in the epithelia of reproductive accessory glands during the period of the treatment[7,8]. The metaplasia in the animals of the present experiment was still visible 11 months after the last injection of estrone. Further, the processes of the squamous stratification seem to be independent of the presence of testes and adrenals and their steroid hormones, although cornification might be affected by the removal of these endocrine glands. Thus this metaplasia appears to be caused by the neonatal treatment of a high dose of estrogen acting at least initially upon the epithelial cells of the reproductive accessory glands and might be permanent. It should be mentioned that permanent hyperplastic lesions in the vagina and uterus produced by neonatal estrogen treatment was recently reported in mice and rats[9-13].

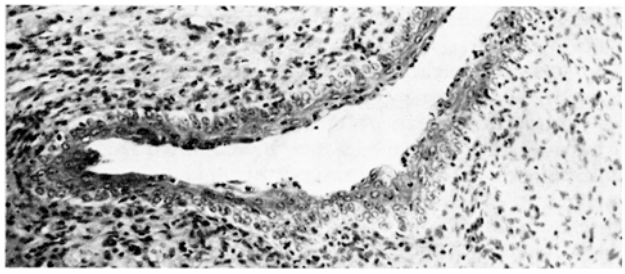

Fig. 3. Seminal vesicle of a neonatally estrone-treated rat sacrificed at 360 days of age. Epithelium stratified but no cornification. × 150.

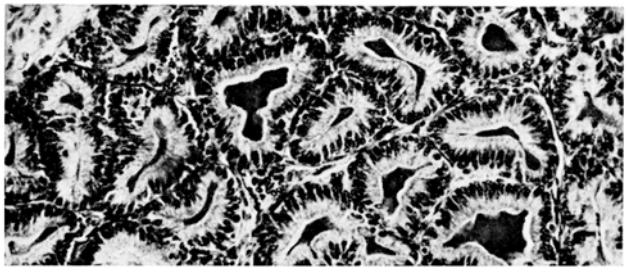

Fig. 4. Seminal vesicle of a rat which received estrone treatment from day 21–50. Epithelium normal. × 150.

Résumé. L'injection de larges doses d'estrone à des rats mâles au début de la période postnatale cause une stratification dans l'épithélium des glandes de coagulation et dans les vésicules séminales. Cette métaplasie a persisté 11 mois après la dernière injection d'estrone.

Y. Arai[14]

Institute of Brain Research, School of Medicine, University of Tokyo (Japan), 8 September 1967.

[7] E. Allen, F. L. Hisaw and W. U. Gardner, in *Sex and Internal Secretions*, 2nd edn (Ed. E. Allen; Williams and Wilkins Co., Baltimore 1939), p. 564.

[8] D. Price and H. G. Williams-Ashman, in *Sex and Internal Secretions*, 3rd edn (Ed. W. C. Young; Williams and Wilkins Co., Baltimore 1961), p. 366

[9] N. Takasugi, Endocrinology *72*, 607 (1963).

[10] N. Takasugi, H. A. Bern and K. B. DeOme, Science *138*, 438 (1962).

[11] N. Takasugi and H. A. Bern, J. natn. Cancer Inst. *33*, 855 (1964).

[12] K. Takewaki, Proc. Japan Acad. *40*, 42 (1964).

[13] Supported by research grants from the Ministry of Education of Japan. I thank Prof. T. Kusama and K. Takewaki, University of Tokyo, for valuable advices during the cource of the experiments.

[14] Present address: Department of Anatomy, Juntendo University School of Medicine, Hongo, Tokyo, Japan.

Radiation-Induced Resistance to Dodine in *Hypomyces*

Resistance to agricultural fungicides in plant pathogenic fungi has not so far created problems comparable to bacterial resistance to antibiotics, or to resistance to certain organic insecticides in insects. Even under favourable laboratory conditions, well defined resistance to the majority of important fungicides has not been achieved[1]. The non-specific mode of action of most antifungal compounds in use, is considered as the most important factor for this rarity of resistant strains. There are, however, mechanisms by which resistance to non-specific toxicants can arise. Hence, suitable mutagenic treatments should be able to induce resistance to many important fungicides. This view is supported by our recent successful use of UV- and γ-radiation to obtain resistance to *n*-dodecylguanidine acetate, commonly known as dodine or Cyprex. This compound is one of our most successful agricultural fungicides and particularly effective against *Venturia inaequalis*. Like most of the present day fungicides, dodine is not known to act by specific enzyme inhibition. Its fungitoxic effect seems to be due to blocking vital anionic sites at the cell surface or inhibiting important enzymes located there[2,3]. This communication reports the development of resistance to dodine in *Hypomyces solani* f. *cucurbitae*, a plant pathogenic pyrenomycete suitable for genetic work, and gives some first data on its nature.

