In vitro studies on the metabolism of aflatoxin B$_1$ and aldrin in testes of genetically different strains of *Drosophila melanogaster*

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Abstract. As *Drosophila melanogaster* occupies an important position within the test battery for mutagens and carcinogens, it is of interest to study the xenobiotics metabolism of this insect. Likewise, the genetic control of these important enzyme systems falls within this interest.

Our attempt was to get new strains, which show changes in their xenobiotics metabolism. This was done by a mutagenization and selection procedure for the second chromosome. The 44 fertile homozygous inbred strains produced by this selection were first tested for DDT resistance. Some of them showed LT50 values which were remarkably higher than that of the original strain Berlin K.

Aflatoxin B$_1$ metabolism in two of the new strains (H349 and H362), Berlin K, and Hikone-R was compared, whilst aldrin epoxidase activity was compared in strains H349, H362, Berlin K, vestigial, and Karsnäs-R. The metabolism studies were carried out in vitro with testes tissue of the different strains. The metabolism in testes is of specific interest because this tissue is most often used in mutagenicity testing.

In the AFB$_1$ assays of the up to 12 observed metabolites three could be identified as AFB$_{2a}$, AFM$_1$, and AFR$_o$. Hikone-R produced mostly AFR$_o$ (3.43% of the initial AFB$_1$ concentration) and small amounts of AFM$_1$ (0.59% AF) and AFB$_{2a}$ (0.36% AF). The strain Berlin K showed only a low production of AFB$_{2a}$ (0.48% AF), while the strain H349 formed AFR$_o$ (6.02% AF) and AFM$_1$ (0.75% AF). The AFM$_1$ appeared in even higher amounts than with Hikone-R. On the other hand, H362 showed the lowest activity in AFB$_1$ metabolism. With this strain none of the determined metabolites could be detected in levels significantly higher than the control. The difference between H349 and the original strain Berlin K was highly significant. The production of AFR$_o$ and the binding of aflatoxin to macromolecules show a linear correlation. In both parameters measured, the strain H349 yielded the highest results. The determination of aldrin epoxidase activity gave the following results (in pmol die-drin · mg$^{-1}$ protein · min$^{-1}$): H349: 0.74; Karsnäs-R: 0.57; vestigial: 0.57; Berlin K: 0.32; H362: 0.27. Again the difference between H349 and Berlin K was statistically significant. The measured activities match values obtained with extrahepatic tissue of mammals.

It is concluded that the line H349 is a mutant in the xenobiotics metabolism. For the strains Hikone-R, Karsnäs-R, and H349 AFR$_o$ could be confirmed to be the main metabolite of AFB$_1$. The metabolism pattern was shown to differ strongly from strain to strain.

Key words: Aflatoxins — Aldrin — *Drosophila* — Xenobiotics metabolism — Testes

Introduction

*Drosophila melanogaster* offers a variety of assay systems for the detection of chemical mutagens and carcinogens (Sobels 1972, 1974; Vogel and Sobels 1976; Würgler et al. 1984; Würgler 1980). Systems are available for the detection of point mutations, chromosome aberrations, nondisjunction and somatic mutations and recombination. The best validated of these assays is the sex-linked recessive lethal test, which detects mutants inducing point mutations and small deletions. The mutations are observed based on their recessive lethal phenotype. This test is easy to perform and relatively fast and cheap.

It was found that over 83% of known carcinogens are detected as mutagens in the *Drosophila* sex-linked recessive lethal assay (Vogel et al. 1980; Lee et al. 1983).

An important advantage of *Drosophila* is its capacity to metabolize xenobiotics in a manner similar to mammals. Therefore, using *Drosophila* based assay systems, it is possible to detect pro-mutagens and pro-carcinogens (Vogel and Sobels 1976).

The xenobiotics metabolism of mammals is most active in the liver (Bentley and Oesch 1982). These enzyme systems have been studied extensively during the last decades. For insects, especially for *Drosophila*, most data available were obtained only recently. Initially the metabolism of insecticides was of primary interest (Casida 1969; Wilkinson and Brattsten 1972; Perry and Agosin 1974; Wilkinson 1976). These studies demonstrated the presence of xenobiotics metabolizing systems in insects, including flies. Microsomes obtained from whole insects or particular organs had metabolic activities similar to those of mammalian liver microsomal preparations. Most of the enzyme activity in insects is located in the gastrointestinal tract, the Malpighian tubules and the fat body (Casida 1969). The first indications that *Drosophila* activates pro-mutagens were obtained by Clark (1959, 1960, 1963) and Pasternak (1962, 1963, 1964). They discovered the strong
mutagenic activity of pyrrolizidine alkaloids and of nitroso-
amines. In the last few years the biochemistry of the metabolism of
pro-mutagens was studied in Drosophila (Kulkarni et al. 1976; Baars 1980; Baars et al. 1977, 1979; Holzer et al. 1978; Holzer 1981). These studies indicate that Drosophila has
potent enzyme systems, most of which are comparable to those of
mammals. In modified Ames tests, crude microsomal fractions from

In earlier studies with insecticides (Georgiou 1965) and in
recent studies (Nigsch 1978; Holzer 1981; Hällström et al. 1982), the second chromosome of Drosophila was shown to
carry genes important in the control of xenobiotics metabo-
lism. Therefore, Drosophila offers the opportunity to study the
genetic control of the enzyme system(s) active in xenobiotics
metabolism. By isolating flies which most probably carried a
second chromosome mutation, and by characterizing the testes of
homzygous carriers with respect to their metabolic activities, we have investigated the genetic control of the
metabolism of aldrin and aflatoxin B1.

