*Biochemical Pharmacology*, Vol. 33, No. 1, pp. 13–20, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00


# GENETIC VARIATION IN CYTOCHROME P-450 AND XENOBIOTIC METABOLISM IN *DROSOPHILA MELANOGASTER*

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(*Received* 9 *March* 1983; *accepted* 1 *July* 1983)

**Abstract**—A marked genetic variation in the capacity to perform xenobiotic metabolism was observed in microsomal fractions from the seven *Drosophila* strains studied. A 1,5 to 2-fold variation was found in the content of cytochrome P-450 and in the NADPH-cytochrome c reductase activity. The two insecticide-resistant strains Hikone R and Oregon R differed markedly when compared to sensitive strains by having a 3 to 17-fold higher *p*-nitroanisole (PNA) demethylase activity and biphenyl-3-hydroxylase activity. SDS-polyacrylamide gel electrophoresis of the microsomes also revealed an increased content of a protein band with an apparent mol. wt of 54,000 in the resistant strains. The 4-hydroxylation of biphenyl was also 2–7-fold higher in the Oregon R strain, and the band with a mol. wt of 56,000 had a higher protein content compared to the other strains. The biphenyl-4-hydroxylation was several-fold lower in the strain Berlin K. 2-OH-biphenyl was formed only in trace amounts by all strains. 7-Ethoxycoumarin (EC) deethylase activity and benzo(a)pyrene (BP) monooxygenase activity was 2–6-fold lower in the Hikone R strain. An increased amount of the protein with a mol. wt of 58,000 was noted in the Canton S strain. No concomitant increase in any enzyme activity was observed. A genetic variation between the strains was observed after phenobarbital (PB) treatment in the content of cytochrome P-450 and in the various enzyme activities, varying from non-responsiveness to a 4- to 5-fold increase. Aroclor 1254 (PCB) was less efficient in enhancing the activities. It caused maximally a 3-fold increase, had often no effect and in some cases even decreased the metabolism. β-naphthoflavone (BNF) caused only marginal increases in the activities in most strains. The only significant effects were an increased formation of 3-OH-biphenyl in Berlin K and an enhanced NADPH-cytochrome c reductase activity in Lausanne-S. In conclusion, the variations observed in this study provide a basis for further studies on the genetic regulation of the cytochrome P-450 system in *Drosophila*. Furthermore, similarities in the regulation when compared to mammals indicate that studies on this genetically well characterized organism might contribute to the general understanding of the genetics of xenobiotic metabolism.

The activation of indirect carcinogens is primarily performed by the cytochrome P-450 enzymes [1–3]. The level of the different enzymes is determined by the individuals' genetic constitution [4–6] as well as by exposure to enzyme-inducing substances in the environment. Genetic differences in the capacity of bioactivation might cause an enormous variation in sensitivity to toxic and carcinogenic effects of xenobiotics between different species, strains and individuals [4, 7].

*Drosophila melanogaster*, the classical organism in genetic investigations, offers unique possibilities for studying genetic regulation of drug metabolism. The presence of an inducible cytochrome P-450 system has been established [8–12] as well as genetic variation in metabolism [10–13], toxicity [14] and sensitivity to mutagenesis [15] by indirect carcinogens. In the present study 7 *Drosophila* strains are compared by means of several enzymatic activities in subcellular fractions, response to pretreatment with typical enzyme inducers and SDS gel electrophoresis pattern of the hemoproteins. Some of the data have been previously reported [11, 16]. The study covers 1 wild type strains of different geographic origin, two strains resistant to insecticides like DDT and carbamates, and one aflatoxin $B_1$ ($AFB_1$)-tolerant strain. The cytochrome P-450 system contributes to the metabolism of the xenobiotics mentioned.

## MATERIALS AND METHODS

### *Chemicals*

Benzo[a]pyrene (BP) was obtained from Sigma Chemical Co (St. Louis, MO), Aroclor 1254 (PCB) from Monsanto Chemical Co. (St. Louis, MO) and β-naphthoflavone (BNF) from Aldrich Chemical Co. Inc. (Milwaukee, WI). Other chemicals, obtained from local commercial suppliers were of analytical grade.

### Drosophila *strains and pretreatments*

The wild type strains Berlin K (Germany), Canton S (U.S.A.), Florida-9 (U.S.A., aflatoxin $B_1$-sensitive) and Karsnäs 60 (Sweden) were used in this study. They were compared to Oregon R (U.S.A.), selected since 1952 for high tolerance to DDT (kindly provided by Dr. D. J. Merrel, Minnesota, U.S.A.), Hikone R (Japan), cross-resistant to several classes of insecticides (obtained from Dr. E. Vogel, Leiden, The Netherlands) and the aflatoxin $B_1$-resistant strain Lausanne-S (kindly provided by Dr. J. Chin-

nici, Virginia, U.S.A.). The Karsnäs $60_w$ is an isogenic strain, synthesized from the original Karsnäs 60 strain by brother–sister crosses (provided by Professor K. G. Lüning, Stockholm). This strain carries the markers $w_{(X)}$ and $B^S_{(Y)}$.