Conidia (*Fusarium* type) were obtained from potato dextrose agar slant cultures kept at room temperature and in diffuse daylight. For the UV-irradiation of the spore suspensions a 15 Watt Philips TUV germicidal lamp was used. This provided a flux of 20 ergs mm^{-2} sec^{-1} at the target distance employed. Eight ml of suspension containing approximately 32×10^5 of conidia were placed in an open Petri dish and agitated during irradiation. Exposure for 6 min was required to give around 95% lethality. In other attempts spore suspensions in small test tubes were exposed to 180,000 rads of γ-radiation from a 'Gammacell 200' Cobalt-60 source of approximately 3000 curies. This irradiation reduced colony formation by 90%. For the selection of mutants a potato dextrose agar medium of pH 5.1–5.4 containing about 5.0 μmoles dodine/100 ml was used. This concentration was 1.5 times the concentration required to prevent formation of colonies by wild type conidia plated at very high

[1] S. G. Georgopoulos and C. Zaracovitis, Ann. Rev. Phytopath., in press.

[2] I. F. Brown and H. D. Sisler, Phytopathology *50*, 830 (1960).

[3] E. Somers and R. J. Pring, Ann. appl. Biol. *58*, 457 (1966).

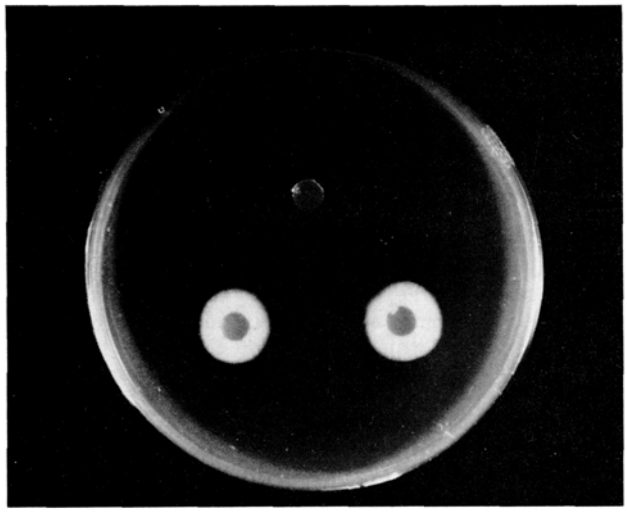

Fig. 1. Five-day-old growth of 2 dodine resistant mutants of *Hypomyces solani* f. *cucurbitae* on potato dextrose agar containing 20 µmoles dodine/100 ml. Blocks (6 mm in diameter) from water agar cultures were transferred to this plate. The picture shows that a similar transfer from the original wild type did not produce a colony. × 0.66.

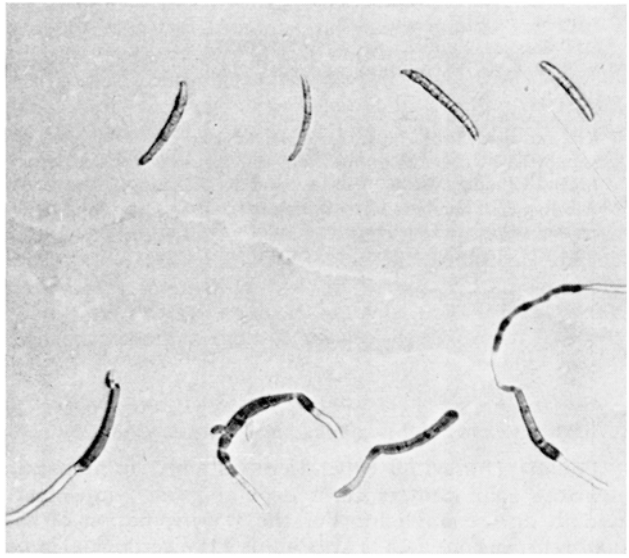

Fig. 2. Conidia of a dodine resistant mutant of *Hypomyces solani* f. *cucurbitae* placed opposite to conidia of the original wild type by micromanipulation. The medium contains 2 µmoles dodine/100 ml. Incubation for 18 h at 25 °C. × 350.

