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INTERMEDIARY METABOLISM OF NORMAL AND TUMOROUS TISSUE OF *XIPHOPHORUS* (TELEOSTEI:POECILIIDAE)

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Abstract—1. The *Xiphophorus* melanoma system is one of the few well established and genetically well understood *in vivo* models in experimental carcinogenesis. However, data describing features of intermediary metabolism of the genetically caused melanoma or the different inducible neoplasia, as well as that of the different transformed cell lines of *Xiphophorus*, are still lacking. For this reason we initiated a comparative study of enolase-, pyruvate kinase-, lactate dehydrogenase- and malate dehydrogenase-activities and pyruvate and lactate levels in transformed as well as normal tissues of *Xiphophorus*.

2. We observed tissue specific and age dependent activities of the different enzymes and substrate levels.

3. Enzyme activities and substrate levels from all tumors analyzed differ from that of any normal tissue. They are dependent on the tumor sections analyzed, the histiotype and the etiology of the tumors.

4. Analysis of enzyme activities from different *in vitro* cultured fish cell lines and the human Hela cell line revealed dependency of the intermediary metabolism on oxygen supply, on the proliferative state of the cells and on the cell types.

5. We could not find a correlation between our data and the expression of the c-*src* gene of *Xiphophorus* and no genotype-dependent changes in enzyme activities were detected.

INTRODUCTION

Since the critical findings of Warburg (1930) that tumor cells may produce high amounts of lactate in the presence of O_2, the metabolism of tumorous and non-tumorous tissue has been studied in many laboratories. Differences in the activities of more than 80 enzymes of metabolic pathways such as the Krebs cycle, glycolysis, glutaminolysis, nucleic acid and protein synthesis and proteolytic processes were observed. Changes in the uptake and transport of different substrates like glucose, glutamine, phosphate etc., were ascertained. Alterations of isozyme composition were also detected. Some biochemical alterations were found to be related to the neoplastic phenotype or to the degree of malignancy of the tumor. Other alterations proved to be tumor histiotype-related (for review see McKeehan, 1982; Weinhouse, 1982; Weber, 1983; Weber *et al.*, 1984). Although these data gave an insight into the metabolism of transformed cells *in vitro* and of solid tumors, they provided little information about the biochemical changes underlying the process of neoplastic transformation itself.

Experimental systems more suitable for studies on the biochemical changes related to neoplastic transformation are, for example, cells infected by temperature sensitive mutants of different acutely transforming retroviruses. Shifting the cells to the permissive temperature allows the study of immediate biochemical alterations accompanying the process of transformation (Radke and Martin, 1979), mediated by the viral oncogene (v-*onc*). It has, however, been reasoned that only a very small percentage of human and mammalian neoplasia are caused by retroviruses and that the mechanisms of viral transformation may be completely different from the mechanisms of transformation leading to any nonviral tumor (for review see Duesberg, 1985). Both tumors of viral and non-viral etiology often show several common features, including activation of one or several oncogenes (Cooper and Lane, 1984; Land *et al.*, 1983). In some instances it was shown that the principles elucidated for viral transformation may also hold true for transformation by cellular oncogenes (c-*onc*) (for review see Klein and Klein, 1985). Most of these experiments were performed *in vitro* with cell cultures, since the number of well established *in vivo* systems for studying oncogenes and neoplastic transformation is very small. One of these *in vivo* systems

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Abbreviations used—EN, enolase; LA, lactate; LDH, lactate dehydrogenase; MDH, malate dehydrogenase (reaction from malate to oxalacetate); MDH* (reaction from oxalacetate to malate); PK pyruvate kinase; PY, pyruvate; RSV, Rous sarcoma virus; c-*src*, cellular homologue of the RSV transforming gene v-*src*; $pp60^{v\text{-}src}$, gene product of v-*src*; $pp60^{c\text{-}src}$, gene product of c-*src*; *Tu*, tumor gene complex of *Xiphophorus*; R, regulating gene specific to *Tu*; *X. hell*, *Xiphophorus helleri*; *X. mac.*, *Xiphophorus maculatus*.

is the *Xiphophorus* melanoma system (Anders and Anders, 1967, 1978; Anders *et al.*, 1984).