Adult flies (2–5 days post eclosion) were pretreated either with PB (1 mg/ml), PCB (0.5 mg/ml) or BNF (5 mg/ml) dissolved in water or corn oil and mixed in the ordinary corn-agar medium for 18 hr as described previously [10]. Prior to the treatment the flies were kept on empty vials for 4 hr.

*Preparation of microsomes*

The flies were homogenized in ice-cold 0.1 M sodium phosphate buffer pH 7,5 containing 1 mM EDTA (0.1–0.2 g flies/ml) in a glass–Teflon Potter–Elvehjem homogenizer. All subsequent preparation procedures were carried out at 2–4°. The homogenate was centrifuged at 15,000 × *g* for 10 min. The supernatant was filtered through nylon cloth and layered over 0.3 M sucrose in the phosphate buffer by the method of Arrhenius [17] and centrifuged at 115,000 × *g* for 60 min. The pellet was resuspended in buffer (about 10 mg microsomal protein per ml, 1 mg corresponds to about 200 flies), kept on ice and used for enzymatic assays within 1 hr or rapidly frozen at −70°, a procedure that caused a negligible decrease in enzyme activity during storage up to 2–3 months.

*Assays*

The reduced, CO-bound cytochrome P-450 difference spectra was recorded according to Omura and Sato [18] (0.5–1 mg microsomal protein), NADPH-cytochrome c reductase was measured by the method described by Mazel [19] (0.25–0.5 mg microsomal protein) and protein was determined according to Lowry *et al.* [20].

BP monooxygenase activity was measured by the fluorimetric method of Dehnen *et al.* [21]. Incubation conditions were as previously described [10]. For the determination of *p*-nitroanisole the incubation mixture contained 1–3 mg microsomal protein, 4 μmol sodium isocitrate, 0.8 μmol $NADP^+$, 4 μmol $MgCl_2$, 40 μmol Tris–HCl buffer (pH 7.8) and isocitrate dehydrogenase with a reducing capacity of 1.24 μmol $NADP^+$/min in a final vol of 2 ml. After preincubation for 2 min at 37°, the reaction was started by the addition of 20 μl 1 M PNA in acetone, incubated for 10 min and stopped with 0.5 ml ice-cold 10% (v/v) acetic acid. To decrease background absorption the sample was extracted with ethylacetate/hexane 1:1 (v/v; 2.5 ml) and the organic phase was reextracted with 1 ml 1 M $Na_2CO_3$ before quantitation of the *p*-nitrophenol was performed by measuring the absorption at 400 nm (according to Dr. D. Hultmark, personal communcation).

The O-deethylation of 7-ethoxycoumarin was determined directly in the fluorescence cuvette at 37° according to Ullrich and Weber [22] with 0.25–0.5 mg microsomal protein, 2 mg bovine serum albumin, 100 mmol Tris–HCl buffer (pH 7.6) and 50 pmol 7-ethoxycoumarin in a total incubation vol of 1 ml. The reaction was started by the addition of 100 nmol NADPH.

For the determination of biphenyl hydroxylation 0.2–0.5 mg microsomal protein, 1.8 μmol NADPH, 1.4 μmol NADH, 4.5 μmol $MgCl_2$ and 1 mg albumin were mixed in a total vol of 1.5 ml Tris–HCl buffer (0.05 M, pH 8.5) and were preincubated at 37° for 2 min. The reaction was started by the addition of 0.3 mg biphenyl in 25 μl acetone. After incubation for 10 min the reaction was stopped by the addition of 0.5 ml 2 M HCl. The analysis was performed as a modification of the method of Rehnberg [23], consisting of extraction with hexane (2 ml), reextraction with 0.5 M NaOH (2 ml), and benzoylation of the phenolic metabolites formed with 25 μl 10% pentafluorobenzoylchloride in toluene, after the addition of 1 ml hexane and 3 ml 1 M NaH $CO_3$ to the aqueous NaOH-phase. The organic phase was shaken with a fresh 2 ml portion of 0.5 M NaOH for 30 min and recentrifuged prior to the quantitation. The biphenyl metabolites were quantitated on an Varian Model 3700 gas chromatograph with $^{63}Ni$ electron capture detector, 25 M quarts glass capillary (i.d. 0.32 mm) coated with SE 54 (1% vinyl, 5% phenyl methyl silicone).

For the fluorescence and absorbance measurements a Shimadzu RF-5102C and UV-500, respectively, were used.

Glutathione S-transferase activity of the 115,000 × *g* supernatant protein was determined by the method of Habig [24] with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

*SDS-polyacrylamide gel eletrophoresis*

SDS-polyacrylamide gel electrophoresis was performed according to the method described by Laemmli [25]. A total amount of 25 μg microsomal protein was applied to each well. The stacking gel contained 4.5% and the separating gel 9% polyacrylamide. After staining the gels for protein with Coomassie Brilliant Blue (CBB) densitometric tracing was performed at 550 nm.

