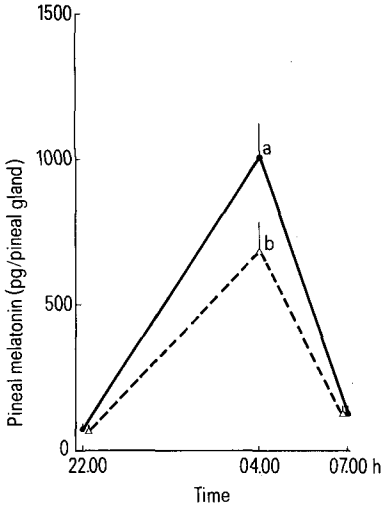
light filter, Kodak) for approximately 6 sec prior to decapitation. Melatonin content of individual pineal glands was quantitated by radioimmunoassay[8]. The validity of utilizing antiserum R1055 (9/16/74) of Rollag and Niswender[8] for quantification of melatonin in hamster pineal gland homogenates was established previously[9].

Results and discussion. Pineal melatonin contents of unoperated hamsters sacrificed at 22.00, 04.00 and 07.00 h were 71±6 (SEM), 1005±106, and 132±18 pg/pineal gland, respectively. Following the removal of the Harderian glands, pineal melatonin levels in hamsters sacrificed at 22.00, 04.00 and 07.00 h were 70±10, 689±94, and 123±28 pg/pineal gland, respectively. Thus, compared to that in unoperated controls the nighttime levels of pineal melatonin in hamsters lacking their pineal gland were significantly depressed (figure).



Pineal melatonin levels in unoperated (●) hamsters and in hamsters from which the Harderian glands had been removed (△). Animals were maintained in a light:dark cycle of 14:10 and killed at 22.00, 04.00 and 07.00 h. Values represent the mean±SEM; *a* vs *b* $p < 0.05$.

In blinded suckling rats, the Harderian glands may mediate the effects of light on pineal serotonin[5] and on pineal HIOMT, an enzyme involved in the synthesis of melatonin[6]. In adult rats, however, these glands do not appear to be essential for the light-induced rhythms in the acetylating enzyme[10]. In the presently reported study, peak pineal melatonin levels, which are normally present in male hamsters at 8 h after the onset of darkness in light:dark cycle of 14:10[9], were significantly decreased following the removal of Harderian glands ($p < 0.05$). However, the peak pineal melatonin levels were still significantly elevated at 04.00 h when compared to daytime melatonin values for the same group ($p < 0.01$). It is possible that the removal of Harderian glands merely shifted the time of occurrence of peak pineal melatonin concentrations and thus the 04.00 h value does not represent the true peak level in these animals. Regardless of whether peak melatonin levels were shifted or depressed, it appears as though the Harderian glands may have a role in mediating changes in pineal melatonin metabolism in the hamster.

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The presence of ecdysteroids and the variations of their level during the first adult stage of the myriapod *Hanseniella ivorensis* Juberthie-Jupeau and Kehe (Symphyla)

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Summary. Adult individuals of the Symphyla *Hanseniella ivorensis* had their antennae cut off at the beginning of the first adult stage; this had the effect of triggering the following molt, which occurred 7.5 days after the treatment. We determined, by a radioimmunoassay method, that ecdysteroids were present in the Symphyla throughout the period studied. They were composed mainly of β-ecdysone but small amounts of α-ecdysone and of high polar products were also detected. During the artificially-induced molting process, the ecdysteroid level showed a smooth peak which took place 5 days after the amputation of the antennae. This indicates that ecdysones probably control the molt in the Symphyla as they do in insects or in crustacea. However, the increase in the hormonal level appears to be not the primary response to the treatment.

Ecdysone level variations during the developmental stages of insects have been well-charted (see Delbecque et al.[2] for review); they also have been studied in some crustaceans[3-9] and in one arachnid[10]. In myriapods, on the other hand, ecdysone determination have been neglected; in fact, no one has even noted the presence of ecdysteroids in these animals. Nevertheless, it may reasonably be assumed that molting is controlled by the same, or similar, hormones as in other arthropods, since ecdysone injections in chilopods induce the molt[11]. However, no one has been able to pinpoint a glandular organ as controlling the molting process in any myriapod. In the present study, we sought to detect the presence of ecdysteroids in a Symphyla and to determine their variations during the first adult stage. Adult Symphyla molt periodically and, in addition to the tegumentary modifications, the intermolting period is characterized by a renewal of the mesenterum and by a spermatogenesis cycle in males[12]. Molting can be triggered artificial-

ly by amputating the antennae at the start of the stage[13]; this fact allowed us to synchronize the development of the experimental animals.

Materials and methods. The Symphyla under study were adults of the first stage of *Hanseniella ivorensis*. This very prolific tropical species has a high rate of development. It should be noted that during the first adult stage no vitellogenesis occurs in females. The antennae were cut off 1 day after ecdysis and the animals were left unfed.