Xiphophorus, including the species *X. maculatus* (platyfish) and *X. helleri* (swordtail), is a viviparous fish genus from Central America. Fish bred from wild populations are highly insusceptible to neoplasia. In contrast, interspecific, interracial and interpopulational hybrids are highly susceptible. They develop a large variety of epithelial, mesenchymal and neurogenic tumors, either "spontaneously" or "induced" by initiators and/or tumor promotors. Most of the knowledge on neoplasia in *Xiphophorus* is based on studies of melanoma. Neoplastic transformation of pigment cells is mediated by a cellular oncogene complex, designated as *Tu* (Anders *et al.*, 1984). *Tu* is normally controlled by different compartment specific regulatory genes ($\acute{R}$-genes). Interspecific crossing leads to a deviation of these two gene complexes and deregulation of *Tu* corresponding to Mendelian laws. Transformed cells are phenotypically recognizable either as small population-specific spots or as benign or malignant melanoma (Anders and Anders, 1978; Anders *et al.*, 1984). The expression of *Tu* has been shown to be correlated with the expression of the proto-oncogene c-*src* (Schartl *et al.*, 1982), the cellular homologue of the Rous sarcoma virus transforming gene v-*src*. As in other systems, the gene product of c-*src* from *Xiphophorus* is a tyrosine specific proteinkinase ($pp60^{c\text{-}src}$) (Barnekow *et al.*, 1982).

Based on this knowledge and on the fact that the product of v-*src*, the $pp60^{v\text{-}src}$ kinase, phosphorylates the glycolytic enzymes phosphoglycerate mutase, enolase and lactate dehydrogenase at tyrosine residues (Cooper *et al.*, 1983, 1984; Eigenbrodt *et al.*, 1983), we began an investigation of the intermediary metabolism in normal and tumor bearing fish. The activities of enolase (EN, EC 4.2.1.11), pyruvate kinase (PK, EC 2.7.1.40) and the level of pyruvate (PY) as parameters of the glycolytic pathway were studied. The activity of lactate dehydrogenase (LDH, EC 1.1.1.27), the level of lactate (LA) and the ratio of malate dehydrogenase (MDH, EC 1.1.1.37) to LDH were determined as a measure of anaerobic metabolism in MDH-activity and its ratio to the reverse reaction (MDH*) were determined to estimate the relative activities of the enzymes involved in the Krebs-cycle and in the malate–aspartate-shuttle transport system.

The present paper aims to elaborate some basic data that could be useful to describe biochemical changes in tumors of *Xiphophorus* and to investigate possible correlations between oncogene activities and these changes.

MATERIALS AND METHODS

Experimental fish

Experimental animals were taken from the following crosses (Fig. 1): crosses of a spotted platyfish (A) with a swordtail (B) result in F1 hybrids (C) developing benign melanoma. Backcrossing of the F1 with the swordtail generate three types of segregants. Twenty five percent of the BC_1 (D) develop benign melanoma like that of the F1-hybrid, 25% (E) develop malignant melanoma, whereas 50% (F,G) develop neither spots nor melanoma. Further backcrossing of the benign melanoma bearing BC hybrids with the swordtail reveals a similar segregation. In the experiments described here we used specimens from A, B, E and F/G. In addition, albino fish bearing amelanotic melanoma were studied (genotype H, Fig. 2). Another source of experimental animals was a cross similar to that outlined in Fig. 1, in which due to a certain regulatory gene linked to *Tu*, no melanoma develops. The tumor free backcross animals carrying *Tu* and the linked *Tu*-gene are, however,