## RESULTS

The content of cytochrome P-450 and the BP-monooxygenase activity of microsomes isolated from the different strains are shown in Table 1. The level of cytochrome P-450 in control animals varied by a factor of two between the strains, with the highest values for Canton S and the insecticide-resistant strain Oregon R. Pretreatment of the flies with PB or PCB increased the cytochrome P-450 content in all strains except Canton S; the increases varied from 1.2 to 2-fold when compared to the control. BNF pretreatment caused no or only marginal changes in cytochrome P-450 content. No strain differences were observed with respect to the location of the absorption maximum wavelength, being 450 nm in all strains. Furthermore, it was not changed as a response to the different pretreatments (data not shown).

BP monooxygenase activity was nearly six-fold lower in the Hikone R strain than in the strain with the most efficient BP-hydroxylation, Karsnäs 60. It was also significantly lower (3 to 4-fold) than in all other strains. The monooxygenase activity was increased in all strains after PB pretreatment, the increase ranging from 1.5 to 5-fold. PCB was less

Table 1. Effects of enzyme-inducing agents on the content of cytochrome P-450 and BP monooxygenase activity in microsomes isolated from adult flies of 7 different *Drosophila melanogaster* strains*

| Strain | Cytochrome P-450 nmol/mg microsomal protein | | | | BP monooxygenase activity pmol product formed/mg microsomal protein per min | | |
|---|---|---|---|---|---|---|---|
| | Control | PB† | PCB | BNF | Control | PB | PCB | BNF |
| Karsnäs $60_w$ | 0.19 ± 0.01 | 0.37 ± 0.03§ | 0.33 ± 0.01§ | 0.22 ± 0.02 | 241 ± 17 | 621 ± 53‖ | 318 ± 14‡ | 241 ± 30 |
| Canton S | 0.28 ± 0.02 | 0.28 ± 0.02 | 0.29 ± 0.03 | 0.25 ± 0.01 | 188 ± 20 | 467 ± 52‖ | 297 ± 36‡ | 160 ± 10 |
| Berlin K | 0.17 ± 0.02 | 0.29 ± 0.01‖ | 0.26 ± 0.02‡ | 0.21 ± 0.02 | 125 ± 18 | 305 ± 30‖ | 163 ± 22 | 143 ± 20 |
| Florida-9 | 0.18 ± 0.03 | 0.26 ± 0.03 | 0.22 ± 0.04 | 0.21 ± 0.03 | 176 ± 18 | 776 ± 83‖ | 220 ± 36 | 168 ± 9 |
| Lausanne-S¶ | 0.18 ± 0.02 | 0.24 ± 0.02 | 0.21 ± 0.01 | 0.18 ± 0.03 | 141 ± 25 | 642 ± 19‖ | 143 ± 27 | 170 ± 15 |
| Hikone R** | 0.21 ± 0.03 | 0.29 ± 0.03 | 0.28 ± 0.04 | 0.23 ± 0.03 | 44 ± 4 | 65 ± 7‡ | 69 ± 6§ | 41 ± 5 |
| Oregon R** | 0.33 ± 0.02 | 0.37 ± 0.03 | 0.41 ± 0.03‡ | 0.29 ± 0.02 | 148 ± 13 | 251 ± 29§ | 204 ± 13‡ | 137 ± 12 |

* Each value represents mean ± S.E. of at least four experiments.
† The inducer was mixed in the diet and the flies pretreated for 18 hr with PB (1 mg/ml), PCB (Aroclor 1254, 0.5 mg/ml) or BNF (5 mg/ml).
‡ $P < 0.05$ when compared with control values.
§ $P < 0.01$ when compared with control values.
‖ $P < 0.001$ when compared with control values.
¶ Resistant to AFB.
** Resistant to insecticides.

efficient in enhancing BP monooxygenase activity, causing maximally a 1.6-fold increase in the Canton S and Hikone R strain. BNF did not, in contrast to the results on larvae [11], significantly affect the activity in any strain.

The capacity of the microsomes from the various strains to perform the *O*-deethylation of 7-ethoxycoumarin and *O*-demethylation of *p*-nitroanisole are shown in Table 2. Hikone R had a much lower 7-ethoxycoumarin deethylase activity when compared to the other strains. PB and PCB enhanced the deethylation 1.5 to 2.5-fold in all strains, except for the low response to PCB pretreatment in strains Florida-9 and Lausanne-S. BNF slightly increased the activity in some strains, but there was a significant deviation from the control only in Hikone R. The *p*-nitroanisole-*O*-demethylase activity was 2.5 to 4.5-fold higher in untreated flies of the two insecticide-resistant strains Oregon R and Hikone R when compared to the other strains. In these resistant strains no response to the different pretreaments was observed, while in all other strains the PNA-demethylase activity was significantly increased by PB, with the most marked effect, a 3-fold increase, in Lausanne-S. PCB and BNF did not significnatly change the activity.

