Thirdly, histopathological lesions observed by both light and EM indicated the presence of mononuclear cell infiltrates in and among the epithelial components (acinar and ductal) of the lacrimal gland. Confirming evidence that an autoimmune state had indeed been induced in the rat lacrimal gland must await further experimentation employing adoptive immunity. This could be accomplished by the passive transfer of immune cells and/or serum into nonimmunized syngeneic rats.

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## Plasma triiodothyronine, thyroxine and thyrotrophin levels in germfree rats[1]

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*Summary.* Plasma $T_3$, $T_4$ and TSH levels in developing germfree rats were high, low and normal as compared with those in conventional counterparts. The high $T_3/T_4$ ratio in germfree rat plasma was lowered by cholestyramine feeding.

Thyroid function in germfree animals has been studied by determining the metabolic rate, the iodine uptake and the circulating thyroxine ($T_4$) etc., leading to the hypotheses of a hypo-thyroidal function in the young germfree rat and a hypo- or euthyroidal function in the adult germfree rat[2-4]. However, little information is available on triiodothyronine ($T_3$) in germfree rats except our preliminary report[5]. The significance of $T_3$ in maintenance of euthyroid status has recently been recognized[6]. In addition, circulating $T_3$ and $T_4$ levels are known to be affected by their enterohepatic metabolism at different rates[7].

The present study was undertaken to evaluate the economy of thyroid hormones in germfree rats, which have large pools of cholesterol[8] and bile acids[9] in contrast to those in conventional rats. We describe plasma $T_3$, $T_4$ and thyrotrophin (TSH) levels in germfree and conventional rats and lowering of the plasma $T_3/T_4$ ratio by cholestyramine feeding in germfree rats. *Materials and methods.* Young (30-day-old) and adult (75-day-old) germfree and conventional male rats[10] were used. In experiment 1, the young rats were fed for 5 days a cholesterol diet containing 0.35% cholesterol[8] or a cholestyramine diet containing 0.35% cholesterol and 5% cholestyramine[11]. Aortic blood was drawn from the fed rats under hexobarbital anesthesia. Plasma was separated and stored at −20 °C until use for the radioimmunoassay of $T_3$, $T_4$[12] and TSH[13]. In experiment 2, the adult rats on a normo-cholesterol diet (CL-2)[14] were decapitated after a 20-h fast. Other procedures were done in the same way as described above.

*Results.* Plasma $T_3$ levels in young germfree rats on a cholesterol diet were higher than those in conventional counterparts, while plasma $T_4$ levels in the former were lower than those in the latter (table 1). Cholestyramine feeding reduced the mean $T_3$ level from 125.5 to 75.3 ng/100 ml and elevated the mean $T_4$ level from 2.53 to 3.40 μg/100 ml in young germfree rats, but did not significantly change these levels in conventional counterparts.

Adult germfree rats on a normo-cholesterol diet had also higher $T_3$ and lower $T_4$ levels in plasma, in contrast to those in conventional counterparts (table 2). However, plasma TSH levels were not significantly different between germfree and conventional rats.

*Discussion.* The present data indicating a lower plasma $T_4$ level in the germfree rat are consistent with the results on serum $T_4$ in 30- and 60-day-old germfree rats obtained by Sewell et al.[4]. However, they observed slightly higher $T_4$ levels in 100-day-old germfree rats, and suggested a delayed maturation of thyroid function in the young germfree rat. Although a delayed maturation has been reported on the pituitary-adrenal axis[15] and the testicular function[16] in germfree rodents, this may not account for a higher $T_3$ level in germfree rat plasma.

The high $T_3/T_4$ ratio in plasma was always associated with normal plasma TSH level in the germfree rat (table 2),

Table 1. Plasma $T_3$ and $T_4$ levels in 30-day-old germfree (GF) and conventional (CV) rats fed a 0.35% cholesterol diet (CONT) or a 0.35% cholesterol-5% cholestyramine diet (CHOL)

| Group of rats | | $T_3$ (ng/100 ml) | $T_4$ (μg/100 ml) | $T_3/T_4$ (%) |
|---|---|---|---|---|
| CONT-GF | (4) | 125.5 ± 14.0[a] | 2.53 ± 0.06[b] | 5.0 ± 0.6[a] |
| CONT-CV | (4) | 63.8 ± 2.4 | 3.08 ± 0.08 | 2.1 ± 0.1 |
| CHOL-GF | (3) | 75.3 ± 5.4[c] | 3.40 ± 0.17[d] | 2.3 ± 0.3[d] |
| CHOL-CV | (4) | 66.5 ± 5.3[e] | 2.90 ± 0.01[e] | 2.3 ± 0.2[e] |

Mean ± SE for number of rats indicated in parentheses. [a,b]Different from the CONT-CV group at 1% and 0.1% levels, respectively. [c,d]Different from the CONT-GF group at 5% and 1% levels, respectively. [e]Not significantly different from the CONT-CV group and the CHOL-GF group ($p > 0.05$).

