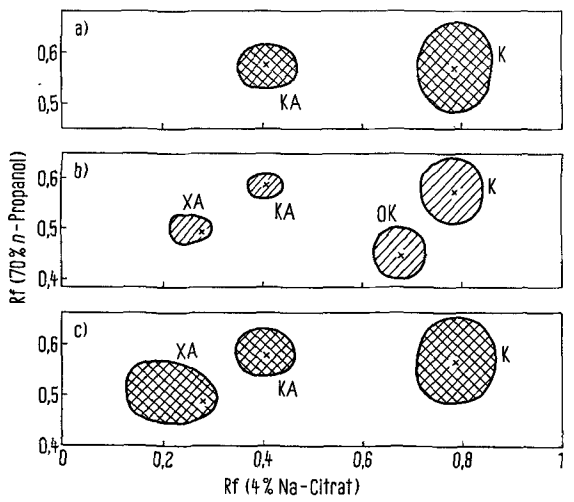

Chromatographic separation (paper: SCHLEICHER and SCHÜLL 2043b) of kynurenine (K), 3-hydroxykynurenine (OK), kynurenic acid (KA) and xanthurenic acid (XA) in an extract of 2 *Habrobracon* prepupae. (a) Orange-eyed mutant, (b) wild type, (c) orange-eyed mutant fed with 3-hydroxykynurenine (CALBIOCHEM).

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.

F. LEIBENGUTH

Zoologisches Institut,
Abteilung Entwicklungsphysiologie der Universität,
D-74 Tübingen (Germany), 15 December 1969.

[11] A. WESSING and D. EICHELBERG, Z. Naturforsch. *23b*, 376 (1968).
[12] D. EICHELBERG, Z. Naturforsch. *23b*, 547 (1968).

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

[1] W. T. EBERSOLD, Am. J. Bot. *43*, 408 (1956).
[2] R. A. EVERSOLE, Am. J. Bot. *43*, 404 (1956).
[3] N. W. GILLHAM, Genetics *52*, 529 (1965).
[4] R. LOPPES, Molec. gen. Genetics *104*, 172 (1969).
[5] G. ZETTERBERG, Hereditas *48*, 371 (1962).
[6] R. R. ARDITTI and G. A. SERMONTI, Genetics *47*, 761 (1962).

Table I. Forward mutations induced by UV, MNNG and EMS

Mutagenic treatment	UV (3 min)	UV (3 min)	UV (6 min)	MNNG (30 min)	MNNG (60 min)	EMS (2 h)	EMS (2 h)
No. of colonies replicated	2820	1181	2299	3381	3002	2546	11,222
Survivors (%)	37	18	0.52	25	14	51	85
No. of mutants	19	4	7	18	6	9	32
Mutants (%)	0.67	0.34	0.30	0.53	0.20	0.35	0.29

All data are from independent experiments.

Table II. Types of biochemical mutants induced with UV, MNNG and EMS

Metabolite required	*p*-Aminobenzoic acid	Thiamine	Nicotinamide	Arginine	Carbon source	Unidentified	Total
UV	3	0	9	2	13	3	30
MNNG	4	3	3	2	8	4	24
EMS	3	4	13	6	15	–	41

puzzling is the fact that higher mutation frequencies were obtained with UV or MNNG in conditions of high survival. This phenomenon has been observed in other experiments and would deserve further study.

The comparison of the types of biochemical mutants induced with the 3 mutagens (Table II) does not reveal any marked difference in the mutation spectra. The lack of thiamine mutants after treatment with UV cannot be considered as significant owing to the fact that this type of mutant has been frequently obtained with UV in recent years[2,7].

However, the important feature lies in the finding of 4 arginine-requiring mutants after treatment with UV and MNNG. These mutants were shown to be NH_4^+-sensitive as EMS-induced arginine-requiring mutants described previously[4]. Furthermore, no arginine auxotroph was ever recovered in our laboratory, after treatment with EMS, on media containing a high concentration of NH_4Cl[8,9].

These results strongly suggest the specificity of mutations leading to an arginine requirement not to be related to the mutagenic agent used. This would mean that the specificity is determined at a very late stage in the mutation process, long after treatment with the mutagen, at the time of mutation expression.

It therefore seems that the culture medium is of crucial importance in that it either allows or does not allow the survival of the arginine auxotrophs. This specificity, however, is not absolute, since the 4 arginine auxotrophs isolated previously in other laboratories are insensitive to NH_4^+ ions. This problem will be discussed in another paper.

Résumé. Des mutations biochimiques ont été induites par le méthane sulfonate d'éthyl (EMS), la N-methyl-N-nitro-N'-nitrosoguanidine (MNNG) et l'ultraviolet (UV) chez l'algue verte unicellulaire *Chlamydomonas reinhardi*. Des mutants auxotrophes pour l'arginine ont été isolés, après traitement par les 3 agents, sur un milieu contenant de l'extrait de levure comme unique source d'azote.

R. Loppes[10]

Université de Liège, Laboratoire de Génétique, 15, rue Forgeur, Liège (Belgium), 6 December 1969.

[7] W. T. Ebersold, R. P. Levine, E. E. Levine and M. A. Olmsted, Genetics *47*, 531 (1962).
[8] R. Loppes, Molec. gen. Genetics *102*, 229 (1968).
[9] R. Loppes, unpublished data.
[10] Chargé de Recherches du Fonds National Belge de la Recherche Scientifique.

Test-Tube Fertilization of Ovules in *Melandrium album* Mill. with Pollen Grains of *Datura stramonium* L.

The technique of test-tube fertilization provides new possibilities of overcoming incompatibilities in plants[1–4]. Little attention has been devoted to the study of the process of pollination and fertilization in those cases when pollen grains and ovules belong to species of different families. The present report contains the results of experiments carried out with the ovules of *Melandrium album* pollinated in vitro with pollen grains of *Datura stramonium*.

Female flower buds of *M. album* were bagged 3 days before pollination. Pistils were sterilized in saturated chlorine water for 15 min and then rinsed several times with autoclaved water. Later the ovary wall was removed