PRETREATMENT OF A *Drosophila simulans* MUTANT LINE WITH BENZ(a)PYRENE INDUCES AN ABERRANT ISOFORM OF CYTOCHROME P-450 WITH INCREASED ABILITY TO METABOLIZE BENZ(a)PYRENE

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The basic condition for the toxic, mutagenic, and carcinogenic action of polycyclic aromatic hydrocarbons (PAH) and aromatic amines (AA) on mammalian cells is their activation to proximal highly mutagenic metabolites in the system of microsomal monooxygenases by a special isoform of cytochrome P-450, induced by these metabolites [2]. Unlike in mammals, in Drosophila this system is virtually not inducible under the influence of PAH themselves, and for that reason their metabolism takes place with the aid of basal forms of these enzymes, which form predominantly phenols and other nonmutagenic products [4, 9]. These facts are linked with the low sensitivity of *D. melanogaster* to the mutagenic action of PAH and AA, and this is one of the principal problems in the screening of procarcinogens on Drosophila [4, 14]. This problem has virtually not been studied in relation to the species *D. simulans*, related to *D. melanogaster*. The writers; previously isolated a mutant line of *D. simulans* (line 364 yv), which is sensitive in the larval stage to the toxic and mutagenic action of benz(a)pyrene (BP) [3].

The aim of this investigation was a comparative study of the system for xenobiotic metabolism in line 364 yv and in the wild-type resistant line Turku.

EXPERIMENTAL METHOD

Lines *D. simulans* 364 yv and Turku were cultured under standard conditions. At the age of 5-6 days after flight the insect was transplanted on to fresh nutrient medium containing or not containing inducers — phenobarbital (PB) in a concentration of 1 mg/ml and BP in a concentration of 0.5 mg/ml ("Fluka AG") for 3 days. Microsomes were isolated by differential centrifugation [1]. The supernatant and residue of microsomes thus obtained, resuspended in 0.05 M Tris-HCl buffer (pH 7.5), were kept on ice and used for further study in the course of 1 h. The protein concentration in the microsomes and supernatant was measured by Albro's method [5]. The concentration of cytochrome P-450 was determined by the method of Omura et al. [11]. Activity of benz(a)pyrene hydroxylase (BPH) and epoxide hydrolase (EH) was measured fluorometrically by the methods of Dehnen and co-workers [7] and Dansette and co-workers [6]. Glutathione-S-transferase (GT) activity was determined by the method of Habig and co-workers [8]. For the spectrophotometric and spectrofluorometric investigations, conducted at 25°C, the Aminco DW2a and Hitachi-850 instruments were used respectively.

Electrophoresis of microsomal proteins in the presence of SDS was carried out by the method described by Laemmli [10]. Hemoproteins were identified by the method of Sinclair and co-workers [12].

The results were subjected to statistical analysis by Student's t test.

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TABLE 1. Effect of Inducers on Enzymes of Xenobiotic Metabolism in Imgao of *D. simulans*

Line	Pretreatment	Content of cytochrome P-450, nmoles/mg protein	DPH activity		EH activity	GT activity
			nmoles/mg protein/min	nmoles/mole cytochrome P-450/min	nmoles/nmole cytochrome P-450/min	
364yv	Control (7)	0,23±0,02	158±17	0,77±0,15	1,67±0,04	51±3
	BP (6)	0,14±0,02*	197±16	1,45±0,09*	1,48±0,13	40±6
	PB (4)	0,49±0,02**	1369±63**	3,31±0,45**	11,74±0,91**	132±12**
Turku	Control (7)	0,21±0,02	178±11	0,91±0,07	1,61±0,11	53±3
	BP (6)	0,26±0,03	160±14	0,67±0,11	1,53±0,06	58±3
	PB (4)	0,42±0,01**	1490±148**	3,69±0,38**	11,55±0,32**	164±13**

Legend. Values of M ± m are shown; number of experiments in each group indicated between parentheses. Significance of differences from control: *$p < 0.01$; **$p < 0.001$.

EXPERIMENTAL RESULTS

The effect of PB and BP on the content of cytochrome P-450 and on activity of the test enzymes, in the case of lines 364 yv and Turku is illustrated in Table 1. Table 1 shows that despite the different sensitivity of the larvae of these lines to BP, differences expressed in relation to these parameters were not present in the microsomes of intact flies. The results were simi lar with data in the literature on *D. melanogaster* [9]. Just as for individuals of this species, for the fly *D. simulans* PB was found to be the most powerful inducer, for it enhanced activity of enzymes of phases I and II of xenobiotic metabolism sharply and equally in both lines. Pretreatment of the flies with PB led to doubling of the cytochrome P-450 content, increased specific BPH activity by 8.4-8.7 times, molecular BPT activity by 4 times, EG activity by 7 times, and GT activity also by 2.5-3 times.

Meanwhile, after pretreatment of the flies with BP, significant differences were found in line 364 yv, namely a significant decrease in the cytochrome P-450 content and a twofold increase in molecular activity of BPH compared with the control and a 2.5-fold increase compared with that in Turku flies pretreated with BP. The first result is probably connected with the fact that BP metabolites, stabilizing mRNA or protein of the cytochrome P-450 isoform responsible for their metabolism, as has been shown for acetone or isosafrol [13], can accelerate the breakdown of mRNA of other isoforms. These highly reactive metabolites can also lead to death in situ of the intestinal cells in which the monosomal monooxygenase system is the most active. In this situation, during isolation of microsomes from whole-body homogenate, the content of cytochrome P-450 must be reduced, for most of it in Drosophila is found in cells of the intestine. In flies of the Turku line, whose larvae are resistant to the toxic action of BP, this effect is absent, probably on account on the different structure of the BP metabolites formed. The most probable cause of the observed increase in molecular activity of BPH in the 364 yv flies, treated with BP, may be induction of a more active isoform of cytochrome P-450 [2].