In this way, the whole stage lasts about 7 days with apolysis of the anterior tergal tegument taking place 3 days after the amputation. During this time span, samples taken from 12 to 25 insects were pooled and made ready for assays (Symphyla weigh between 0.2 and 0.26 mg). Up to the middle of the 6th day, samples were taken at intervals of not more than 24 h. The loosening of the tegument from the epidermis under the claws of the 12th pair of legs, its detachment and the formation of the new claws are used as chronological criteria. The secretions of the tergal tegument are studied by ultrastructure preparations, during the intermolt cycle.

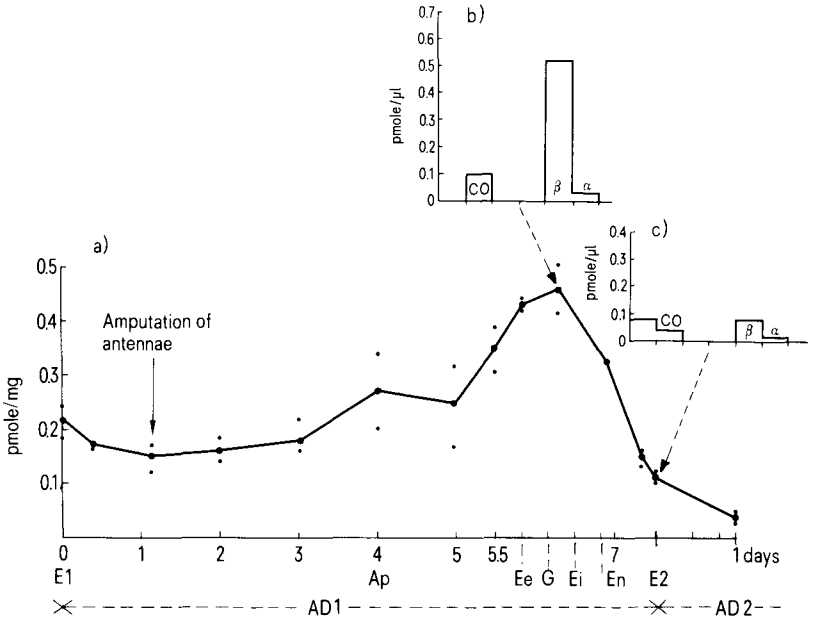
Frozen sample pools stored at $-30\,^{\circ}C$ were homogenized by sonication in 75 µl of methanol. After being left to stand 1 h at 40 °C, they are centrifuged; the supernatant was evaporated and the dry extract was dissolved in 30 µl of 0.1 M citrate buffer, pH 6.2. The final solution was incubated in a microdialysis apparatus[14] for ecdysone assay[15].

Moreover, the ecdysteroid extract of certain sample pools composed of 40 to 100 animals was chromatographed on thin-layer plates (silica gel Merck, 60 F 254) in a solvent of chloroform:methanol (80:20, v/v) (2× 1 h). After elution, the gel was scrapped in 1 cm wide strips along the path of migration. The ecdysteroid content of each of them was then measured by the radioimmunoassay. The respective positions of α- and β-ecdysone were known by the migration of standard solutions.

Results. Radioimmunoassay shows that ecdysone is present in the Symphyla *H. ivorensis* throughout the period under study (figure, A). At the time of amputation, i.e. 24 h after ecdysis, the hormonal concentration is 0.15 pmoles/mg of fresh tissue. This value starts to increase during day 3 and reaches its maximum, 0.50 pmoles/mg, around day 6. Thereafter, during the 12 h prior to molting, the ecdysteroid level drops; by the time of ecdysis, it has fallen to 0.2 pmoles/mg. In the next 24 h, the level continues to decrease a little, down to 0.05 pmoles/mg.

Thin-layer separation combined with radioimmunoassay indicates that at its peak on day 6, the radioimmune activity is mainly due to β-ecdysone, the concentration of which is 15 times greater than that of α-ecdysone (figure, B). A quantitatively small part of the activity, not more than 10% of the total, is seen to come from strongly polar substances. This is likely to be a result of ecdysteroid conjugates.

Discussion. This is the first attempt to identify ecdysones in myriapods. The results of the experiments made after artificially-induced ecdysis show that, like in other arthropods, molting in these animals is ecdysone-dependent. It was seen that the total ecdysteroid level is at a maximum on the 6th day of the stage, i.e. 2 days after apolysis of the dorsal tegument and at the time when the claws of the 12th pair of legs are beginning to form. The epicuticles of the new tegument are also laid at this time. It should be noted, however, that endocuticle synthesis starts shortly before ecdysis and continues for 1 day after molting, thus taking place at a time when ecdysone level is low. Moreover, it is noteworthy that the increase of ecdysone level during the first adult stage is relatively limited; the ratio between the highest and lowest values is 3:1, in comparison to 3 or 6:1 in a Collembole[16], or 10:1 in a spider[10]. In insects the ratio can be greater; it is over 100:1 in Pterygotes. Lastly, ecdysone level does not immediately increase after the amputation of antennae. Indeed, the hormone response to amputation came 2 days later, after apolysis had already occurred. This would indicate that, while ecdysones control the molting process in the Symphyla studied, their synthesis is not the initial response to the treatment.



