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THE ROLE OF DROSOPHILA IN CHEMICAL MUTAGENESIS TESTING*

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INTRODUCTION

An important question facing our society is the impact of numerous chemical insults on the health of man and his environment. Faced with a staggering array of chemicals and enormous testing costs, only a few chemicals can be tested for possible carcinogenic effects. Recent results with the *Salmonella*/mammalian microsome mutagenesis assay developed by Ames (2) demonstrating a striking correlation between carcinogenicity and mutagenicity of many chemical compounds offer the possibility that mutagenesis assay systems can provide a quick identification of potential carcinogens. Results from microbial assays can serve as a guideline for further mutagenesis testing as well as identify those compounds requiring more extensive analysis in mammalian systems.

Unquestionably, man is more closely related to other mammals than bacteria and information regarding pharmacokinetics can only be obtained from mammals. Detection of point mutations and small deletions in mammals, however, requires enormous costs and considerable time and labor; thus the number of chemicals that can be investigated is

restricted. Other mammalian assay systems which rely solely on chromosome breakage do not suffer from these disadvantages, but their utility as diagnostic tests are questionable in light of recent results obtained in Drosophila. Vogel (10) has shown that many chemicals are very effective in producing point mutations and small deletions but do not produce chromosome breakage effects at all, while others produce chromosome breakage, but only at concentrations much higher than that required to produce point mutations. Such compounds would appear safe in any assay which measured only chromosome breakage.

Reliance on the results from a single mutagenic assay system is rather risky. It would seem preferable, in our opinion, to use a battery of tests (the tier approach) which would include the rapid microbial assays as well as mammalian systems. Also the use of Drosophila as a bridge between the microbial and mammalian assays has many desirable features as will be discussed in the following paragraphs.

Advantages of Drosophila as a Test Organism

As a mutagenesis test organism, Drosophila is not as economical nor as rapid a screen as the microbial assays but few higher organisms offer the economy and short generation time that can be achieved with Drosophila. Drosophila mutagenic assays can be used in pre-screening

tests but perhaps the most useful approach is to use them as a confirmation of results obtained in microbial assays and extension of the anlaysis to include genetic end-points which are unattainable in the microbial systems.

Due to the availability of a wealth of tester strains, the assessment of a variety of induced genetic changes is readily obtainable in Drosophila. Genetic end-points easily scored cover a wide spectrum including point mutations and small deletions, translocations, chromosome loss, non-disjunction and genetic recombination. Thus mutagenic assays in Drosophila can detect genetic damage due to both point mutation and chromosome breakage.

In many mutagenesis screening programs, method of exposure is often an important parameter. In those cases, the advantage of using Drosophila again becomes apparent as the chemical compound may be administered via feeding, injection, inhalation or direct treatment of sperm. Feeding and injection are the most commonly used methods but inhalation of a gas or aerosol is also very effective. A serious disadvantage of the aerosol method is that a considerable volume of the chemical agent is required; a disadvantage which is overcome by the injection technique where only microliter quantaties are needed. For a more thorough discussion of the advantages and disadvantages, see

the review by Lee (8). Whatever method is chosen it should be kept in mind that a negative result may be due to the particular method of exposure (17), and in these cases an alternate route of administration should be used.

Chemical mutagens often show a cellular specificity (3) and failure to detect mutagenic activity may result from a stage-specific response. In Drosophila the mutational response to a chemical insult in different germ cell stages may be studied by the brood pattern analysis (a technique whereby the mutaion frequency of successive mating is obtained). Though somewhat more time consuming the additional information gained can give a more detailed picture of the mutagenic activity of a chemical. The method of brood pattern analysis developed for use in radiation genetics works equally well with chemicals except that with chemicals one has to consider the lingering effect of chemicals which remain in the body and which results in exposure of germ cells over a longer period of time.

Another feature which adds to the utility of Drosophila is the presence of a mixed function oxidase system which is similar to that of the mammalian liver in its ability to activate indirect mutagens. In recent years considerable attention has been focused on the metabolism of certain drugs and pesticides by insects and it has been shown that

the crucial step in such metabolism is a oxidative attack by mixed function oxidases which can be isolated as a microsomal fraction (1, 5). Although Drosophila are insects the evidence that they also possess microsomal activities similar to the mammalian liver was indirect. It was based largely on the fact that some forty-fifty compounds that require metabolic activation are, when tested in Drosophila, effective in inducing recessive lethals (11). These compounds fall into several different groups with widely differing structures. From these types of studies one can conclude that the Drosophila enzyme systems are similar to the mammalian systems in the versatility and lack of substrate specificity. Recently, Baar et al. (4) have presented evidence that isolated Drosophila microsomes possess cytochrome P450 and aryl hydrocarbon hydroxylase activity and Nix et al. (9) have shown that Drosophila microsomes are capable of activating numerous promutagens when they are substituted for rat liver microsomes in the Salmonella histidine reversion assay.