Table 2. Plasma $T_3$, $T_4$ and TSH levels in 75-day-old germfree (GF) and conventional (CV) rats fed a normo-cholesterol diet

| Group of rats | | $T_3$ (ng/ml) | $T_4$ (μg/100 ml) | $T_3/T_4$ (%) | TSH (ng/100 ml) |
|---|---|---|---|---|---|
| GF | (4) | 90.0 ± 7.1[a] | 2.97 ± 0.05[b] | 3.1 ± 0.2[b] | 292.0 ± 7.3[c] |
| CV | (5) | 64.0 ± 4.0 | 4.00 ± 0.06 | 1.6 ± 0.1 | 294.0 ± 8.7 |

Mean ± SE for number of rats indicated in parentheses. [a,b] Different from the CV group at 2% and 0.1% levels, respectively. [c] Not significantly different from the CV group ($p > 0.05$).

suggesting that the high ratio may be of peripheral events. This concept is supported by the facts, such as lowering of the plasma $T_3/T_4$ ratio by cholestyramine feeding in the germfree rat (table 1), and better intestinal absorption of $T_3$ than $T_4$ observed in the conventional rat[17]. The reabsorption of $T_3$ is thought to be enhanced by an increase of intestinal bile concentration[7], which is higher in the germfree rat[9,18].

To elucidate the mechanism of the high $T_3/T_4$ ratio in germfree rat plasma, further studies would be necessary on the thyroidal release of $T_3$, the hepatic conversion[19] from $T_4$, a prohormone[20], to $T_3$ and the biliary excretion or intestinal reabsorption of $T_3$ in germfree rats. However, our present study suggests that the enterohepatic circulation of thyroid hormones may have more significance in regulating the plasma $T_3/T_4$ ratio in the germfree rat than in the conventional. In addition, we assume that the high plasma $T_3$ level in the germfree rat may be a result of adaptation.

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## Effect of adrenaline and adrenergic active drugs on growth hormone secretion in immature cockerels

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*Summary.* In immature cockerels adrenaline administration lowered the levels of plasma growth hormone. Both alpha and beta adrenergic receptor agonists also depressed the circulating growth hormone levels. In the presence of beta blockade, the suppressive effect of adrenaline on growth hormone secretion was not observed.

In primates there is considerable evidence that adrenergic pathways are involved in the control of growth hormone secretion[1-4]. In particular it is known that many of the stimuli that cause growth hormone release (e.g. insulin hypoglycaemia, exercise, arginine) act via dual adrenergic mechanisms; being suppressed by alpha blockade and enhanced by beta blockade[4]. Large doses of adrenaline itself have also been found to stimulate growth hormone secretion in the monkey[5] although in man a stimulatory effect of adrenaline on growth hormone secretion is only seen in the presence of beta blockade[6], an effect which is blocked by alpha blockade[7]. In contrast, adrenaline has been found to inhibit growth hormone secretion in the rat and this effect is not influenced by alpha or beta adrenergic blockade[8]. Adrenaline has also been found to inhibit growth hormone release in sheep[9] and mice[10] although it has no effect in pigs[11] and cattle[12]. In birds very little is known of the factors controlling growth hormone secretion and the possibility that adrenaline or adrenergic mechanisms might affect the circulating levels of plasma growth hormone has not been assessed. However, in the domestic fowl growth hormone secretion is affected in some stressful conditions[13] when endogenous adrenaline might be expected to be released. Therefore the aim of the present communication was to investigate the effect of adrenaline and adrenergic agents on the levels of plasma growth hormone in the domestic fowl.

*Materials and methods.* All the birds used in this study were 6-8-week-old cockerels (Thornber 909's). They were bled by brachial vein venipuncture and 1-1.5 ml blood taken. The needle was left in situ and the test substances i.v. injected. A 2nd blood sample was taken 20 min later. In 1 experiment groups of 8-week-old cockerels were i.v. injected with adrenaline (L-epinephrine, Sigma Chemical Co. Ltd.) at doses of 0-10 mg/kg b.wt in a volume of 1 ml/kg. Subsequently groups of 6-week-old cockerels were treated with freshly dissolved adrenaline (1 mg/kg), alone or together with various adrenergic receptor-active drugs: phentolamine mesylate (Ciba Ltd) an alpha blocker, 1 mg/kg; L-phenylephrine hydrochloride (Sigma Chemical Co. Ltd) an alpha stimulator, 1 mg/kg; DL-propranolol hydrochloride (Sigma Chemical Co. Ltd) a beta blocker, 1 mg/kg and DL-isoproterenol hydrochloride (Sigma

Table 1. Effect of adrenaline on the levels of plasma growth hormone in immature cockerels

| Dose of adrenaline | Plasma growth hormone (ng/ml ± SEM, N = 5) | | % Pretreatment level (means ± SEM, N = 5) |
|---|---|---|---|
| | Pretreatment level | Posttreatment level | |
| 0 | 55 ± 12 | 63 ± 13 | 127 ± 24 |
| 0.01 | 44 ± 5 | 47 ± 12 | 111 ± 29 |
| 0.10 | 91 ± 19 | 34 ± 8* | 37 ± 9** |
| 1.00 | 58 ± 15 | 18 ± 1* | 41 ± 10** |
| 10.00 | 63 ± 16 | 16 ± 1* | 23 ± 4*** |

Significantly different from pretreatment level, *$p < 0.05$ (paired t-test). Significantly different from saline controls, **$p < 0.01$; ***$p < 0.001$ (ANOVA analysis of variance).