feedback control cannot start. This physiopathological situation is due to the block of thyroid hormone synthesis by the high level of thyroid iodide during this initial phase[9]. From day 4 to day 8, thyroid hormone neosecretion appeared very intensively for T_3, and more slowly for T_4. Therefore, between days 4 and 8, with a normal T_3 concentration, plasma TSH levels do not change because the concentration of plasma T_4 has not yet changed significantly; it is only when T_4 increases significantly on day 12 that TSH starts to decrease with the same T_3 concentration. Thus the normal level of plasma T_3, by itself, is not able to induce the negative feedback control.

Fukuda et al.[10], using a similar protocol, did not find such a latency; plasma TSH concentrations decreased as soon as iodide refeeding started. This discrepancy is probably due to the fact that these authors used a less severe iodide deficiency without PTU. A defect in peripheral T_4 deiodination[11] cannot explain the delay in thyroid hormone action, since the absence of feedback control is temporary. The possible role of a decreased number of pituitary T_3 nuclear receptors as demonstrated in thyroidectomized rats[12] is unlikely since it has been shown that as little as 1 h after a T_3 injection, the concentration of nuclear receptors returned to normal and TSH secretion was inhibited[12].

In the present study, the inhibition of TSH secretion only started between 8 and 12 days of iodide refeeding, when plasma T_4 concentration also began to increase significantly, reaching a high enough level to provide the thyrotrope cells with further amounts of T_3 from T_4 deiodination[6]. The role of T_4 is also confirmed by the fact that a highly significant correlation exists between plasma TSH and plasma T_4 concentrations, but a less significant one between circulating levels of TSH and T_3. This experimental hormonal pattern could explain in human pathology why normal T_3 and low T_4 plasma levels are associated with high TSH plasma concentrations[13].

In conclusion, in iodide-deficient rats, the return to a normal level of circulating T_3 alone appears not to be able to inhibit the TSH secretion. The negative feedback mechanism is established only when an adequate T_4 level, not necessary as high as the normal value, B reached in addition to a normal T_3 concentration; this probably results in the provision of a higher T_3 concentration at the thyrotrope cells after T_4 deiodination.

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Identification of a testosterone-dependent unique volatile constituent of male mouse urine: 7-*exo*-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]-3-octene

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Summary. Investigations regarding the chemical composition of the volatiles in male mouse urine have recently enabled the structural elucidation of a hitherto unreported urinary component, 7-*exo*-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]-3-octene. This compound's uniqueness to mouse urine and its dependence on testosterone levels in the male suggest its probable role as a mouse pheromone or pheromone adjuvant.

Chemical messengers appear to play an important role in the overall social behavior and reproductive function of *Mus musculus*, the common house mouse[4-7]. Although the mouse has a variety of known and potential sources of chemical messengers, such as various glands, urine has been the most extensively studied source of primer pheromones and other intraspecific chemical signals. Estrus synchronization[8,9], puberty acceleration[10], pregnancy block[11-13], group and individual recognitions[7], sexual attraction[4,11,15], aggression[4,5,16], fear and stress[17,18], histocompatibility-related mating preference[19], etc., now appear to be well documented and traced to urine as a source.

Production of the primer pheromones in the male mouse is under androgen control. Because of certain similarities in the 3 primer effects, i.e., estrus synchronization, puberty acceleration, and the pregnancy block, Bronson[4] suggested that they could be mediated by the same substance(s). However, some disagreement about volatility of these messengers exists. Transport of estrus-synchronizing activity (Whitten effect) through a wind tunnel[20] and detection of the pregnancy-blocking activity (Bruce effect) in a condensed urine vapor by Hoppe[21] support the thesis of pheromone's volatility, whereas Marchlewska-Koj[22] associates both types of activity with larger (non-volatile) urinary constituents. Vandenbergh et al.[23,24] also demonstrated that the puberty-accelerating principle was due to a non-volatile fraction of the male mouse urine.

Studies in our laboratory[25] seem to indicate that the results of the above investigators may not be as contradictory as they appear to some. Further separation and chemical characterization[25] of the fraction responsible for puberty acceleration in female mice revealed that a) the active fraction contains non-volatile peptides, as suggested by Vandenbergh et al.[23]; and, b) it is associated with smaller molecules and retains the 'mousy' odor. Thus, the possibility of a volatile pheromone associating with a larger non-volatile adjuvant molecule needs to be considered.

While investigating volatile urinary profiles of the male mice, we have identified structurally a hitherto unreported constituent, 7-*exo*-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]-3-octene. This compound is unique to mouse urine[26] and is contained in the fraction active for puberty acceleration[25]; it displays a strong dependence on testosterone levels in the male mouse as shown in figure 1. The properties suggest a probable role as a pheromone or pheromone adjuvant.

The structure shown in figure 2 was deduced from chromatographic studies and 3 sources of spectral data and verified by synthesis.

Male mice, 1-6 months old, of BALB/cWT strain were maintained on a standard diet. Urine collections were performed with groups of 4-5 animals at the same time, using plastic metabolism cages. The urine collection vessels were cooled by dry ice, so that the specimens were immediately frozen. Collections from the same animals were obtained before and after castration, and for 20 days following the testosterone implantation (4 mm plastic tubes containing the hormone[27] grafted under the back skin).

Spectral information

Technique	Data
GCMS (quadrupole)	Integral mass (relative intensity)
	154 (12), 136 (8), 125 (26), 121 (8),
	111 (52), 97 (21), 96 (29), 95 (48), 83 (26),
	81 (23), 57 (32), 43 (100), 41 (22)
GC-HRMS	Exact mass (elemental composition)
(double focusing)	154.0986 ($C_9H_{14}O_2$)
	136.0914 ($C_9H_{12}O$)
	121.0685 (C_8H_9O)
	111.0817 ($C_7H_{11}O$)
	97.0620 (C_6H_9O)
	96.0537 (C_6H_8O)
	95.0466 (C_6H_7O)
	83.0473 (C_5H_7O)
	81.0322 (C_5H_5O)
GC-FTIR (vapor phase)	Major IR band frequency
	3051 cm^{-1}
	2970
	1454
	1394
	1254
	1196
	1045
	1026
	972
	860