Materials and methods

a) Chemicals and materials. DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] was purchased from Fluka, Buchs, Switzerland; EMS (ethyl methanesulfonate) from K+K, ICN Pharmaceutical Inc., Life Sciences Group, Plain-
view, NY; dieldrin and aldrin as Pestenal (HEOD) and Pestenal (HHDN) from Riedel-DeHaen AG, Seelze, Han-
hoever, FRG; all aflatoxin standards (AFB1, AFB2, AFM1, and AFRe) from Senn Chemicals, Dielsdorf, Switzerland.

[14C]-aflatoxin B1 was produced biosynthetically by the
method of Hsieh and Mateles (1970) with Aspergillus flavus
ATC 15517 grown on medium containing [1-14C]-sodium acetate (purchased from New England Nuclear, Boston, MA; specific activity 57.7 mCi/m mole). The radioactivity of the product was 30.4 mCi/m mole.

A modified Bio-Rad assay was used for all protein
determinations (Bradford 1976; Foerster 1979).

b) Drosophila strains. The following strains were used: Berlin K (wild type; from E. Vogel, Dept. of Radiation Genetics and
Chemical Mutagenesis, State University Leiden, The Nether-
lands), DTS-91 (a strain with a dominant temperature-sensi-
tive mutation with the following genetic constitution: Berlin K*/Berlin K*), this cross was again performed under
standard conditions (25°C). Homozygous offspring of any such inbred cross is homozygous for the second chromosome (Berlin K*) and is potentially homozygous for EMS-induced mutations.

d) DDT tests. The DDT tests were used as a first screening for
physiological changes in strains homozygous for a mutagenized second chromosome compared to the original strain Berlin K.
We tested the DDT-resistance in the short term test developed by
Boehning (1954) as modified by Nigsch (1978). For each test
1–2-day-old flies were used. They were placed in groups of 10
females and 10 males into petri dishes coated with DDT (0.2
mg per dish) or into control dishes without DDT. Flies which
were lying on their backs were counted hourly for up to 8 h for
both sexes separately. All tests were repeated at least twice.
The data were analyzed with computer programs based on the
multinomial distribution (BerkGilrd 1982).

e) Preparation of testes tissue. For each preparation at least
1,500 adult males were collected during the 2 days before the
experiment and kept in bottles with standard medium and live
yeast. All steps of the preparation were performed on ice with
precooled media and material. Portions of 100–200 flies were
immobilized by cooling and pressed through a collector (Bellco
Glass Inc., Vineland, NJ) with sieve nr. 2 (860 μm within
mesh) and the sieve rinsed with cold autoclaved Ringer’s
solution (Ephrussi and Beadle 1936). The suspension obtained
by this step was collected in a 200-ml beaker. This step was
repeated until all flies were prepared. The resulting suspension
was sedimented at 1 g for 5 min and the volume reduced to
20 ml by aspirating the major part of the supernatant. This
supension was then filtered through sieve no. 3 (520 μm within
mesh) and the sieve rinsed with an excess of fresh Ringer’s
solution. This suspension was sedimented at 1 g for 20 min and
the supernatant was again discarded. The pellet was trans-
ferred to a petri dish and the testes were collected with small
paster pipettes under stereomicroscopes. The testes were
immobilized by cooling and pressed through a number 3
(sieving no. 3) or 4 (520 μm) sieve. All steps of the preparation
were performed on ice with precooled media.

f) Aflatoxin assay. For all assays, freshly isolated testes tissue
was used. For each strain duplicate dishes with intact and
heat-inactivated tissue were studied. All dishes were preincubated for 10 min at 35°C in a shaking water bath (75 stpm).
The incubation was started by adding 20 μl [14C]-aflatoxin B1
(1.8 × 103 dpm, 2.67 mmole) dissolved in methanol and stopped after 60 min by transferring the tubes into ice water
and adding 5 ml dichloromethane. Each charge was extracted
three times with 5 ml dichloromethane. The organic fractions
were pooled, and dried on sodium sulfate, evaporated in a N2
stream and redissolved in 20 μl benzene. All four charges of
one strain were then applied individually to the corners of a
HPTLC plate (10 × 10 cm, Kieselgel 60 F254, Merck, Darm-