20% in sample L_1 to more than 80% in sample L_8. In the fraction of sulfoconjugated steroids, consisting primarily of lipophile steroid sulfatides[6], the same metabolites accounted for little more than 40% of isolated ^{3}H-labelled compounds. 5-androstene-3β,17β-diol proved to be the most important metabolite, followed by 17β-hydroxy-4-androstene-3-one (testosterone) and 4-androstene-3,17-dione. The formation of labelled estrogens from sulfoconjugated DHEA in human lung tissue is not surprising in view of the observations of BRAMBILLA[12], who reported elevated estrogen levels in a case with a respiratory infection. As far as quantitative aspects are concerned, the direct metabolism[13] of sulfoconjugated DHEA by far exceeded the conversion of the substrate by indirect pathways, e.g. after hydrolysis of the conjugate. Although peripheral plasma and 24-h urine contained ^{3}H-labelled steroids, indicating their escape into the general circulation in the course of the experiment, the contribution of peripheral metabolism to the aforementioned results may be considered minor. For only 0.35% and 0.01% of infused ^{3}H-activity were detected in the fractions of urinary steroid sulfates and glucurono sides respectively. In contrast hereto, after the peripheral i.v. injection of the same substrate, 15–30% of administered ^{3}H-activity can be recovered from the 24-h urine[14].

Zusammenfassung. Bei der in-vivo Perfusion menschlichen Lungengewebes mit 7α-^{3}H-DHEA-^{35}S-sulfat zeigte es sich, dass im Lungengewebe sowohl Sulfatase- wie auch Sulfokinaseaktivität enthalten ist. Als Metaboliten konnten neben dem vorherrschenden 5-Androsten-3β,17β-diol auch Testosteron, Androstendion, Androsteron, Ätiocholanolon sowie Oestrogene nachgewiesen werden.

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Concerning Non-Darkening of Mutant *Habrobracon (Bracon hebetor)* Eyes as Consequence of a New Chromogen-Reducing Mechanism in Insect Larvae

The phenotypes of non-autonomous mutants are generally changed to wild-type if they feed on or receive through injections those substances of the normal pathway which lie behind the genetic block. Consequently, the eyes of *v*- and *cn*-pupae of *Drosophila* are found to be normally coloured when the larvae are fed with an extract from wild types[1]. Contrarily, the eyes of the orange-eyed mutant[2–4] of the parasitic wasp, *Habrobracon juglandis* (= *Bracon hebetor*), which cannot synthesize 3-hydroxykynurenine, do not darken although their larvae feed on the caterpillars of wild *Ephestia kühniella*. Present investigations have been aimed at providing an explanation for this unusual behaviour.

Haemolymph and fat body of *Ephestia* larvae contain small amounts of 3-hydroxykynurenine[5]. However, even when the hydroxykynurenine level of the *Ephestia* larvae is elevated by injecting the synthetic substance, no eye-darkening occurs in the parasite. Extraction of 3-day-old feeding larvae of *Habrobracon* (30 °C) reveals that hydroxykynurenine has not been completely metabolized by the host itself, because it was found in the gut and also to some extent in the haemolymph of *Habrobracon*. Moreover, compared with hydroxykynurenine, a high concentration of xanthurenic acid was found in haemolymph and gut.

After 2 days, eye pigmentation by ommochrome deposition begins in the wild-type prepupa. Extraction of the mutant prepupa, fed on hydroxykynurenine, reveals total absence of hydroxykynurenine in the haemolymph. Evidently within 2 days it is transaminated to xanthurenic acid and is excreted during moulting to the prepupa, leaving no hydroxykynurenine for ommochrome synthesis. In contrast to the wild type, the mutant spinning larvae and prepupae not fed with hydroxykynurenine accumulate kynurenine and kynurenic acid (Figure a, b). Figure c shows the third chemotype: the feeding and spinning larvae metabolized all hydroxykynurenine to xanthurenic acid before ommochrome synthesis in the eye began.

Now the question is why there is such a strong transamination in feeding and spinning larvae of *Habrobracon*. It is assumed that whenever toxic amounts of tryptophan are set free during development of insects they must be reduced to a compatible level[6,7], either by excretion[8] or by conversion into kynurenine and 3-hydroxykynurenine. These substances seem also to be incompatible in higher concentrations and hence are further metabolized to the ommochromes, specially in the pupal eyes of all insects after having induced, perhaps, the synthesis of the structural component of the ommochrome synthesizing apparatus[9]. In other stages or organs where structurally and enzymatically ommochrome synthesis is possible, colouring is always preceeded by chromogen accumulation, e.g. in the hibernating eggs of the silkworm[10], in larval skin and gonads of *Ephestia*[5], in haemolymph and fat body of *Cerura*[6] and in the MALPIGHIAN tubes of

