Sponsored by the Environmental Mutagen Society

CHEMICAL MUTAGENS

Principles and Methods for Their Detection

Volume 9

Edited by Frederick J. de Serres

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Preface

Volume 9 of *Chemical Mutagens* consists mainly of chapters discussing the development and validation of short-term assays to detect the mutagenic effects of environmental chemicals. These chapters include an assay with the grasshopper neuroblast, a comparison of mutagenic responses of human lung-derived and skin-derived diploid fibroblasts, a forward-mutation assay in *Salmonella*, a multigene sporulation test in *Bacillus subtilis*, a specific locus assay in mouse lymphoma cells, a study of the induction of bacteriophage lambda, and the granuloma pouch assay. In addition, there are two chapters on the identification of mutagens in cooked food and in human feces.

Frederick J. de Serres

Research Triangle Park, North Carolina

Contents

CHAPTER 1

The Grasshopper Neuroblast Short-Term Assay for Evaluating the Effects of Environmental Chemicals on Chromosomes and Cell Kinetics

Mary Esther Gaulden, Jan C. Liang, and Martha J. Ferguson

1. Introduction

The grasshopper neuroblast (GHNb) is a newcomer to the library of tests available for evaluating the mutagenicity of environmental chemicals. Most of the current tests have been in use since the beginning of the present era of active research on the identification of environmental mutagens and carcinogens, which began to attain international momentum in the late 1960s.[28,56] Why, then, did we recently develop another assay? First, the neuroblast (Nb) of the grasshopper *Chortophaga viridifasciata* (De Geer) has been shown to be very sensitive to X rays (the effects of doses as low as 1 rad on chromosome breakage and on mitotic

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rate can be detected[42,43]), so we reasoned that it might also be very sensitive to chemical mutagens. Second, the fact that the spontaneous chromosome aberration frequency in the GHNb is zero means that significant data on mutagens can be obtained with a minimum number of cells. Third, the GHNb has a short cell cycle[44] with a number of well-defined phases, and thus, much information about the effects of agents on cell progression can be obtained. This aspect of environmental mutagen action has received relatively little attention and is of considerable relevance to teratogenesis.[36] The short cell cycle (*Chortophaga*, 4 hr; *Melanoplus sanguinipes*, 2 hr, 38°C) is also advantageous for testing chemicals with short half-lives. Fourth, the GHNb is a simple, fast, reproducible, and inexpensive eukaryotic test system. No single assay developed to date is ideal for estimating the risks of environmental chemicals for humans, so a battery of systems is required, and the need to search for good ones is still with us.

Grasshopper cells have long been used for chromosome studies (see Ref. 39). Initially, germ cells were the focus of attention and were used by McClung[79] to first show that specific chromosomes determine sex. Later, J. G. Carlson, a student of McClung, undertook a study of the somatic cell chromosomes of grasshoppers, which led him eventually to work on Nbs. His first studies were done at the Cold Spring Harbor Laboratory in New York State, and *Chortophaga* was the only adult species available in the field when he arrived in early summer. This species is one of the few that is multivoltine (produces several broods a year). In other words, the embryo of this species does not have a diapause phase, i.e., a genetically determined cessation of development [128] that is usually broken by prolonged exposure to low temperature. Thus, *Chortophaga* embryos develop straight through to hatching, thereby enabling an investigator to obtain 4–6 generations a year in the laboratory.[23] Subsequently, Carlson, his colleagues, and his students have studied extensively the chromosomes and cell cycle of the living as well as the fixed Nb of *Chortophaga*, with emphasis on radiation effects. These studies provide a valuable data base as background for chemical mutagen studies.*

One attribute of the GHNb that commends it for testing is its embryonic origin. Of all the systems currently employed for mutagen testing, only one of the more widely used involves a cell of primary embryonic origin (dominant lethal test with early mammalian embryo), and it is time-consuming. In the life history of an organism, embryonic cells are among the most sensitive to ionizing radiation and probably

* A complete list of references for these studies is available from the senior author.

to chemical mutagens. Further, in the embryo and fetus there is evidence that different cell types have different sensitivities to mutagens, with Nbs (stem cells for the nervous system) being among the most sensitive, including those of the human.[40,45,59] A detailed rationale has been presented for the view that exposure of Nbs *in vivo* to small doses of mutagens may give rise, by chromosome aberration induction, to subtle teratogenesis of the central nervous system (CNS) in humans, resulting in functional defects.[36,40] The results obtained with a short-term mutagen test on the sensitive embryonic Nbs of the grasshopper may, therefore, have relevance to the hazards of environmental chemicals to human embryos with respect to teratogenesis as well as to mutagenesis and carcinogenesis.

The purpose of this chapter is to provide the detailed information that an investigator, unfamiliar with GHNb methods, needs in order to obtain data on chemical mutagens with a minimum of startup time. Recent work in our laboratory has shown that good rearing conditions for a grasshopper colony are essential for a constant supply of normal embryos with no spontaneous chromosome aberrations, so rearing methods are described. Previous reviews provide some of the Nb techniques[16,20,39]; details of methods pertinent to the exposure of Nbs to chemical mutagens will be given here not only for *Chortophaga*, but also for a nondiapausing strain of *Melanoplus sanguinipes*[98] that we have recently begun to study. In addition to the methods for examining chromosome aberrations and cell cycle effects, those that permit detection of other endpoints in the GHNb are also described, namely, spindle abnormalities, unscheduled DNA synthesis, and effects on normal DNA, RNA, and protein synthesis. A summary of some of the data obtained with chemicals is included.