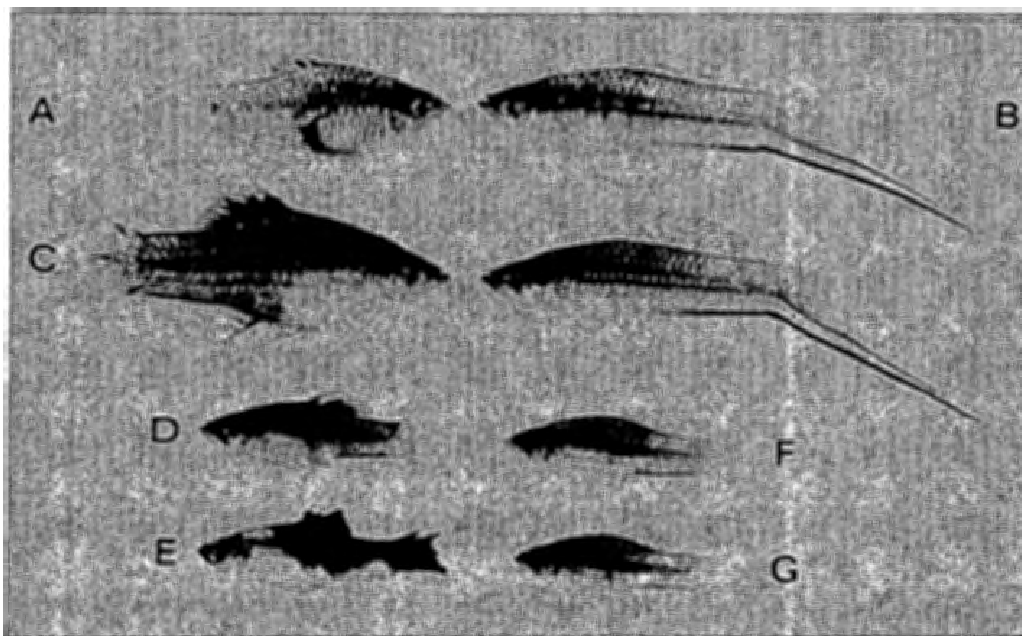

Fig. 1. Crossing scheme of the platyfish (A) and the swordtail (B); (C) F1-hybrids; (D) BC_1-hybrids developing benign melanoma; (E) BC_1-hybrids developing malignant melanoma; (F,G) melanoma free BC_1-hybrids.