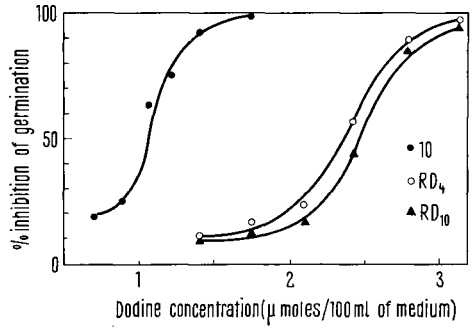

Fig. 3. Dodine dosage-response curves of conidial germination of a wild type strain of *Hypomyces solani* f. *cucurbitae* and 2 dodine resistant mutants obtained from it. Incubation for 18 h at 25 °C.

density (2×10^5 conidia per Petri dish). Putative resistant colonies could be observed between 5 and 10 days after plating.

Two wild type strains, 10 (mating type a) and 14 (mating type A), were used and a few millions of conidia were irradiated in each case. So far 13 strains resistant to dodine have been obtained. Of these 10 developed after UV- and 3 after γ-irradiation. No resistant strains were obtained from equally high numbers of non-irradiated conidia plated and incubated similarly. The resistant strains obtained after irradiation have been transferred to fungicide-free media without loss of dodine resistance. Some of these strains have been crossed to wild types of the opposite mating type and random ascospores as well as tetrads have been obtained as described previously[4]. Analyses for dodine resistance have shown that in each case resistance resulted from mutation of a single chromosomal gene. Whether all mutants carry the same gene for resistance is now under investigation. Evidence of linkage to the mating type locus has been obtained.

Dodine resistance in *Hypomyces solani* f. *cucurbitae* is very clear-cut and easy to recognize in the laboratory. Mass transfers from water agar cultures of the resistant mutants give good size colonies on concentrations of dodine on which similarly transferred wild type cannot grow at all (Figure 1). Scoring for resistance can also be done very effectively with conidial germination tests. For such tests we have used extensively plates of potato dextrose agar containing approximately 2.0 µmoles dodine/100 ml of medium. On these plates wild type conidia are unable to start germination while those of the mutants germinate normally and practically 100% (Figure 2). By varying the concentration of the fungicide, the dodine dosage response of various strains has been studied. The results of a typical experiment are shown in Figure 3. Strains RD_4 and RD_{10} represent UV-induced mutations from wild type 10.

It is known[3,5] that dodine is rapidly concentrated from ambient solutions by fungal spores so that the toxic effect of a certain external concentration largely depends upon the amount of spores/unit medium. In our spore germination experiments, either on the surface of poisoned agar or in dodine-containing liquid media, the toxic effect to a particular strain was also different for different spore densities but, on the basis of ED_{50} (median effective dose) values in each experiment, the mutant conidia were usually 2–3 times more resistant than the wild type conidia. Such differences in sensitivity are not necessarily unimportant from the practical viewpoint[1].

Studies on the physiology and pathogenicity of dodine resistant mutants of *Hypomyces solani* f. *cucurbitae* are under way.

Résumé. Les auteurs ont étudié la résistance de *Hypomyces solani* f. *cucurbitae* au fungicide Dodine. La résistance a été induite en soumettant les spores à l'irradiation UV ou γ, et contrôlée génétiquement. Les mutants étaient environ 2 fois plus resistants au Dodine que les souches sauvages correspondantes.

A. KAPPAS and S. G. GEORGOPOULOS

Nuclear Research Center 'Democritus', Aghia Paraskevi, Athens (Greece), 5 August 1967.

[4] S. G. GEORGOPOULOS, Phytopathology *53*, 1086 (1963).
[5] L. P. MILLER, Phytopathology *50*, 646 (1960).