Res. Ltd (London). Free and bound fractions were separated using charcoal-dextran. Intra- and inter-assay coefficients of variation were 8% and 17%, respectively.

Statistical analysis were performed with Student's t-test or with a two-way analysis of variance. All data were log, transformed before their statistical evaluation.

Results. The results of the first experiment are indicated in table 1. No effect of the number of animals per cage on body and testes weights was found. Likewise, both experimental groups showed the same basal serum testosterone. Analysis of variance revealed a significant effect of hCG administration ($p < 0.001$) but not of previous housing conditions on serum testosterone (fig. 1).

The results of the second experiment are depicted in table 2. Mice housed in groups of 24 per cage showed lower body weights than mice housed in groups of four per cage ($p < 0.05$). Neither adrenal nor testis weights were altered by housing conditions. Figure 2 shows the effect of acute noise on circulating testosterone in the three housing conditions. A significant effect of acute noise exposure ($p < 0.01$) but not of previous housing conditions on testosterone secretion was found.

Discussion. Our results indicate that the number of animals per cage during the post-weaning period did not alter either testis weight or testosterone secretion in male mice. In the first experiment both basal serum testosterone and its response to hCG were the same in both housing conditions. Therefore, no evidence for an altered responsiveness of Leydig cells to gonadotropin was found. These results were confirmed in the second experiment in which three different population densities were used. Neither basal testosterone nor its fall after acute noise exposure differed in the three experimental groups. Recently, it has been reported that the developmental pattern of testosterone secretion was altered in mice raised in high population densities; however, neither fertility nor the growth of the reproductive organs was affected at adulthood[7].

Although it is well accepted that crowding induces inhibition of reproductive processes and testosterone secretion[1-4], these effects are probably restricted to the post-puberal period in which dominance and hierarchies would be established. Thus, adult mice housed in groups together with non-familiar mice show frequent signs of physical injuries and these are exacerbated by increasing population densities. In contrast, we have not observed any sign of physical injuries in mice crowded in the post-weaning period.

Acute noise depressed serum testosterone in all mice irrespective of their housing conditions, suggesting that sensitivity to stress-induced inhibition of testosterone secretion was not modified by crowding. Hampered testosterone secretion after acute stress is a well known fact[9-13] and could be due to the inhibition of Leydig cells responsiveness to gonadotropin[14].

In conclusion, we have found that post-weaning crowding did not affect either the weight of adrenals and testes or serum testosterone. Using the same experimental design and animals of the same strain, age, and sex we have found that crowding neither modified adrenal weight and basal serum corticosterone nor increased fighting, in accordance with the results obtained in the present work. However, an increased corticosterone response to some additional acute stresses was found in crowded mice, indicating that post-weaning superpopulation affects some endocrine systems. A reevalution of the effects of crowding on the endocrine system is needed taken into account both the age at which crowding was started and the housing conditions before crowding.

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Inhibition of TSH-stimulated thyroid hormone release and potentiation of TRH-stimulated TSH release by indomethacin in perifusion systems of rat thyroids and pituitaries

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Summary. Using indomethacin (Ind), a prostaglandin synthesis inhibitor, in vivo experiments in rats and in vitro experiments with perifusion systems of rat thyroids and pituitaries were conducted. After 35 days of intragastric infusion of Ind, serum TSH levels were markedly increased, the thyroid was swollen and, as a consequence, T_3 and T_4 levels were normal. The T_3 release from perifused rat thyroids under continuous stimulation with 10 mU/ml TSH was inhibited significantly ($p < 0.01$) by 1.0×10^{-6} M Ind. On the other hand, the TSH release from perifused rat pituitaries under TRH stimulation was enhanced conspicuously by Ind. It was concluded that Ind decelerated thyroid hormone release from the thyroid and accelerated TSH release from the pituitary in perifusion systems.

Key words. Rat thyroid; rat pituitary; thyroid, rat; pituitary, rat; thyroid hormone release; indomethacin.