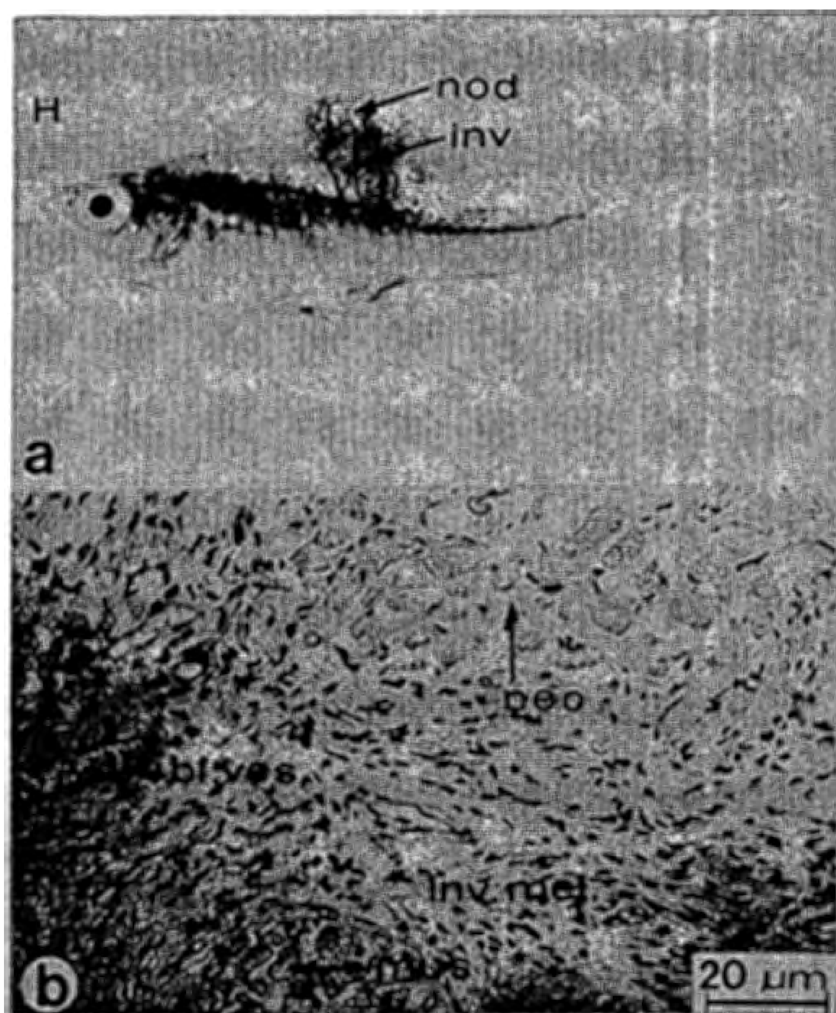

Fig. 2. Backcross hybrid bearing an amelanotic melanoma (a) and histological image of the melanoma showing different tumor sections: bl. ves., blood vessels; inv., invasive section; inv. mel., invasive ingrowing melanoma cells; mus., muscle; nec., necrotic sections of the melanoma; nod., nodular section of the melanoma.

highly sensitive to carcinogen treatment (genotype I, Fig. 3). Both nodular and invasive areas of the tumors were, if possible, dissected and analyzed separately (see Fig. 2).

Treatment of fish and tumor diagnosis

N-methyl-*N*-nitrosurea (MNU) and ethylnitrosurea (ENU) were administrated by exposing fish of genotype I (Fig. 3) to a 10^{-3} M solution of the carcinogen in the aquarium water, 4 times for 1 hr at 2-weekly intervals. Fish of genotype F and G were treated once with 0.02 mM KCN in aquarium water as a toxical control for the treatments of *in vitro* cell cultures. For a complete description of the etiology and histopathology of the different fish tumors see Schartl *et al.* (1985).

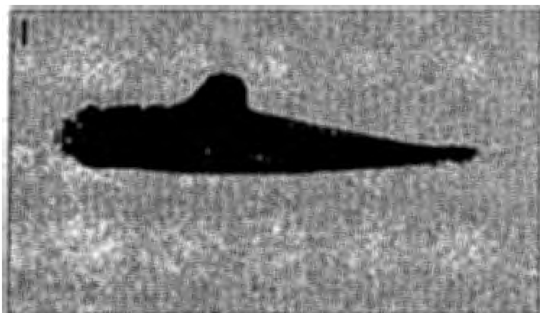

Fig. 3. Primary tumor free backcross hybrids, highly sensitive to carcinogens (genotype I), bearing an induced melanoma.

Cell culture

We used an uncloned spontaneously transformed cell line (A2) derived from embryos of *X. xiphidium* by Kuhn *et al.* (1979) and an uncloned cell line (PSM) derived from a malignant amelanotic melanoma of a *Xiphophorus* hybrid, isolated by Wakamatsu *et al.* (1984). In addition, the human Hela cell line was used for comparative studies. Fish cells were cultured at 28°C in two different media. Medium 1:M199 (Biochrom KG, Seromed, Berlin) containing 25 mM Hepes, and medium 2:MEM (Biochrom KG, Seromed, Berlin), containing 1.25 g $NaHCO_3$/l. Cells cultured in medium 2 were degased with air containing 10% CO_2. Hela cells were cultured in medium 2 at 37°C. Cells were normally harvested after reaching confluency. A2 cells were treated either with 2 mM KCN for 20 hr or were degased with N_2 for 72 hr. For one series of experiments PSM cells were harvested after having grown to different degrees of cell density and were then analyzed for enzyme