The Use Of Drosophila In The Mutagenic Analysis Of Complex Mixtures

Potential health effects of existing, as well as new, fuel technologies have beomce of increasing concern. Epler et al. (6, 7) have used the Salmonella/mammalian-microsome test system to

assay environmental effluents and crude products from the synthetic fuels technology. Complex mixtures were fractionated, and each fraction was tested for possible mutagenic activity. Such procedures identified several fractions as mutagenic and as candidates for further biological testing. Experiments described here represent an attempt to extend their observations to a eucaryotic organism and to identify other genetic effects. In addition we describe the isolation of a crude Drosophila microsome fraction and the use of such fractions in the Salmonella mutagenicity test system.

Oregon-R wild-type males and Muller-5 [In (1) $sc^{sl}L\,sc^{8}R+S$, $sc^{sl}\,sc^{8}\,w^{a}B$] males and females were collected as needed from the Oak Ridge stock collection. The Salmonella strain used was TA98 (hisD3052, uvrB, rfa, frameshift plus R factor), obtained from Dr. Bruce Ames, Berkeley, California.

Synthetic fuel fractions were dissolved in DMSO and then diluted with a sterile sucrose solution to a final concentration of 2% sucrose and 4% DMSO. A glass-fiber filter paper was placed into an empty glass vial and then saturated with 175 microliters of the appropriate test solution. Wild-type (Oregon-R) males, 1-2 days old, were starved for 5 h, placed in the vials containing the test solution (25 males per vial), removed after 24-48 h and mated to virgin Muller-5 females. In the brood-pattern analysis treated males were mated for five

successive 3-day broods. F_1 females were mated and progeny scored for the presence of X-linked recessive lethals.

In the Salmonella/microsome mutagenicity tests the standard procedures given by Ames et al. (2) were employed except that Drosophila microsomes were substituted for rat-liver microsomes. Concentrations of buffer and cofactors were as previously described by Ames.

For the isolation of Drosophila microsomes, wild-type (Oregon-R) flies were grown on standard media which contained no live yeast. Adults were collected 7-10 days after emergence, etherized, and placed on ice. Two volumes (w/v) of ice-cold potassium phosphate buffer (pH 7.5) was added and flies were homogenized by gently pounding in a mortar until a smooth brei was formed (approximately 120-150 stokes with the pestle). The homogenate was filtered through four layers of cheesecloth and the filtrate was spun at 750 g. The resulting supernatant was spun two times at 10,000 g and after the final spin the supernatant was immediately in the Salmonella system; the remainder was frozen at -70°C.

Our primary concern in the assay of the mutagenic effects of the synthetic fuels was to confirm the results in a higher organism and then if possible to extend the analysis to include other genetic effects.

For this purpose we selected the X-linked recessive lethal assay as it has been shown to be the most sensitive in Drosophila. Vogel (10) has carried out a comparative study of the frequency of induction of recessive lethals, dominant lethals, and chromosome loss by various concentrations of different mutagens. For all mutagens studied the recessive lethal assay was the most sensitive. We find a similar result for a series of cyclic nitrosoamines as shown in Table 1. In addition we find a very close correlation between mutagenicity as measured by the X-linked recessive lethal assay and carcinogenicity in rats.

Since the crude synthetic fuel is toxic to Drosophila, only selected fractions could be tested. The results of a brood pattern analysis is shown in Table 2. Fractions 7 and 9 are ineffective in including X-linked recessive lethals in broods 1-3, although fraction 9 seems to be slightly mutagenic for spermatogonial cells. Using fraction E, the acetone soluble portion of a more highly purified subfraction of the combined basic fractions from the Stedman fractionation scheme, (6, 7), we find a significant increase in the frequency of lethals in broods 1 and 2 but not brood 3. This suggests that fraction E is an effective mutagen for mature sperm and spermatids but not meiotic cells. With this in mind, we then fed fractions 7, 9, and 14 at several different concentrations and monitored the production of X-linked recessive

Table 1

Induction Of X-linked Recessive Lethals And Sex Chromosome Loss In Drosophila By A Series Of Cyclic Nitrosoamines

Compound	Mutagenicity in Drosophila		Carcinogenicity in rats*
	X-linked recessive lethals	Chromosome loss	
Nitrosopiperidine (NP)	+	-	+
2,6-Dimethyl NP	-	-	-
2-Methyl NP	+	-	+
4-Methyl NP	+	-	+
3,4-Dichloro NP	+	-	+
Nitrosopipecolic acid	-	NT	-
Dinitrosopiperazine	+	NT	+
2,3,5,6-Tetramethyl-dinitrosopiperazine	-	NT	-
Nitrosomorpholine	+	NT	+

*The carcinogenicity data was kindly provided by Dr. W. Lijinsky

NT = Not tested.