It has been suggested that prostaglandins (PGs) might mediate the effects of some hormones on the adenylate cyclase activity of their target organs[1,2]. It is well known that PGs in high concentration exist in the human thyroid[3], and that levels of endogenous PGs are lowered by PG synthesis inhibitors such as indomethacin (Ind) and aspirin[4-6]. However, the mechanisms of action of PGs on the thyroid function have not yet been clearly characterized.

In the present study, we administered Ind to rats and conducted perifusion experiments with rat thyroids and pituitaries to ascertain the effects of endogenous PGs on the release of thyroid hormones (T_3 and T_4) and TSH.

Materials and methods. Male Sprague-Dawley rats (190–240 g) which were fed laboratory chow were used in all experiments of the present study. TRH was obtained from Tanabe Pharmaceutical Co. (Osaka, Japan). Ind, bovine serum albumin, T_3 and T_4 were purchased from Sigma Chemical Co. (St. Louis, MO). TSH (Thytropar) was a product of Armour Pharmaceutical Co. (Chicago, IL). All other reagents used were of analytical grade.

Experiments in vivo. In one group of 4 rats, 1.25 or 5.0 mg/kg B.W. of Ind suspended in 0.5% acacia was infused into the stomach through a tube once a day for 35 days. In the control group, 0.5% acacia alone was used. The animals were laparotomized under ether anesthesia and blood was drawn from the iliac vein after five weeks of Ind treatment. Serum samples were stored at −20 °C until used. The experiment was carried out between 09.00 and 12.00 h.

Experiments in vitro. Thyroid perifusion was performed by the same system as reported previously[7]. The method of pituitary perifusion was almost identical with that of thyroid perifusion. In brief, the pituitary, taken out under ether anesthesia, was divided into two pieces and was preincubated for 60 min. Two sections of pituitary tissue were put in a chamber with a capacity of 0.3 ml. Krebs-Ringer bicarbonate buffer containing 0.1% glucose and 0.3% bovine serum albumin (KRBG), pH 7.4, was infused at a rate of 5.7 ml/h, and perifusates were obtained at intervals of 10 min. 20 min of stimulation with 2.76×10^{-8} M to 2.76×10^{-5} M TRH was repeated pulsatively. Continuous infusion of 1.0×10^{-6} M Ind was initiated 30 min after the start of perifusion.

Serum T_3 and T_4 were assayed by the method delineated previously[7] and TSH by the RIA method using a kit kindly supplied by the NIAMDD, NIH. The statistical significance of the data obtained was determined by Student's t-test.

Results. Effect of Ind in vivo. Serum T_3 and T_4 levels were both within normal limits after five weeks of Ind treatment, except that in rats given 5 mg of Ind, serum T_4 levels were significantly lowered (fig. 1). On the other hand, serum TSH levels were markedly elevated, and the thyroid gland was slightly swollen. We have observed that serum T_3 and T_4 levels tended to decrease 24 h after Ind injection (not shown), and some authors previously reported the inhibition of thyroid hormone release by Ind[6,8,9]. It was reasonably assumed that the marked increase of TSH was compensatory for the fall of serum thyroid hormone levels and, as a consequence, serum T_3 an T_4 levels were normalized. The present observations support the general concept that the swelling of the thyroid can be ascribed to the enhancement of TSH secretion[10].

Effect of Ind in vitro. Our thyroid perifusion system[7] was utilized to clarify the effect of Ind on thyroid hormone release. As seen in figure 2, the release of T_3 under continuous stimulation with 10 mU/ml of TSH was significantly inhibited by 1.0×10^{-6} M Ind at 200, 220, 300, 340 and 360 min. The total output of T_3 recorded during 6 h with and without addition of Ind more clearly showed that the inhibitory effect of Ind was remarkable (table).