Table 1. Enzyme activities and substrate levels from normal tissue of *Xiphophorus*: *IU/mg protein ± standard deviation, †substrate levels in μmol/mg wet wt, ( ), number of samples; n.d., not detectable

Tissue	EN*	PK	PY†	LDH*	LA†	MDH*/LDH	MDH*	MDH*	MDH*/MDH
Brain	1.551 ± 0.161 (4)	3.303 ± 0.243 (7)	0.084 ± 0.023 (4)	1.716 ± 0.122 (9)	3.32 ± 0.44 (4)	3.8 ± 0.3 (9)	6.274 ± 0.297 (9)	1.997 ± 0.144 (9)	3.2 ± 0.2 (9)
Testis	0.761 ± 0.123 (5)	1.093 ± 0.113 (7)	u.d. (2)	0.590 ± 0.065 (7)	1.43 ± 0.40 (7)	6.5 ± 0.2 (7)	3.770 ± 0.392 (7)	1.214 ± 0.141 (7)	3.1 ± 0.1 (7)
Spleen	n.d.	n.d.	n.d.	0.088 ± 0.021 (7)	n.d.	4.7 ± 0.3 (7)	0.393 ± 0.092 (7)	0.124 ± 0.023 (7)	3.1 ± 0.2 (7)
Skin	2.717 ± 0.253 (5)	1.711 ± 0.128 (7)	0.095 ± 0.009 (7)	2.345 ± 0.254 (11)	6.30 ± 0.63 (7)	1.7 ± 0.2 (11)	3.650 ± 0.283 (11)	1.051 ± 0.086 (11)	3.5 ± 0.2 (11)
Liver	3.192 ± 0.192 (5)	0.156 ± 0.038 (7)	u.d. (8)	0.834 ± 0.093 (8)	1.53 ± 0.16 (8)	5.3 ± 0.6 (8)	4.079 ± 0.23 (8)	1.192 ± 0.109 (8)	3.5 ± 0.2 (8)

activities. Medium in all cases was changed every second day.

Preparation of extracts, determination of enzyme activities and substrate levels

All preparation steps were carried out at 4°C. Fish were decapitated and relevant tissues removed. All tissues, embryos and postnatal fish were immediately frozen in liquid nitrogen. Cell cultures were washed 3 times in Ca^{2+}/Mg^{2+} free PBS solution containing 5.8 g NaCl, 0.15 g Na_2HPO_4 and 0.15 g KH_2PO_4 per l and were harvested with a rubber policeman. For enzyme activity determinations, nearly 20 mg tissue, 10 embryos of a particular developmental stage, single postnatal fish or $1\text{–}2 \times 10^6$ cells were homogenized (10 strokes in a tight-fitting Dounce homogenizer) in 1 ml of extraction buffer (for description of the different extraction buffers see Bergmeyer, 1970; Büchner and Pfleiderer, 1955; Eigenbrodt *et al.*, 1983; Rübsamen *et al.*, 1982). For substrate determinations, 100 mg of tissue was employed. Samples extracted for pyruvate and lactate determination were centrifuged for 10 min at 9000 *g*. The supernatants were neutralized with KOH (50% w/v) and centrifuged again for 10 min at 9000 *g*. Samples extracted for enzyme activity determinations were centrifuged for 15 min at 1500 *g*. All supernatants were then centrifuged for 1 hr at 100,000 *g* and stored at −80°C. No changes in enzyme activity and substrate amounts after storing up to 14 days were observed. MDH*-, LDH-, EN- and PK-activities were determined as described by Büchner and Pfleiderer (1955); Eigenbrodt *et al.* (1983) and Rübsamen *et al.* (1982), lactate and pyruvate according to the method described in Bergmeyer (1970). MDH-activity was measured in 90 mM $Na_4P_2O_7$; 33.3 mM L-malate; 4 mM NAD at pH 10.8. All measurements were carried out in a spectrophotometer at 340 nm at room temperature by recording the rate of NADH turnover during the enzyme-reaction. Protein determination was carried out either on trichloro acetic acid (TCA) precipitated aliquots according to the method of Lowry *et al.* (1951), or by way of a commercially available protein assay (Bio-Rad laboratories, California, USA). Enzyme activities were calculated in IU/mg protein and substrate levels in μmol/10^6 cells for cell culture samples. For comparing the different parameters, values were expressed as percentages relative to the corresponding values obtained from normal adult skin (Table 1). For statistical analysis we employed the *t*-test (Sachs, 1968). The level of significance was fixed at $P = 0.05$ (5%).

