clearly observed for the unique differential bands of $S_6$ (fig. 1B) and $S_7$ (fig. 1C), as well as for the multiple bands of $S_{14}$ (labeled a, b, c, and d in fig. 1D).

In order to characterize the S specific bands further, they were analyzed by SDS-PAGE. In one set of experiments, native 2-dimensional electrophoresis was carried out by electrophoresing the basic portion of the 1st-dimension IEF gel into an SDS gel. The silver stained second-dimension pattern is shown in figure 2A for $S_6$ stigmas. The $S_6$-specific glycoprotein is resolved into several components, all clustered in the 60–66 k mol. wt region and well separated from the other dozen or so lower mol. wt protein species seen on the gel. All components indicated by the arrow (fig. 2A) can also be detected in self-compatible bud stigmas and exhibit the developmental change in concentration described above. In a 2nd set of experiments, the differential glycoprotein bands of the $S_6$, $S_7$, $S_{13}$ and $S_{14}$ homozygotes were analyzed by SDS-PAGE of eluates from unstained IEF gel slices corresponding to the bands in question. In all cases, the eluates of what appear on IEF gels as single glycoprotein bands were shown by silver staining to consist of several species on SDS gels (fig. 2B). For each genotype, the major components consist of several species all of which stain with ConA-FITC. The 4 unique bands (a, b, c, d in fig. 1) revealed by IEF in $S_{14}$($S^c_{14}$ are no exception. They too are each resolved into what appear to be identical complexes of several mol. wt species. Based on the intensity of staining with silver nitrate, 2 bands are prominent within each cluster; their estimated mol.wts are: for $S_6$, 65 k and 63 k; for $S_7$, 59 k and 57 k; for $S_{13}$, 63 k and 61 k; for $S_{14}$, 64 k and 62 k. Other workers[12] have estimated a mol.wt value of 57 k for an $S_7$ specific molecule, although no mol.wt heterogeneity was reported by them for this genotype.

The functionally related S-allele specific molecules that we have discussed here are also related in their molecular structure. Although differing greatly in pI points, from the very basic $S_6$, $S_7$ and $S_{13}$ molecules (pI > 9.0) to the slightly acidic and neutral multiple IEF bands of $S_{14}$ (pI 6.2–7.2), they are all resolved into several glycoprotein components of similar mol.wt on SDS gels. As this molecular heterogeneity may be the result of differences in amino acid and/or carbohydrate composition, it is not clear at this stage whether the SDS-resolved components are different subunits of a native multimeric S molecule or intermediate forms of the processing modification of a core polypeptide chain. Since the components in question are glycoproteins as demonstrated by their binding to ConA, post-translational modification by glycosylation must occur in any event. The question is thus raised as to whether the S locus codes for the core protein or controls the modifying enzymes. The answer to this question is clearly significant for the understanding of the molecular basis of the genetically determined S specificities and of cell-cell interactions in plants.

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## Effects of thyroxine on the reproductive organs and growth of young chickens

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*Summary.* Male chicks of an egg-laying strain were injected with 0, 1, 2 or 4 µg/100 g b.wt thyroxine s.c. daily from 7 to 12 weeks of age. Increasing dose suppressed testicular development and puberty was completely blocked by the highest level. Live weight gain was enhanced by thyroxine treatment. Similar treatment of females prevented normal development of the ovaries at puberty.

It has been widely reported that administration of thyroid hormones, or their analogues, to mature males or females of several species, including the domestic chicken, causes regression of gonads and reduction of spermatogenesis and oogenesis[2]. There is also an inverse relationship between thyroid secretion and gonadal development during natural annual cycles in the domestic duck[3] and the Canada goose[4]. There is little information in the literature, however, of effects of thyroxine administered to young chicks on their sexual development, including histological studies of endocrine organs.

We have, therefore, studied the size, structure and development of gonads and other endocrine organs in chickens given several doses of thyroxine from 7 to 12 weeks after hatching.

*Methods.* Chicks of an egg-laying strain (Thornber 404, Mytholmroyd Hatcheries, Hebden Bridge) were penned in groups from the day after hatching and offered chick starter crumbs and water ad libitum in an environmental temperature of 35 °C and continuous light. From 3 weeks of age they were caged individually and offered a standard grower ration (Linton Mill Ltd, Wintringham, Malton) in a room at 22–25 °C with a 15.25 L:8.75 D lighting pattern, the light phase starting at 07.00 h.