Variations of the titer of ecdysones (RIA activity) during the first adult stage of *Hanseniella ivorensis*. The RIA activity is expressed as pmoles of β-ecdysone-equivalent per mg of fresh tissues. A: Variations of the total RIA activity. B and C: RIA activity of thin-layer chromatography fractions, showing the respective contribution of α- and β-ecdysones and of conjugates. E1: Larval-adult molt. AD1: 1st adult stage. E2: 1st adult molt. AD2: 2nd adult stage. Ap: Apolysis of the dorsal tegument. Ee: Secretion of the external epicuticle. Ei: Secretion of the internal epicuticle. En: Secretion of the endocuticle. G: Retraction of the epidermis under the claws of the 12th pair of legs. CO: Ecdysone conjugates.

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TSH-Receptor antibodies, HLA B8 and thyroid autoantibodies in patients with Graves' disease in therapeutically induced euthyroidism

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Summary. The prevalence of TSH-receptor antibodies and of thyroid autoantibodies was studied in 48 HLA-typed patients with Graves' disease, who were in an euthyroid state after antithyroid therapy with methimazole. TSH-receptor antibodies, which were found in 35% of the patients, did not correlate with the positivity of HLA B8. By contrast the persistence of thyroid microsomal antibodies was significantly associated with HLA B8.

Several studies have shown that Graves' disease is an HLA-associated autoimmune disorder[1-8] characterized by an interaction between the endocrine and the immune systems, which results in the formation of autoantibodies to TSH receptor. TSH-receptor antibodies, which are detectable in about two thirds (ranging from 50 to 70%) of untreated patients with Graves' disease[9-15], are assumed to mimic the stimulatory effect of TSH by binding to TSH-receptor and activating adenylate cyclase. Recently an association between HLA B8 positive thyrotoxic patients and prevalence[3,16] or persistence[8] of thyroid microsomal antibody formation was found. Furthermore, findings previously observed in patients with Graves' disease indirectly suggest a possible influence of HLA B8 on the persistence of thyroid stimulating antibodies[8]. Therefore we studied the prevalence of TSH-receptor antibodies and of thyroid autoantibodies in 48 HLA-typed patients with Graves' disease, who were in an euthyroid state after antithyroid therapy with methimazole.

Material and methods. 48 patients suffering from Graves' disease were studied; as a result of previous methimazole treatment all patients were in an euthyroid state at the time of investigation. For determination of TSH-receptor antibodies a modification[11] of a radioligand receptor assay described by Smith and Hall[17] was used. The assay was considered to be positive if the binding of 125 J-TSH to thyroid membrane was below the mean value minus the double SD in the presence of control IgG. Thyroid antibodies were measured by standard procedures with the tanned red-cell haemagglutination method for thyroglobulin and indirect immunofluorescence technique for microsomal antibodies. HLA A, B and C locus antigens were determined by the NIH microlymphocytotoxicity technique.

Results and discussion. HLA B8 was found in 15 of the 48 patients (31%) vs. in 81 of the 450 (18%) controls ($p < 0.03$), which confirms the previously reported association between Graves' disease and HLA antigens[1-8]. Detectable TSH-receptor antibodies were observed in 35% of the patients in therapeutically-induced euthyroidism, which is in accordance with data of Graves' patients treated by antithyroid drugs studied by other authors[12-14]. Interestingly, the incidence of TSH-receptor antibodies did not differ in B8 positive and B8 negative patients (table). Likewise, the prevalence of thyroglobulin antibodies was almost similar in the respective groups (table). By contrast the persistence of thyroid microsomal antibodies was significantly correlated with HLA B8, which is analogous to the islet-cell antibody persistence in insulin-dependent diabetes[18,19].

The present study demonstrates a relatively high incidence of TSH-receptor antibodies in patients with Graves' disease in euthyroidism after treatment with antithyroid drugs. Although the course of the disease seems to be genetically determined[6-8], persistence of TSH-receptor antibody production was not correlated with the HLA system. Interestingly, TSH receptor antibodies are also found in patients with Hashimoto's thyroiditis[15,20,21], ophthalmic Graves' dis-

TSH-Receptor antibodies, HLA B8 and thyroid autoantibodies in patients with Graves' disease in therapeutically induced euthyroidism

Thyroid autoantibodies	All thyrotoxic patients* (N = 48)	B8 positive thyrotoxic* patients (N = 15)	B8 negative thyrotoxic* patients (N = 33)
TSH-Receptor antibodies	N = 17 (35%)	N = 6 (40%)	N = 11 (33%)
Thyroid microsomal antibodies	N = 19 (40%)	N = 10 (67%)	N = 9 (27%)**
Thyroglobulin antibodies (> 1:250)	N = 10 (21%)	N = 4 (27%)	N = 6 (18%)

* All patients were euthyroid after treatment with methimazole. ** $p = 0.01$; χ^2-test.