RESULTS

Non-tumorous tissue

In order to characterize enzyme activities and substrate levels of normal tissue of *Xiphophorus*, heart, muscle, eyes, skin, brain, testis, spleen and liver of adult fish ($N = 7$) were analyzed. Values were expressed as percentages relative to the corresponding values obtained from normal adult skin (Table 1) for comparing the different parameters. The pattern of both enzyme activities and substrate levels was found to be tissue specific. Brain (see Table 1) and heart (see Fig. 4) display relatively high EN- and PK-activities, a relatively low LDH-activity and a high MDH*/MDH ratio, as well as the highest MDH*- and MDH-activities, indicating an essentially aerobic intermediary metabolism for these tissues. Conversely, muscle (see Fig. 4) although having high EN- and PK-activities, PY-levels, a high LDH-activity and LA-level has the lowest MDH*/LDH ratio and relatively low MDH*- and MDH-activities, indicating an anaerobic intermediary metabolism. Enzyme activities obtained for the other tissues revealed either more aerobic (eyes, Fig. 4; liver, spleen, testis, Table 1) or anaerobic (skin, Table 1) features of intermediary metabolism.

We investigated whether enzyme activities of normal tissue from tumorous fish are influenced by the different genetic backgrounds and/or are influenced by the elevated expression of *c-src*. Brain, eyes, muscle and livers of fish of the genotypes D (inter-

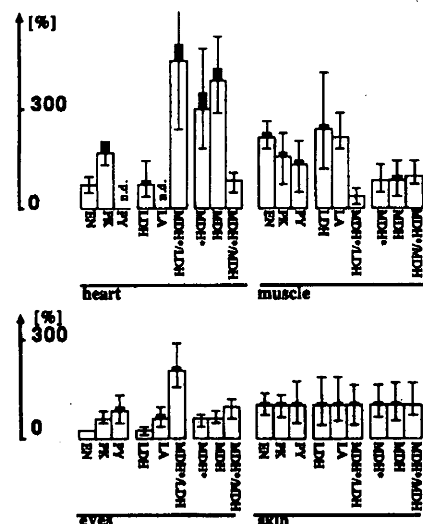

Fig. 4. Enzyme activities and substrate levels from normal tissues of *Xiphophorus* given as percentages relative to the corresponding values from normal adult skin (Table 1). ■, S.D.; I, extremest deviations; n.d., not done. Experiments were repeated 6–10 times.

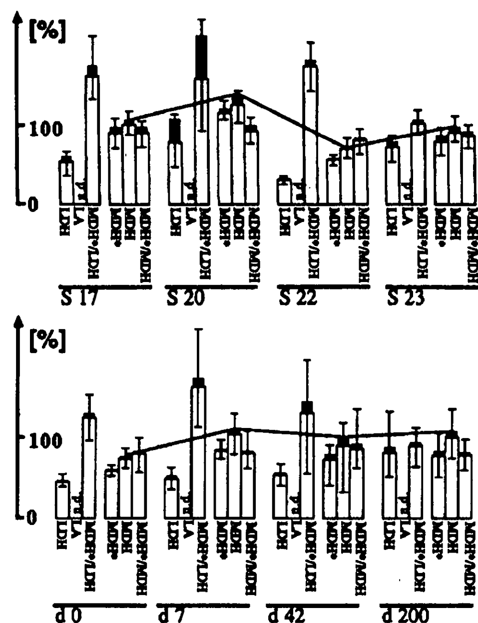

Fig. 5. Enzyme activities from embryos of different development stages (S) and postnatal fish of different age (d = day after birth). Values are expressed in percentages relative to the corresponding values from normal adult skin (Table 1). ■, S.D.; I, extremest deviations; n.d., not done. The present data reflect at least one reduplication of the experiment.