Two experiments were carried out: a) 8 male birds aged 7 weeks were treated for 36 days, after which they were killed

by cervical dislocation and several organs were weighed. Plasma taken from heparinized blood was assayed for glucose concentration using an auto-analyzer (Technicon, AA2, Tarrytown, NY). Samples of gonads, pituitary, thyroid and adrenal glands were fixed in 10% buffered formalin. Paraffin wax-embedded sections were prepared and stained with hemotoxylin and eosin, b) 8 female birds aged 7 weeks were treated for 36 days. At slaughter tissues and samples were taken for histological study.

In each experiment ¼ of the birds were given each of the following doses of thyroxine (3,3′,5,5′-tetraiodo-L-thyronine; Sigma Chemical Corp., St. Louis): 0, 1, 2, 4 µg/day/100 g b.wt. The thyroxine was dissolved in 0.9% saline with the addition of a few drops of N/10 sodium hydroxide solution and was administered s.c. at approximately 10.00 h daily. The data were subjected to 1-way analysis of variance and the standard error and least significant difference for each parameter were calculated from the residual mean square[8].

*Results.* Weight gains and measurements made at the end of experiment 1 are shown in the table. There were significant, dose-related increases in live weight gain, food intake, carcass weight, intestinal length, weight of skin and feathers, head and legs and plasma glucose. Thyroid, testis and liver weights were depressed by increasing doses of thyroxine.

Microscopic examination of testes showed little effect of 1 µg thyroxine/100 g b.wt compared with controls (fig. 1). With the 2-µg dose there was obvious arrest of spermatogenesis at the primary or secondary spermatocyte stage, while birds given 4 µg/day (fig. 2) showed complete inhibition of

Weight gain, organ weights, plasma glucose concentrations and food intakes of male chickens treated with thyroxine for 36 days

| | Daily dose of thyroxine | | | | | |
|---|---|---|---|---|---|---|
| | 0 µg | 1 µg | 2 µg | 4 µg | SE[a] | Significance[b] |
| Weight gain (g/day) | 19 | 22 | 27 | 28 | ± 0.4 | ** |
| Carcass weight (g) | 625 | 796 | 821 | 895 | ± 40 | * |
| Thyroid weight (mg) | 90 | 87 | 52 | ND | ± 1.4 | *** |
| Intestine length (m) | 1.56 | 1.81 | 2.05 | 2.23 | ± 0.07 | ** |
| Skin + feather wt (g) | 230 | 248 | 270 | 283 | ± 7.1 | * |
| Head and legs wt (g) | 120 | 133 | 137 | 143 | ± 3.0 | * |
| Liver weight (g) | 44 | 37 | 33 | 27 | ± 1.3 | *** |
| Testis weight (g) | 26.6 | 24.8 | 22.2 | 3.8 | ± 2.1 | |
| Food intake (g/day) | 101 | 106 | 124 | 116 | ± 1.5 | * |
| Plasma glucose (mg/100 ml) | 214 | 256 | 267 | 294 | ± 10 | ** |

[a] Standard error of a treatment mean from analysis of variance, derived from the residual mean square. [b] Significance of the linear regression of response against dose of thyroxine: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ND, Thyroid glands could not be detected in these birds.

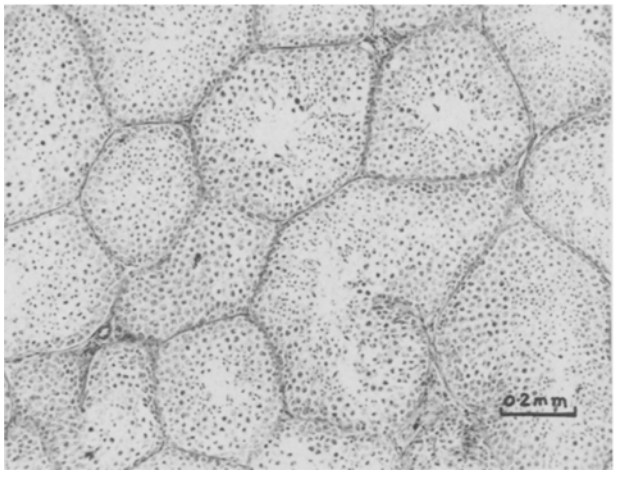


Figure 1. Section of testis of bird given carrier only.