Effect of Ind on T_3 release from perifused rat thyroids under continuous stimulation with TSH

	(n)	T_3 (pg/mg thyroid · 6 h)[a]	
Control	(9)	805.4 ± 95.1[b]	
TSH (10 mU/ml)	(24)	2170.7 ± 135.0	$p < 0.001$ vs. control
TSH plus Ind (1.0×10^{-6} M)	(5)	1635.9 ± 153.7	$p < 0.01$ vs. TSH

[a] Each value represents the total output of T_3 during 6-h infusion of KRBG buffer containing TSH and/or Ind.; [b] Mean ± SE. The experimental procedure was the same as in figure 2.

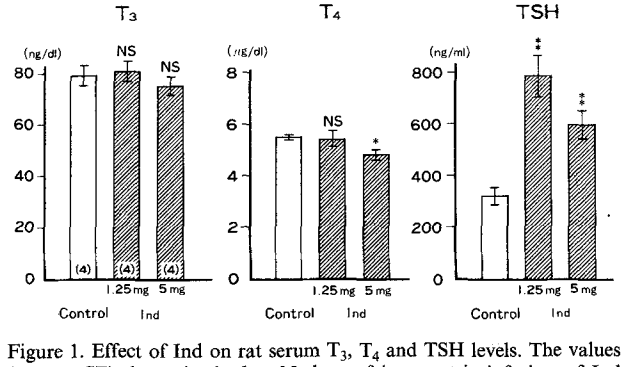

Figure 1. Effect of Ind on rat serum T_3, T_4 and TSH levels. The values (mean ± SE) determined after 35 days of intragastric infusion of Ind (1.25 or 5.0 mg/kg b.wt·day) are shown. A numeral given in parentheses represents the number of animals examined. NS, not significant *, $p < 0.05$; **, $p < 0.01$ vs control (see Materials and methods).

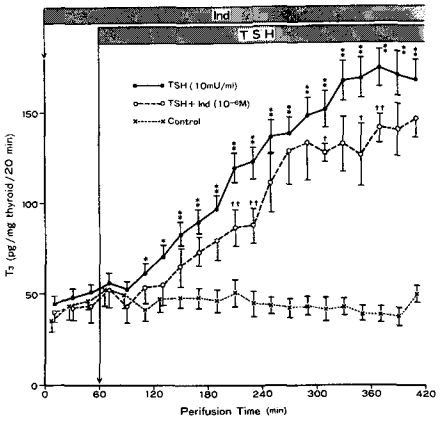

Figure 2. Effect of Ind on T_3 release form perifused rat thyroids under continuous stimulation with TSH. KRBG was used as an infusion buffer for the initial period of 60 min and, subsequently, the buffer containing 10 mU/ml of TSH was used (●——●, n = 24). In the test group (○------○, n = 5), the buffer containing 1.0×10^{-6} M Ind was used initially and was switched over to that containing TSH plus Ind at 60 min. In the control group (× ------×, n = 9), KRBG buffer was infused throughout 7 h of perifusion. Each value given is a mean ± SE. *, $p < 0.05$; **, $p < 0.01$ vs control, +, $p < 0.05$, ++, $p < 0.01$ vs TSH.

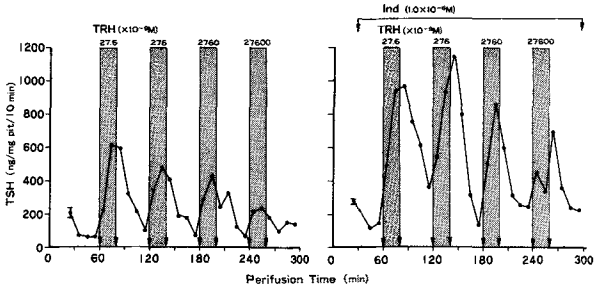

Figure 3. Effect of Ind on TSH release from perifused rat pituitaries during 20 min TRH pulses. Left panel: KRBG was used initially for 60 min and replaced by the buffer containing 2.76×10^{-8} M TRH for the subsequent 20-min period for stimulation. Thereafter, 20-min pulsative stimulation with 2.76×10^{-7} to 2.76×10^{-5} M TRH was performed repeatedly every 60 min. Right panel: Infusion of 1.0×10^{-6} M Ind was started at 30 min and continued up to 300 min. The other conditions were the same as those shown in the left panel.