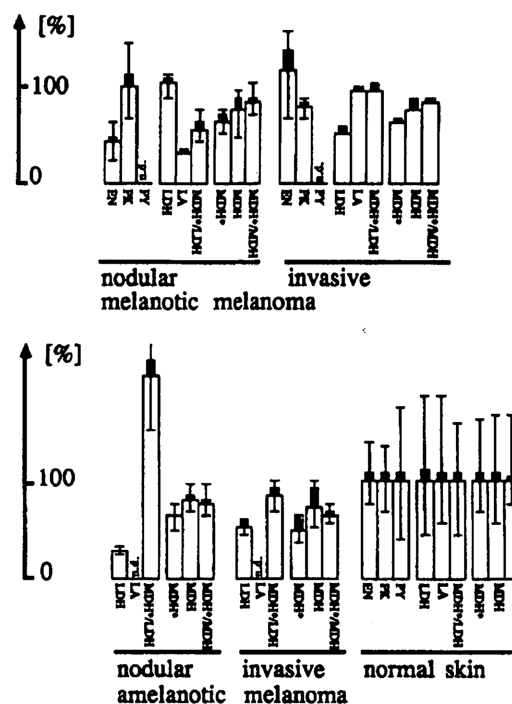

Fig. 6. Enzyme activities from spontaneously developing melanoma expressed in percentages relative to the corresponding values from normal adult skin (Table 1). ■, S.D.; I, extremest deviations; n.d., not done. Most of the experiments were repeated three times.

mediate pp60$^{c\text{-}src}$ activity in brain, $N = 3$); E,H (high pp60$^{c\text{-}src}$ activity in brain, $N = 3$); and I (low pp60$^{c\text{-}src}$ activity in brain, $N = 3$) were analyzed. No significant differences in enzyme activities and substrate levels between the different brains, eyes and muscles were observed. However, livers obtained from fish bearing spontaneously developing melanotic melanoma show 2-fold lower EN- and 2-fold higher PK-activities, and the liver of fish bearing an induced fibrosarcoma show a 2-fold higher LDH-activity, compared to liver of healthy fish (data not shown).

To determine whether enzyme activities vary during normal development of the fish, whole embryos ($N = 30$) of different developmental stages and postnatal fish (genotypes F,G; $N = 5$) were analyzed. Minor changes in the activities of the different enzyme activities and the ratios MDH*/LDH and MDH*/MDH were observed (Fig. 5). All the determined parameters oscillate dependent upon the developmental stage of the fish (compare Fig. 5, MDH-activity).

Tumorous tissue

In order to characterize enzyme activities and substrate levels of neoplastic tissue of *Xiphophorus*, various sections of tumors differing in etiology and histopathology were analyzed. All neoplasia tested so far show a pattern of enzyme activities and substrate levels which differ from that of any normal tissue (see Table 1, Table 2, Fig. 4, Fig. 6).

In fish bearing spontaneously developing melanoma (genotypes E,H; $N = 5$) LDH-activity in the nodular compartments of the melanotic melanomas is increased while EN-activity is decreased compared to the corresponding invasive sections of the tumors (see Fig. 6). LDH- and MDH*-activities determined from nodular compartments of amelanotic melanoma differ from that detected in the corresponding invasive section. In addition the nodular compartments of melanotic and amelanotic melanomas differ in LDH-activity and the ratio of MDH*/LDH. No differences in enzyme activities and the ratios MDH*/LDH and MDH*/MDH between invasive

Table 2. Enzyme activities from induced neoplasia of *Xiphophorus* in IU/mg protein ± S.D., ( ) number of samples