Biphenyl was metabolized to 3- and 4-OH-biphenyl by all strains (Table 3). The capacity to hydroxylate biphenyl in the 3-position differed ten-fold between the strains and the formation was much higher in the two insecticide-resistant strains as compared to the other strains. A significant increase in the metabolism after the different pretreatments was observed only in strain Florida-9. The formation of 4-OH-biphenyl was very low in Berlin K, whereas the insecticide-resistant Oregon R was the most efficient strain in performing this reaction. PB treatment increased the metabolism in all strains except Oregon R and Hikone R, giving maximally a fourfold increase. The formation of 4-OH-biphenyl in Canton S and Berlin K was also enhanced after pretreatment of the flies with PCB. In Lausanne S, PCB markedly decreased the formation of both 3- and 4-OH-biphenyl. The o-hydroxylated metabolite 2-OH-biphenyl was formed in very low quantities in all strains both in control and in pretreated animals. In Florida-9 and Lausanne S it was not possible to detect any formation of this metabolite.

Some strain differences were also indicated in the activity of cytochrome c reductase (Table 4). The control activity ranged from 25–37 nmoles cytochrome c reduced/mg microsomal protein per min in the various strains. In this respect Karsnäs $60_w$ microsomes were the least and Oregon R the most potent. The activity of the phase II-enzyme GSH-S-transferase (Table 4) towards CDNB varies from 77 to 111 nmol/mg protein. The increases in both activities were rather weak after the various pretreatments. Significant increases (P 0.05) in GSH-S-transferase activity were observed only in Lausanne S and Karsnäs 60 (PB), and in cytochrome c reductase activity in Lausanne S (PB, BNF) and Oregon R (PB, PCB).

The results of SDS-polyacrylamide gel electrophoresis of microsomes from the different strains are shown in Figs. 1 and 2. The region that contains

Table 2. Metabolism of 7-ethoxycoumarin and *p*-nitroanisol in *Drosophila* microsomes: genetic variation and effects of enzyme-inducing agents*

| Strain | 7-Ethoxycoumarin deethylase activity pmol 7-OH-coumarin formed/mg microsomal protein per min | | | | *p*-Nitroanisol demethylase activity nmol *p*-nitrophenol formed/mg microsomal protein per min | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | PB† | PCB | BNF | Control | PB | PCB | BNF |
| Karsnäs $60_w$ | 67 ± 7 | 140 ± 16§ | 144 ± 5‖ | 65 ± 12 | 0.48 ± 0.03 | 1.07 ± 0.11‖ | 0.64 ± 0.08 | 0.45 ± 0.05 |
| Canton S | 67 ± 5 | 152 ± 9‖ | 121 ± 13§ | 75 ± 9 | 0.46 ± 0.05 | 0.69 ± 0.05§ | 0.53 ± 0.03 | 0.37 ± 0.01 |
| Berlin K | 70 ± 7 | 102 ± 11‡ | 120 ± 12§ | 91 ± 5 | 0.40 ± 0.05 | 0.59 ± 0.05‡ | 0.47 ± 0.03 | 0.49 ± 0.09 |
| Florida-9 | 73 ± 4 | 113 ± 15§ | 87 ± 7 | 63 ± 8 | 0.32 ± 0.02 | 0.66 ± 0.12‡ | 0.33 ± 0.04 | 0.30 ± 0.06 |
| Lausanne-S[f] | 65 ± 4 | 126 ± 6‖ | 90 ± 6 | 55 ± 7 | 0.28 ± 0.03 | 0.83 ± 0.08‖ | 0.29 ± 0.03 | 0.32 ± 0.05 |
| Hikone R[g] | 36 ± 3 | 84 ± 9‖ | 83 ± 10§ | 54 ± 5‡ | 1.17 ± 0.17 | 1.28 ± 0.22 | 1.03 ± 0.19 | 1.06 ± 0.27 |
| Oregon R[g] | 75 ± 5 | 126 ± 16§ | 115 ± 21‡ | 80 ± 10 | 1.24 ± 0.08 | 1.46 ± 0.09 | 1.46 ± 0.12 | 1.18 ± 0.09 |

* Each value represents mean ± S.E. of at least three experiments.
† Pretreatments were performed as described in Table 1.
‡ $P < 0.05$ when compared with control values.
§ $P < 0.01$ when compared with control values.
‖ $P < 0.001$ when compared with control values.
¶ Resistant to $AFB_1$.
** Resistant to insecticides.