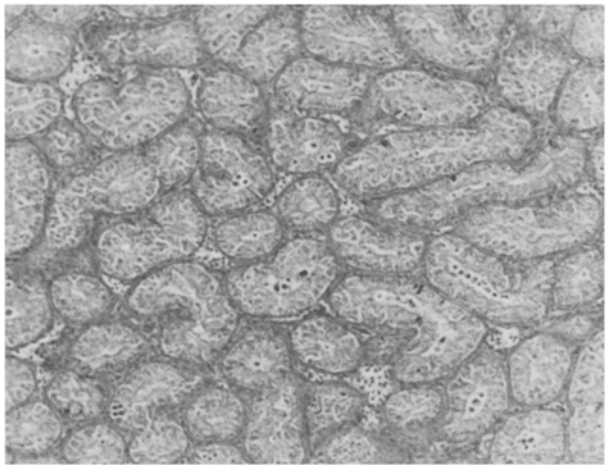

Figure 2. Section of testis of bird given 4 µg thyroxine.

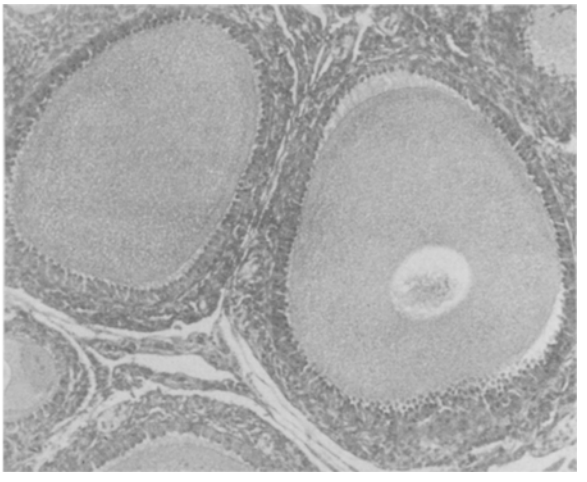

Figure 3. Section of ovary of bird given carrier only.

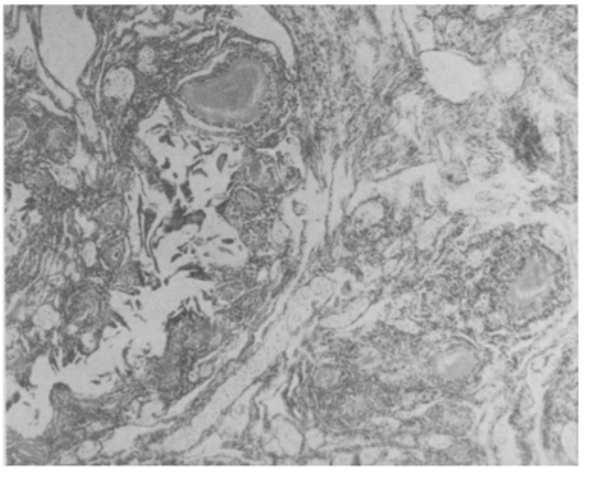

Figure 4. Section of ovary of bird given 4 µg thyroxine per day.