Table 2

Brood Pattern Analysis of X-linked Recessive Lethals Induced In Drosophila melanogaster by Synthetic Fuels

Fraction	Conc. fed (µg/ml)	Brood	Chromosomes tested	Lethals	% Lethals
Control	---	1	1334	3	0.22
		2	1839	4	0.22
		3	1318	1	0.08
		4	803	2	0.25
7*	994	1	1071	0	0.00
		2	1039	3	0.29
		3	984	3	0.30
9*	1059	1	1083	4	0.37
		2	1197	1	0.08
		3	1230	2	0.16
		4	1295	7	0.54
E+	500	1	1661	11	0.66
		2	1686	13	0.77
		3	1780	3	0.17

*Basic fractions isolated from a crude synthetic fuel product by the Stedman fractionation procedure.

+Acetone subfraction of Stedman basic fraction which is further fractionated by LH-20 [Epler et al., these proceedings].

lethals in mature sperm and spermatids. Inspection of Table 3 reveals that fraction 7 is ineffective at all concentrations tested. Fractions 9 and 14, at the two lower concentrations tested, increase the frequency of lethals 2-fold over the spontaneous level but this is not statistically significant. In order to show a significant doubling with a critical region of 0.05 one would need to test 12,000-15,000 chromosomes. From these results we can conclude that the basic fractions (7, 9), which are mutagenic in the "Ames" assay, induce at the most only a 2-fold increase in the frequency of X-linked recessive lethals in Drosophila melanogaster. Further purification of these fractions results in a subfraction which shows a slight mutagenic activity in Drosophila; it induces an increase of 3-4x over the spontaneous level. Thus, we confirm the mutagenic activity of fractionated products of synthetic fuels in a eucaryotic organism but such activity is very low compared to that obtained in the microbial assay.

One of the advantages of Drosophila as a mutagenesis test organism is the presence of metabolic activation system. By substituting isolated Drosophila microsomes for rat liver microsomes in the Salmonella/histidine reversion assay one can correlate mutagenic activity of a chemical compound in vivo with the ability of isolated

Table 3

Induction Of X-linked Recessive Lethals In Mature Sperm And Spermatids Of Drosophila melanogaster by Subfractions Of Synthetic Fuel

Fraction	Conc. fed (µg/ml)	Chromosomes tested	Lethals	% Lethals
Control	--	1753	4	0.23
6	15.02	1214	2	0.16
7	994	1097	0	0.0
	397	1069	3	0.28
	199	1036	1	0.10
9	1059	1346	0	0.0
	423	702	3	0.42
	212	861	4	0.46
14	870	1012	2	0.20
	435	1185	6	0.51
	218	1065	5	0.47

microsomes to activate such chemicals. Results of experiments in which we tested the ability of Drosophila microsomes to activate fractions 7, 9, and E are shown in Figure 1 and Table 4. Instead of Aroclor-induced rat liver fractions, 400 microliters of Drosophila 10,000 g supernatant was used; all other procedures were as described by Epler et al. (6, 7).

In light of the in vivo activity, these results are rather surprising. Figure 1 shows the number of revertants/plate plotted versus concentration. For all three fractions we obtained a linear dose-response curve over the concentrations tested. The slope of each induction curve was determined and these results along with those obtained using Aroclor-induced rat liver are shown in Table 4. It is of interest that Drosophila microsomes are just as effective as Aroclor-induced rat liver microsomes in the activation of all three fractions and is even more effective with fractions 9 and E. We have tested several pure compounds in the Salmonella/Drosophila microsome assay. Of these, 2-acetylaminofluorene and aftatoxin B_1 showed the highest mutagenic activity, 144,000 and 180,000 revertants/mg respectively; thus Fraction E gives almost a 10-fold increase in mutagenic activity over any compound we have thus far tested. In these instances, results with uninduced Drosophila microsomes compare very well with those of induced rat liver. Comparisons based on the number

Table 4

Comparison Of Mutagenic Activity Of Synthetic Oils

Activated By Drosophila And Aroclor-Induced Rat Liver Microsomes

Fraction	Specific activity (rev/mg)	
	Rat liver	Drosophila
7_B*	45,000	30,000
9_B	28,900	85,000
E	222,000	1,300,000

*See footnotes to Table 2.

of histidine revertants per milligram of S-9 protein are even more striking in favor of Drosophila as typical Drosophila microsomes preparations contain about one-fourth the protein of induced rat liver microsomes.

The discrepancy between the in vivo and in vitro mutagenic activity of fractionated complex mixtures is interesting but at this point we have no explanation. One must keep in mind that the metabolism of foreign compounds involve enzymatic pathways which result in toxification as well as detoxification. The balance between the two will often determine whether an active metabolite generated will remain in the cell and exert genetic damage or be detoxified before any damage can be done.