Table 3. Hydroxylation of biphenyl at different positions in 7 *Drosophila melanogaster* strains after pretreatment with phenobarbital, PCB or β-naphthoflavone*

| Strain | pmol product formed/mg microsomal protein per min | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-OH-Biphenyl | | | | 3-OH-Biphenyl | | | | 4-OH-Biphenyl | | | |
| | Control | PB† | PCB | BNF | Control | PB | PCB | BNF | Control | PB | PCB | BNF |
| Karsnäs $60_w$ | + | + | + | + | 28 ± 3 | 28 ± 2 | 15 ± 2 | 26 ± 4 | 109 ± 14 | 238 ± 30‡ | 105 ± 19 | 151 ± 13 |
| Canton S | + | + | + | + | 16 ± 1 | 21 ± 3 | 13 ± 2 | 14 ± 2 | 75 ± 4 | 270 ± 40§ | 131 ± 19‡ | 87 ± 12 |
| Berlin K | + | + | + | + | 9 ± 1 | 18 ± 2‡ | 11 ± 2 | 14 ± 1‡ | 38 ± 6 | 153 ± 34‡ | 112 ± 16‡ | 47 ± 5 |
| Florida-9 | − | − | − | − | 15 ± 1 | 17 ± 3 | 14 ± 2 | 10 ± 1 | 90 ± 10 | 188 ± 26‡ | 93 ± 17 | 71 ± 4 |
| Lausanne-S‖ | − | − | − | − | 12 ± 2 | 13 ± 2 | + | 12 ± 2 | 72 ± 11 | 145 ± 11‡ | 42 ± 9 | 78 ± 13 |
| Hikone R¶ | + | + | + | + | 102 ± 10 | 116 ± 14 | 128 ± 14 | 119 ± 14 | 139 ± 18 | 179 ± 27 | 210 ± 29 | 182 ± 22 |
| Oregon R¶ | 4 ± 1 | 5 ± 1 | 4 ± 1 | 4 ± 1 | 156 ± 23 | 135 ± 16 | 104 ± 11 | 128 ± 21 | 274 ± 41 | 229 ± 33 | 195 ± 29 | 225 ± 17 |

* Each value represents mean ± S.E. of at least three experiments.
† Pretreatments were performed as described in Table 1.
‡ $P < 0.01$ when compared with control values.
§ $P < 0.001$ when compared with control values.
‖ $AFB_1$-resistant.
¶ Insecticide-resistant.
** Trace amounts discernible on the GC chromathogram, but below the detection level (~2 pmol/mg per min) under the experimental conditions described in Materials and Methods.