A further in vitro experiment was performed, utilizing the rat pituitary perifusion system to ascertain the effect of Ind on TSH release. The findings are given in figure 3. The TSH release stimulated with 2.76×10^{-8} to 2.76×10^{-5} M TRH (left panel) was notably potentiated in the presence of 1.0×10^{-6} M Ind (right panel).

Discussion. The present study demonstrated the inhibitory effect of Ind on thyroid hormone release not only in vivo but also in vitro, in a perifusion system with rat thyroids. It is unquestionable that endogenous PG levels in the thyroid are lowered by Ind administration[5,6]. However, conflicting data have been presented concerning the effects of Ind on the release of thyroid hormones and of TSH. Some authors have reported that the thyroid hormone release was curbed by Ind[6,8,9], but others have said that it was not influenced[5,11,12]. Similarly, previous findings on the TSH release are divergent; that is, a deceleration[13,14] or an acceleration[9] of TSH release, or neither phenomenon was observed[5,6,12,15]. Haye et al.[16] claimed that the divergence may be explained by the hypothesis that there are two sorts of intrathyroidal PG pools. Thompson and Hedge[14] observed that therapy with a PG synthesis inhibitor (Ind or aspirin) suppressed the compensatory rise in TSH level following thyroidectomy or enfeebled the pituitary responsiveness to TRH. On the contrary, in our in vivo experiment, long-term Ind administration led to a marked increase of serum TSH and, simultaneously, the thyroid gland was swollen. However, serum T_3 and T_4 levels were both within normal limits. This perfect balance between TSH and thyroid hormones suggested a fully compensatory feedback mechanism.

In order to get data attesting to these effects of Ind observed in vivo, in vitro perifusion experiments were carried out. We obtained the following two characteristic findings: 1) Ind inhibited the TSH-stimulated thyroid hormone release and 2) it strengthened the TRH-stimulated TSH release. These in vitro results suggest independent action of Ind on pituitary and thyroid. Though it is questionable whether these results can be used for the interpretation of the in vivo results it is suggested that PGs play an important role in the pituitary-thyroid axis, especially in relation to thyroid hormone release.

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Estrogen and accessory sex gland lipids[1]

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Summary. Administration of estradiol-17β elicited differential responses on accessory sex glands of rats. In caput epididymis, the estrogen treatment led to an accumulation of glycerides and phospholipids while in cauda epididymis, there was no significant change. However, in seminal vesicles, depletion of phospholipids was observed. In prostate, the treatment, resulted in an accumulation of glycerides.

Key words. Rat, sex glands; estrogen treatment; lipid accumulation; epididymis, caput: epididymis, cauda; seminal vesicle; prostate.

Lipids form the major secretory products of the epididymis, besides sialoproteins, and the concentration of phospholipids is generally higher in caput epididymides of many species[2]. Epididymal lipids have been found to fluctuate during maturation of sperm[3]. It is also well established that the major amount of lipid in semen is contributed by the prostate[4] and that the formation and metabolic turnover of phospholipids in the prostate and seminal vesicles depend on androgenic stimulation[5].

Preliminary studies have shown a significant hormone-lipid interrelationship in male accessory sex glands[6]. In rates, estradiol has been shown to cause regression of the male accessory sex glands in general and prostate in particular[7]. The present study was undertaken to investigate the effect of estrogen on the male accessory sex gland lipids.

Materials and methods. Fourty male albino rats of the Wistar strain (100–110 days old; 190–200 g b.wt) were used in the present investigations. The animals ware divided into four groups of 10 each.

Group I: Control (one day) received peanut oil. Group II: Experimental (1 day) received estradiol 17β (6 µg/100 g b.wt) for a day. Group III: Control (7 days) received peanut oil. Group IV: Experimental (7 days) received estradiol 17β (6 µg/100 g b.wt) for 7 consecutive days.

Groups I and II animals were sacrificed by cervical dislocation 24 h after the treatment. Groups III and IV animals were sacri-