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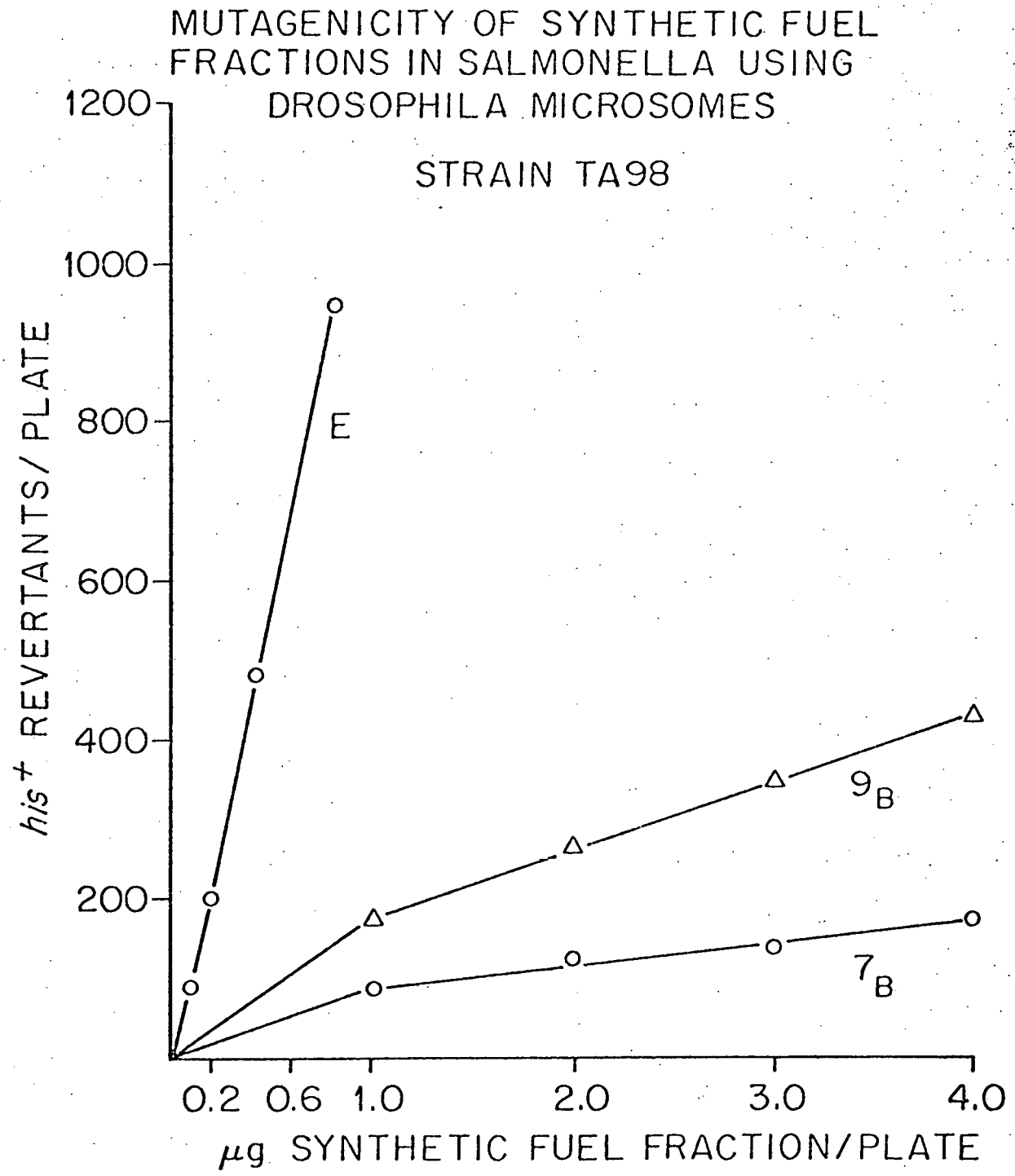
FIGURE LEGENDS

Fig. 1. Effect of increasing concentration of synthetic fuel fractions on *his*$^+$ reversion in *Salmonella* strain TA98. All reagents were as described by Epler et al. (6, 7) except that 400 microliters of *Drosophila* S-9 was substituted for rat liver S-9.

●, 7_B ; Δ, 9_B ; O, E


MUTAGENICITY OF SYNTHETIC FUEL FRACTIONS IN SALMONELLA USING DROSOPHILA MICROSOMES
STRAIN TA98
1200
1000
800
600
400
200
his+ REVERTANTS/ PLATE
E
9B
7B
0.2
0.6
1.0
2.0
3.0
4.0
μg SYNTHETIC FUEL FRACTION/PLATE