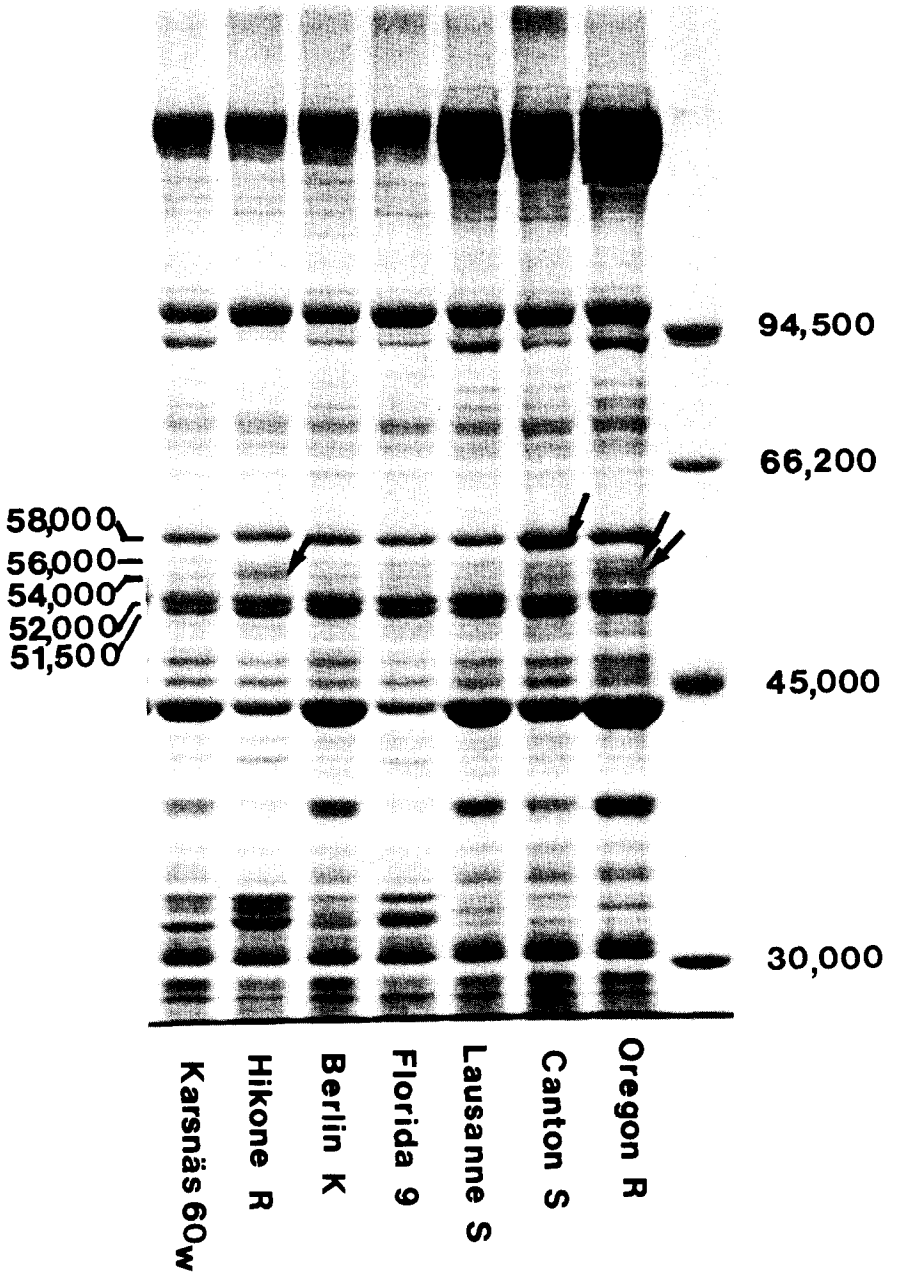


Fig. 1. SDS-polyacrylamide gel electrophoresis of microsomes from different *Drosophila* strains. The gel was stained with Coomassie Brilliant Blue.

possible P-450 species, stains positive for heme and contains inducible protein bands with apparent mol. wt of 51,000–58,000 as described earlier [11]. Within this region several strain differences were observed. The protein band with a mol. wt of 54,000 was markedly more abundant in the two insecticide-resistant strains Oregon and Hikone. The Oregon R strain also had an increased protein content in the 56,000 band, while the intensity of the 58,000 band was higher in Canton S.

## DISCUSSION

The present study has revealed a marked genetic variation between the *Drosophila* strains studied in the capacity to perform xenobiotic metabolism. As expected, the genetic regulation of the cytochrome P-450 enzymes seems rather complicated. Some indications of the underlying genetic mechanisms are however discernible on the basis of the enzyme activities and contents of heme proteins. Marked

Table 4. NADPH-cytochrome c reductase activity and glutathione S-transferase activity in subcellular fractions from different *Drosophila* strains: influence of phenobarbital, PCB and β-naphthoflavone*

| Strain | NADPH-cytochrome c reductase activity nmol cytochrome c reduced/ mg microsomal protein per min | | | | Glutathione S-transferase activity nmol product/mg cytosolic protein per min | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | PB† | PCB | BNF | Control | PB | PCB | BNF |
| Karsnäs $60_w$ | 25 ± 2 | 25 ± 3 | 22 ± 2 | 26 ± 4 | 77 ± 5 | 115 ± 18‡ | 92 ± 18 | 77 ± 5 |
| Canton S | 30 ± 5 | 38 ± 5 | 36 ± 7 | 30 ± 5 | 90 ± 6 | 114 ± 11 | 94 ± 11 | 86 ± 5 |
| Berlin K | 30 ± 5 | 30 ± 4 | 35 ± 4 | 33 ± 5 | 92 ± 11 | 99 ± 14 | 96 ± 8 | 91 ± 13 |
| Florida-9 | 29 ± 1 | 35 ± 3 | 33 ± 2 | 37 ± 6 | 111 ± 4 | 123 ± 3 | 117 ± 15 | 91 ± 16 |
| Lausanne-S§ | 26 ± 3 | 37 ± 3‡ | 33 ± 5 | 42 ± 5‡ | 80 ± 5 | 101 ± 9‡ | 80 ± 4 | 83 ± 4 |
| Hikone R‖ | 32 ± 2 | 32 ± 3 | 33 ± 3 | 33 ± 6 | 100 ± 12 | 108 ± 9 | 94 ± 16 | 105 ± 19 |
| Oregon R‖ | 37 ± 3 | 46 ± 8‡ | 53 ± 3‡ | 33 ± 3 | 102 ± 4 | 110 ± 9 | 99 ± 9 | 100 ± 6 |

* Each value represents mean ± S.E. of at least three experiments.
† Pretreatments were performed as described in Table 1.
‡ $P < 0.05$ when compared with control values.
§ $AFB_1$-resistant.
‖ Insecticide-resistant.