During SDS-electrophoresis of microsomal proteins, with staining for heme, hemoproteins with an apparent molecular weight (M) of about 51.5, 54.56, and 59 kD were found in intact flies of both lines (Fig. 1). Pretreatment with PB also led to the appearance of a hemoprotein with M = 52 kD. In microsomes of intact flies of the Turku strain, the band of the hemoprotein with mol. wt. of 56 kD was more intensely stained than that of the intact 364 yv. However, pretreatment with BP caused an increase in the intensity of this band only in 364 yv flies.

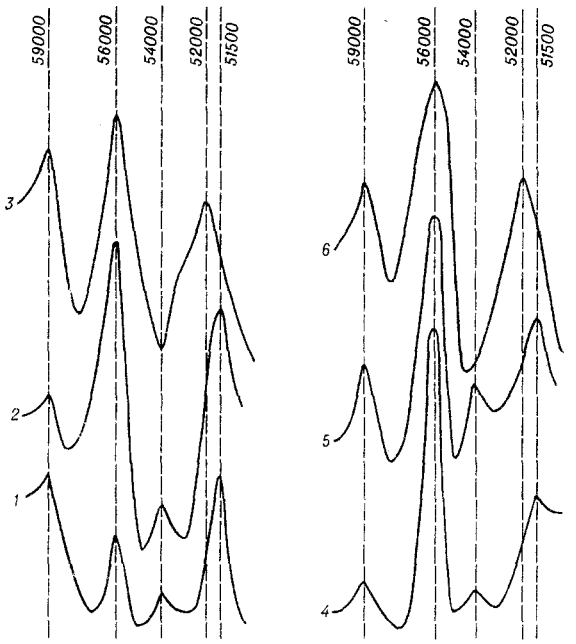

Fig. 1. Densitometry of lanes in gel on SDS electrophoresis of microsomal proteins of *D. simulans* at 550 nm, after staining with Coomassie brilliant blue. Standard proteins with mol. wt. of 94, 67, 45, 30, and 20.1 kD were used as molecular weight markers. 1) 364 yv, control; 2) 364 yv, pretreatment with BP; 3) 364 yv, pretreatment with PB; 4) Turku, control; 5) Turku, pretreatment with BP; 6) Turku, pretreatment with PB.

These data indicate induction by BP of one of the isoforms of cytochrome P-450 in the 364 yv flies. This isoform is probably identical in the 364 yv and Turku flies, for in the latter case it was not induced by BP.

The possibility cannot be ruled out that whereas the aberrant isoform of cytochrome P-450 with mol. wt. of 56 kD was induced by BP not only in the imago, but also in larvae of the 364 yv line, it may be responsible for the formation of highly mutagenic metabolites of BP and, as a result, for the sensitivity of this line to the effects of BP. It must be emphasized that, unlike PB, treatment with BP did not lead to induction of conjugating enzymes, responsible for the detoxication process (Table 1). Very probably in the case with line 364 yv, we were dealing with a quite rare mutation event, in which, as a result of the action of the H-factor of chromosomal instability [3] one of the steps in regulation of the enzyme system for xenobiotic metabolism was modified [3].

Further investigations may be essential both for an understanding of the mechanisms of functioning of this system and for optimization of screening of environmental genotoxic carcinogens on Drosophila.

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ESTABLISHING THE ROLE OF METHYLATION OF DNA CYTOSINE IN HUMAN GENETIC INDIVIDUALITY

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Three main trends of development have become formed in genetic dactyloscopy. The first, and the best studied at the present time, is the discovery of individual structural features of the genotype relative to number and size of mini-satellite DNA fragments [1, 8, 14], the second is establishment of sites of interaction of transcriptionally active chromatin with nuclear regulatory proteins [2, 11], and the third is analysis of the distribution of methyl groups in the promoter region of functioning genes [3, 12]. The data so far obtained suggest that the last two trends of genetic dactyloscopy may be closely interconnected during discussion of their molecular-biological mechanisms, for it has been observed that methylation of guanine bases, in the opposite, complementary, but transcriptionally inactive DNA chain prevents binding of the regulatory protein with its own site-specific region [2]. It must be expected that investigations in the region of DNA-protein interactions and DNA methylation constitute one common direction in the study of the functional features of the genotype of the cell, tissue, organ, or whole organism. The writers pointed out previously [3] that a DNA probe containing the 5′-flank of the human HA-RAS 1 oncogene exhibits polymorphism of the lengths of the restriction fragments (PLRF) of the HA-RAS 1 protooncogene, which has features of individual specificity for each patient with gastric carcinoma studied. The aim of the present investigation was to study molecular-biological phenomena determining this feature of the human genotype.

EXPERIMENTAL METHOD

Altogether 20 patients with carcinoma of the stomach and chronic gastriculcer were investigated. Chromosomal DNA was isolated by the phenol-detergent method [3] from white blood cells (nine samples), gastric mucosa [15], primary carcinomas [16] and metastases of carcinoma of the stomach in regional lymph nodes [7], and cells surrounding a chronic gastric ulcer (three samples). Molecular-genetic analysis of the structure of the HA-RAS 1 proto-oncogene was carried out by methods described previously [3]. Genomic DNA was hydrolyzed by enzymes Msp I and Bam HI ("Ferment," USSR). The EJ 6.6 probe (plasmid pEJ 7.6), carrying the full-scale human HA-RAS 1 oncogene [13], was used in the hybridization reaction. In this experiment hybridization was carried out under mild conditions, determined by the presence of triple SSC and a temperature of 60-62°C. The filters were washed and autoradiography carried out by the standard method [3].

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