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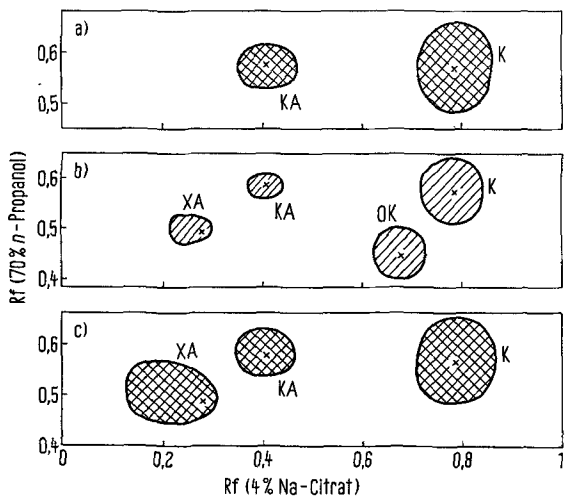

Chromatographic separation (paper: SCHLEICHER and SCHÜLL 2043b) of kynurenine (K), 3-hydroxykynurenine (OK), kynurenic acid (KA) and xanthurenic acid (XA) in an extract of 2 *Habrobracon* prepupae. (a) Orange-eyed mutant, (b) wild type, (c) orange-eyed mutant fed with 3-hydroxykynurenine (CALBIOCHEM).

Drosophila[11,12]. When ommochrome synthesis is occurring only in the pupal eyes, another mechanism must be operating to reduce high larval chromogen contents. In *Habrobracon* this pathway is the transamination of 3-hydroxykynurenine to xanthurenic acid (and to a lesser extent of kynurenine to kynurenic acid). This mechanism has been found to be efficient to such an extent that no fed chromogen is left over for the second reducing mechanism, which is the ommochrome synthesis operating only in the pupal stage.

Zusammenfassung. An Larven der orangeäugigen Mutante der parasitischen Schlupfwespe *Habrobracon juglandis* verfüttertes 3-Hydroxykynurenin führt nicht zur Ausfärbung der Imaginalaugen, da es der pupalen Ommochrombildung durch Transaminierung zu Xanthurensäure entzogen wird.

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Selection of Arginine-Requiring Mutants in *Chlamydomonas reinhardi* After Treatment with Three Mutagens

From the early experiments by EBERSOLD[1] only 4 arginine-requiring mutants have been isolated in *Chlamydomonas reinhardi*[1-3]. In the course of our investigation on the specificity of forward mutations in this organism, several new arginine-requiring mutants have been obtained after treatment of wild-type cells with ethyl methanesulfonate (EMS) and plating on media deprived of mineral nitrogen but containing, as sole nitrogen source, casein hydrolyzate or yeast extract[4]. Reconstruction experiments have shown that the growth of the new strains was rapidly inhibited with increasing concentration of NH_4^+ ions in the culture medium. The causes of inhibition by ammonium have been investigated: the results of this study will be reported elsewhere.

However, another question arose: whether the specificity of these mutations was attributable to a plating medium effect only (and was accordingly independent on the mutagenic agent used) or was due to specific interaction between plating medium and EMS. In the latter case, NH_4^+-sensitive arginine-requiring mutants are not expected to appear, even on ammonium-free medium, after treatment with mutagens other than EMS. Examples of severe specificity of this kind have been reported in the literature. In *Ophiostoma multiannulatum*, histidine-requiring mutants are recovered on both complete and minimal + histidine media after treatment with N-nitroso-N-methylurethan, but on minimal + histidine medium only after treatment with UV-light[5]. In *Penicillium chrysogenum*, manganous chloride suppresses the mutation to azaguanine resistance induced by nitrogen mustard but not by other mutagens[6].

It is the reason why it seemed interesting to compare the spectra of mutations induced with EMS and 2 other mutagens, UV-light and N-methyl-N-nitro-N'-nitrosoguanidine (MNNG) in our forward mutation system.

Material and methods. The wild-type, mt^+, strain 137c obtained from R. P. Levine (Harvard University), was used throughout this study. General methods of culture and mutant isolation procedures were those described previously[4]. All mutants were isolated on a medium without NH_4Cl but supplemented with 4 g/l Difco yeast extract (M − N + YE 4). The treatments were performed on stationary phase cultures grown for 3–4 days in liquid complete medium. After washing the cells were suspended in 0.02 *M* potassium phosphate buffer (pH 6.9) at a concentration of about 10^7 cells/ml, then treated as follows. MNNG: the suspension was treated with 100 mg/l MNNG in buffer at 25 °C for 30 or 60 min. The treatment was interrupted by 2 washings in buffer. The cells were plated at appropriate dilutions on M − N + YE 4 medium and incubated in the light (5000 lux) for 7–10 days, after which the colonies were replica-plated on to minimal and supplemented media. UV: The UV source was a Hanau Sterilamp, type F 2318. 5 ml of the suspension were irradiated in an open petri dish (50 mm diameter) at 35 cm of the source. The suspension was gently agitated on a magnetic stirrer during irradiation. UV-treated cells were manipulated as MNNG-treated ones, except that for the first 12 h of incubation they were kept in the dark to prevent photoreactivation. EMS: The cells were treated with 0.27 *M* EMS in 0.1 *M* potassium phosphate buffer at 25 °C for 2 h as previously described[4].

Results and discussion. Table I shows that MNNG, UV and EMS are effective in inducing forward mutations in *C. reinhardi*. The highest mutation frequencies were observed following treatment with UV or MNNG. Rather

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