differences were observed between the insecticide-resistant (Hikone R and Oregon R) and the insecticide-sensitive strains. The former strains had a several-fold higher capacity for PNA-demethylation and 3-hydroxylation of biphenyl (cf. Table 2 and 3). This is in accordance with the higher metabolism of vinyl chloride [13], as well as with the more marked toxicity [14] and mutagenicity [15] of dimethylnitrosamine in insecticide-resistant strains, indicating a correlation between the genetic regulation of insecticide metabolism and metabolism of the compounds mentioned. This correlation could be due to a structural gene mutation if structural similarities in the substrates permit metabolism by the same enzyme, or to a mutation in a regulating locus. The latter seems most probable, since the high metabolism was coupled to a non-responsiveness to enzyme induction (cf. Tables 2 and 3, [13, 14] and since the behavior corresponded to that of constitutive mutants. Regulator mutations in xenobiotic metabolism has been observed earlier by Nebert *et al.* [4, 5] in mouse, concerning the AH locus and induction with polycyclic aromatic hydrocarbons (PAH). The occurrence in the SDS-polyacrylamide gel electrophoresis of an increased protein content in the band with an apparent mol. wt of 54,000 in Oregon R and Hikone R indicated that one enzyme or several with similar mol. wts could be associated with the resistant genotype. This was further supported by the fact that a strain, carrying the second chromosome from Hikone R and the rest of the genome from Karsnäs 60, had the same strong 54,000-band and shared the metabolic characteristics observed with Hikone R. (unpublished results). The dominant gene responsible for the high vinyl chloride metabolism, dimethylnitrosamine toxicity and insecticide resistance was previously localized to the second of the four *Drosophila* chromosomes, at about 64–66 cM [14, 26]. A genetic localization is in progress in order to analyze whether the enzymes responsible for PNA metabolism and biphenyl 3-hydroxylation are regulated by the same gene. Genetic variation in cytochrome P-450-mediated metabolism depending on a simple Mendelian, dom-

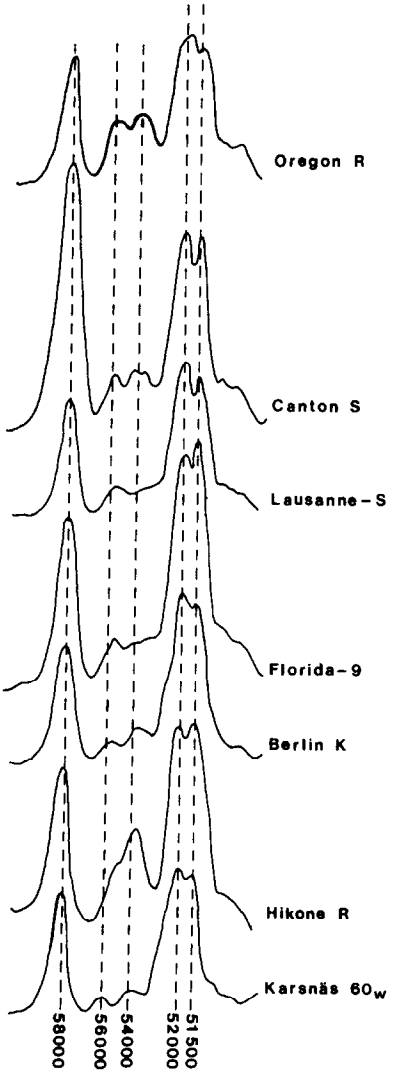


Fig. 2. Densitometric tracing at 550 nm of the gel in Fig. 1.

inant trait has also been shown for many drugs, both in mouse and in man [27–30].

In microsomes prepared from *Drosophila* larvae a nearly four-fold higher cytochrome P-450 content was observed in the Hikone R strain when compared to insecticide-sensitive strains [10]. A high P-450 content was also correlated to insecticide resistance in the house fly, *Musca domestica* [31, 32]. In the present material on adult *Drosophila* no such correlation could be observed; the highest cytochrome P-450 content was observed in the DDT resistant Oregon R strain, but Hikone R did not differ from the sensitive strains. The metabolic differences were also more marked in larvae, where PNA demethylation differed between Hikone R and Karsnäs 60 by a factor of ten (I. Hällström, unpublished data) whereas it was only 2.5-fold higher in adult Hikone R (cf. Table 2). The observation that the metabolism pattern typical for each strain is more marked in larvae indicates that the selection pressure of the insecticides is acting preferentially on the larval stages.

Microsomes from the Hikone R had a lower capacity of BP hydroxylation and 7-ethoxycoumarin deethylation when compared to the other strains (cf. Table 1 and 2). The low metabolism seemed to depend on recessive or semidominant mutations in both cases as judged by preliminary results on reciprocal crosses between Hikone R and Karsnäs 60. It remains to be clarified whether the two enzyme activities are under the same genetic regulations; no conclusions can be drawn on the basis of the present data. BP hydroxylation was 6-fold lower in adult Hikone R microsomes when compared to Karsnäs 60 (cf. Table 1). In contrast, the Hikone R microsomes prepared from larvae were slightly more efficient than preparations from Karsnäs 60 [10]. Lower BP metabolism in Hikone R of both stages were, however, reported by Baars *et al.* and Ziljztra *et al.* [8, 9, 33].

The formation of 4-OH-biphenyl showed a less clear-cut correlation to insecticide-resistance than the 3-hydroxylation (cf. Table 3). The metabolism was highest in Oregon R but nearly as high in Karsnäs 60 as in the other insecticide-resistant strain Hikone R. One explanation is a second high-metabolism trait in Oregon R not associated with the insecticide resistance gene. This was supported by the finding of an increased amount of protein in the 56,000 band in Fig. 1. It is possible that the protein with apparent mol. wt of 56,000 is involved in the 4-hydroxylation of biphenyl, but this has to be demonstrated by further genetical analyses.