The details given here are probably applicable with minor variations to other species of grasshopper. Grasshopper embryo development and Nb characteristics have been shown to be similar for several species, so it can be reasonably assumed that the early embryonic development in other species of grasshopper is essentially the same,[3] except perhaps for the time scale. If this is the case, the widespread distribution of grasshoppers in many parts of the world makes the GHNb technique available to investigators through the use of native species.

It should be noted that eggs of *Chortophaga* and *M. sanguinipes* survive mailing conditions quite well if they are not subjected to extreme temperatures. We will be glad to send a starter supply of eggs from our surplus to investigators who wish to initiate a colony. Dr. J. E. Henry (personal communication) tells us that he will send starter egg pods of *M. sanguinipes* when his laboratory has an excess, or that under

a cooperative agreement, an investigator could be sent eggs at reasonably regular intervals.*

2. Embryo Supply

For mutagen testing, grasshopper embryos are needed year-round, so a constant supply of mature adults is required, the size of the colony being dictated by the number of embryos needed. It is therefore necessary to maintain a laboratory for rearing and maintaining egg-producing animals. With attention to a few details about food, light, temperature, and cleanliness, this can be accomplished with a minimum of time, effort, and expense.

2.1. Species

The two species we use are *Chortophaga viridifasciata* and *Melanoplus sanguinipes* (family: Acrididae; order: Orthoptera). In contrast to *Chortophaga*, few cell data on the Nb, much less other cell types, are available in the literature for *M. sanguinipes* [formerly *M. mexicanus mexicanus* (Sauss.) and *M. bilaturatus* (Walker)], which is the so-called migratory grasshopper of North America. Because of its economic importance to agriculture, *M. sanguinipes* has been much studied in other respects, e.g., embryonic development,[103] fecundity,[97] food preferences,[96] physiology,[106,131] toxic responses,[82] and sensitivity to plant growth hormones.[25] Such information is useful in establishing and maintaining a healthy colony. Of the two species, *M. sanguinipes* is the faster growing and is the more vigorous in the laboratory. Its appetite is also more voracious.

2.2. Origin of Colonies

Chortophaga viridifasciata (subfamily: Oedipodinae†) is found in the wild in eastern North America from southern Ontario to Georgia and is abundant as far west as an area bounded by a line transecting the eastern portions of Saskatchewan, Oklahoma, and Texas (approximately 50 miles east of Dallas). In the southernmost regions of its range,

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† Two additional volumes projected by Otte[94] for a definitive treatise on North American grasshoppers will bring up to date the taxonomy of the Oedipodinae.

Chortophaga produces three generations a year, one each in spring, summer, and fall. Because of availability, we have previously used field animals for embryo supply by bringing nymphs and adults into the laboratory. Recently we have begun to establish a laboratory colony. Dr. Kenya Kawamura of the College of Agriculture in Hokkaido, Japan, informs us that he has a colony of *Chortophaga viridifasciata* in his laboratory derived from animals he obtained while in Tennessee in the late 1950s. Dr. Saralee N. Visscher of Montana State University has recently established a colony in her laboratory (personal communication), also from animals collected in Tennessee. Experience in three laboratories shows, therefore, that even though *Chortophaga* is one of the less hardy grasshoppers,[23] it can be bred satisfactorily under laboratory conditions. To avoid excessive inbreeding, we recommend occasional introduction of animals from the field to the colony.

We obtained eggs of *Melanoplus sanguinipes* (Fabricus) (subfamily: Melanoplinae) in 1980 from Drs. G. B. Staal and M. P. Pener of Zoecon Corp., whose colony was derived from the original nondiapausing strain developed by Pickford and Randell.[98] Species of *Melanoplus* in nature are univoltine; the embryos have an obligatory diapause period. Pickford and Randell had observed that in the laboatory a few eggs developed without pause to hatching after incubation at 30°C with no cooling. By selecting adults from such eggs, they were able over a period of 12 years to establish a vigorous colony of a nondiapausing strain of *M. sanguinipes*. It might be noted that Slifer and King,[119] using the same methods, had previously developed a nondiapausing strain of the much studied *M. differentialis* (Thomas). Dr. Bruce Nicklas had, to our knowledge, the only surviving colony of this strain, but he reports that it is now extinct (personal communication).

2.3. Life Cycle

The life cycle of the grasshopper consists of three phases: egg, nymph, and adult. Under the laboratory conditions for rearing grasshoppers given in Section 2.4, the durations of the egg and nymph phases of *Chortophaga* are 6 weeks each; adults survive for 6–8 weeks. The egg and nymph phases of *M. sanguinipes* are shorter, 3–4 weeks, but the life span of adults is comparable to that of *Chortophaga*.

At the time of hatching, the vermiform larva is enveloped by a membrane, thin and transparent, which serves as a provisional cuticle; it is a real cuticle in that it is acellular and chitinous. As soon as the larva reaches the soil surface, it undergoes its first molt, called the intermediate molt, and sheds the provisional cuticle, which when dry