spermatogenesis with seminiferous tubules lined mainly by Sertoli cells and spermatogonia.
In females there was no noticeable effect of 1 µg thyroxine/100 g b.wt, whilst after 2 µg there was slight suppression of follicular development. The highest dose gave marked suppression of development, with atresia, reduction in follicular size and some evidence of fibrosis (figs 3 and 4).
Treatment caused no obvious effects on the histology of pituitary or adrenal glands, either in males or females, while thyroid cell size was suppressed by increasing dose; the thyroid glands could not be located after the 4-µg treatment.
*Discussion.* Treatment of the male birds in this experiment with thyroxine caused significant increases in weight gains and carcass weight, but prevented normal development of the testes. Ovarian development was also inhibited in female birds. These effects on the gonads show that thyroxine can prevent normal development, as well as causing atrophy in mature birds, which was previously shown by Wheeler and Hoffman[5] amongst others. Shaffner[6] noted a reduction in fertility in cockerels, and a decline in egg production in hens, by feeding thyroprotein. The review of Maqsood[2] shows that equivalent results have been found in mammals.
More recently, Jallageas and Assenmacher[3] have shown that seasonal hyperthyroidism accounts for the onset of the regressive phase of the annual sexual cycle in domestic ducks, thus supporting the idea that endogenous thyroid hormones affect reproduction so that the effects of exogenous treatment in this experiment are likely to be of physiological significance. It is unlikely, however, that the highest dose of thyroxine used in the work reported here was within the physiological range as it had severe effects on the thyroid glands. While it is highly unlikely that they atrophied completely, they were so much reduced in size and/or changed in color that they could not be detected by the naked eye.
A significant stimulation of growth of chickens by thyroxine was observed in experiment 1, which was similar to the results of Singh et al.[7]. Thyroid hormones are known to be synergistic with growth hormone in the control of growth in mammals, and it is possible that chickens do not normally produce sufficient thyroxine to support maximal growth rates. Hyperthyroidism causes glycogen depletion of the liver[9] which accounts for the hyperglycemia and reduced liver weights observed in experiment 1.
Although the results reported here do not offer conclusive proof that thyroid secretions are responsible for the timing of sexual maturation, there is clearly need to follow up this possibility with further work in this area.

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## Diagnostic alleles and systematics in termite species of the genus *Reticulitermes* in Europe

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*Summary.* It was possible to separate species and complexes of species in the *Reticulitermes* genus of Western Europe using esterase 3 and acid phosphatase 2.

Electrophoretic techniques sometimes help taxonomists to differentiate between sibling species on the one hand, and semi-species on the other, morphologically very close in natural populations[1]. In European termites of the *Reticulitermes* genus, morphology gives little information for species discrimination[2]. Research on natural hybridization in the sympatric area is very difficult with classical types of observation (morphology and biometry), but species can be isolated according to their sexual pheromone proportions[3] and cuticular compounds[4].
Luykx[5] described in 1981 an enzymatic polymorphism in one species and we know[6,7] that in Western Europe, enzymatic polymorphism is very marked in *Reticulitermes*. Two loci (esterase 3 and acid phosphatase 2) have diagnostic alleles and allow species discrimination.
*Esterase 3.* 10 loci were distinguished by electrophoresis at 300 V for 4 h at 5 °C in 12.5% hydrolyzed starch at pH 8.25 on Poulik gel buffer[7] or in a 7.5% acrylamide gel at pH 8.9 in tris-glycine or borate electrode buffer (pH 8.6). Worker termites were homogenized in distilled water. An equal mixture of $\alpha$ naphtyl sodium acetate (Sigma) and $\beta$ naphtyl sodium acetate (Sigma) with fast blue R.R. salt (Sigma) on sodium phosphate buffer (0.2 M) discriminated different loci at pH 6.25. Esterase 2 and 6 were pink colored, esterase 7 and 9 red, esterase 1, 3, 4, 5, 8 and 10 black. 7 loci were polymorphic (fig. 1). Esterase 3 is the most polymorphic and overlaps other enzymes. These systems were inhibited by n-propanol (13%) during the staining procedure (fig. 2).
*Acid phosphatase 2.* 2 loci appeared (ACPH1 and ACPH2) when workers were homogenized in distilled water. $\alpha$-Naphthyl acid phosphate (Sigma), 5% n-Propanol and Fast Garnet GBC salts mixed with sodium acetate buffer (0.2 M) at pH 5 were used in the staining procedure. 2 other loci (PAC1 and PAC2) were revealed when termites were homogenized in 1‰ triton X100.
A single pair of reproducers (winged or neotetenic one) was discovered in 6 societies out of 104, and allowed a formal genetic study of the enzyme genes.
A chi-square test confirmed the existence of a monomeric autosomal and co-dominating locus for each enzyme. 8 alleles coded for esterase 3 and 5 for ACPH 2 (table 1).
104 societies from Western Europe were studied. These