The abundancy of the 58,000 band in Canton S (Fig. 1) so far can not be correlated to an increased capacity for specific enzymatic reactions. The existence of such increases seems, however, plausible in view of the high cytochrome P-450 content in this strain.

In the metabolism of aflatoxin $B_1$ the cytochrome P-450 system gives rise to both activation and detoxification products [1, 2]. A metabolic resistance in analogy with the insecticide-resistance should therefore be possible. An increased tolerance could be achieved either by increasing the capacity of the detoxification pathways leading to increased amounts of specific P-450 enzymes as was the case for the insecticide-resistant strains, or by a decreased amount of the activating enzyme(s). The $AFB_1$-resistant strain Lausanne-S shows neither increased nor decreased activities or enzyme contents when compared to the $AFB_1$-sensitive Florida-9 or the other strains, indicating no connection between the resistance and the metabolic characteristics studied in this paper. Very specific changes in amount of or affinity towards $AFB_1$ of one or a few enzymes not detected in this study might still contribute to the resistance. Such differences might be dramatic enough to change the balance of activation and detoxification without marked changes in the total cytochrome P-450 content or changes in the electrophoretic pattern. It can thus not be excluded that the second and third chromosome mutations involved in the $AFB_1$-resistance in Lausanne-S observed by Chinnici [34] are concerned with the cytochrome P-450 system. Studies on the metabolic profile from resistant and sensitive strains are necessary to elucidate this problem.

The activities of GSH-S-transferase and cytochrome c reductase are continuously spread among the strains, showing no simple high-low metabolism traits within this material. Thus, the increased tolerance of the various resistant strains does not depend on increased glutathione conjugation. The GSH-S-transferase activity in the different strains are in good agreement with the results of Baars [9].

Marked genetic variation between the strains studied were observed in response to the different pretreatments. The influence of PB on the different enzyme activities varied from the non-responsiveness in the insecticide-resistant strains with regard to PNA demethylation to increases up to 4- and 5-fold for some strains and pretreatments. Two strains, Florida-9 and Lausanne-S also, in contrast to the other strains, showed no effects following PCB pretreatment on any of the enzyme activities studied, a decrease in biphenyl metabolism being even noted in Lausanne-S. The 3-hydroxylation of biphenyl was the only enzyme activity that was almost unaffected by all the pretreatments in all strains, indicating a constitutive form of cytochrome P-450 being responsible for this reaction. Taken together, these data give further evidence of a multiplicity of cytochrome P-450 isozymes in *Drosophila melanogaster*.

The genetic variation between the strains in response to PB treatment mentioned above (see Tables 1–4) are in accordance with the results of Page *et al.* [35]. They reported a 1.5 to 4-fold range in response to PB treatment in the metabolism of ethylmorphine and aniline in ten strains of rat.

A 2–3-fold increase in aldrin epoxidation after PCB treatment was observed in insecticide-sensitive house flies, while the resistant strains R-Fc and R-Baygon were non-responsive [36]. As discussed above we found a similar non-responsiveness, as measured by several enzyme activities, in insecticide-resistant *Drosophila*.

BNF was shown to be a poor inducer in contrast to the effects in mammals (Tables 1–2). These results were in agreement with the results on house flies, where another PAH, 3-methylcholantrene, was inefficient in increasing microsomal mixed function

oxidases [37]. The low response to BNF pretreatment and the low formation of the 2-OH-metabolite of biphenyl were in agreement with the low formation of dihydrodiols from BP [10] as well as the poor mutagenic effect of polycyclic aromatic hydrocarbons and aromatic amines in *Drosophila* tests [38, 39]. This supports the presence of low, non-inducible amounts of enzymes (e.g. PAH-metabolizing enzymes) known to be inducible in mammals as previously discussed [10, 11].

Genetically determined increases or decreases in cytochrome P-450 enzymes as shown above can be part of a natural variation in response to changes in the environment and represent an important feature for the adaption to toxic exposure. *Drosophila* provides excellent opportunities for genetic analyses of this variation. Furthermore, the short generation time of *Drosophila* makes this organism suitable for the creation of metabolism mutants, through selection for high and low tolerance of different cytochrome P-450 substrates, which could make more refined analyses of the genetics of the P-450 system possible.

*Acknowledgements*—The authors want to thank Professor C. Ramel and Dr. R. Toftgård for valuable discussions and criticism during manuscript preparation. We are also obliged to Professor J.-Å. Gustafsson and Professor S. Jensen for the use of their laboratory facilities for parts of the work. We want to thank Dr. J. Chinnici for interest and discussions. Much thanks are also owed to Miss M. Lienzén for skilful technical assistance and encouragement throughout the work. This work was supported by grants from the Swedish Council for Planning and Coordination of Research.

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