Tissue	LDH	MDH*/LDH	MDH*	MDH	MDH*/MDH
Nodular melanoma	2.706 ± 0.086 (2)	0.9 ± 0.1 (2)	2.467 ± 0.115 (2)	0.977 ± 0.051 (2)	2.5 ± 0.3 (2)
Invasive melanoma	0.674 ± 0.065 (1)	2.3 ± 0.3 (1)	1.557 ± 0.078 (1)	0.588 ± 0.047 (1)	2.8 ± 0.3 (1)
Invasive fibrosarcoma	0.658 ± 0.083 (1)	2.5 ± 0.7 (1)	1.641 ± 0.238 (1)	0.626 ± 0.055 (1)	2.6 ± 0.6 (1)
Nodular rhabdomyosarcoma	2.901 ± 0.121 (1)	0.9 ± 0.1 (1)	2.485 ± 0.116 (1)	0.806 ± 0.022 (1)	3.1 ± 0.2 (1)

compartments of different melanotic and amelanotic melanoma were observed (Fig. 6). These results suggest that the intermediary metabolism of the *Xiphophorus* melanomas is specific for tumor compartments and may depend on the histiotype of the melanoma.

Induced neoplasia (two invasive and two nodular melanomas; one invasive fibrosarcoma; one nodular rhabdomyosarcoma) were analyzed from fish of genotype I. LDH-, MDH*- and MDH-activities and the ratios MDH*/MDH determined from the invasive melanotic melanomas and the invasive fibrosarcoma were found to be identical (see Table 2). Also, no differences in enzyme activities between the nodular melanotic melanomas and the nodular rhabdomyosarcoma was found. All nodular tumors show 4-fold higher LDH- and 1.5-fold higher MDH-activities and a 2.5-fold lower ratio of MDH*/LDH, compared to the invasive tumors (Table 2). While the induced nodular tumors show no differences to the nodular compartments of spontaneously developing melanotic melanomas, the induced invasive tumors differ with an up to 2-fold lower LDH-activity and an approx. 2-fold higher ratio MDH*/LDH from that of the invasive compartments of spontaneously developing melanoma (see Table 2, Fig. 6). These results suggest that the intermediary metabolism of neoplasia of *Xiphophorus* is also dependent on tumor etiology.

Cell lines

To investigate whether enzyme activities of transformed fish cells are influenced by exposure of the cells to different environmental conditions, the following experiments with *in vitro* cultured cells were performed. To determine whether metabolism of the cells is dependent upon oxygen, A2 cells were cultured under an atmosphere containing either 100% N_2 or 10% CO_2, or were treated with 2 mM KCN. Control cells were maintained in normal atmosphere without additional CO_2. Compared to the control, cells maintained under N_2 show drastically decreased MDH*- and MDH-activities and only a minor increase in LDH-activity (Fig. 7). Cells cultured under CO_2 exhibit a more than 4-fold higher LDH-activity while MDH*- and MDH-activities were found to be unchanged (Fig. 7). Cells treated with KCN show no significant changes in LDH-, MDH*-, and MDH-activities and the ratios of MDH*/LDH and MDH*/MDH compared to that of untreated control cells (Fig. 7). In addition no phenotypic alteration or decreased vitality of the treated cells was observed. Adult fish treated with 0.02 mM KCN died within 20–30 min. These results suggest that transformed cells are able to adapt their intermediary metabolism to extreme changes of the environment.

In order to find out how the proliferative state of transformed fish cells may influence their intermediary metabolism, PSM cells were cultured under 10% CO_2 and harvested after growing to different degrees of cell density. Two-fold higher EN-, PK-, LDH- and MDH-activities and three times elevated MDH*-activity were found in cells which have grown up from 1.8×10^5 to 4.8×10^5 cells/ml (subconfluent monolayer, Fig. 8). When cells have grown up to 9.2×10^5 cells/ml (confluent monolayer), PK-,

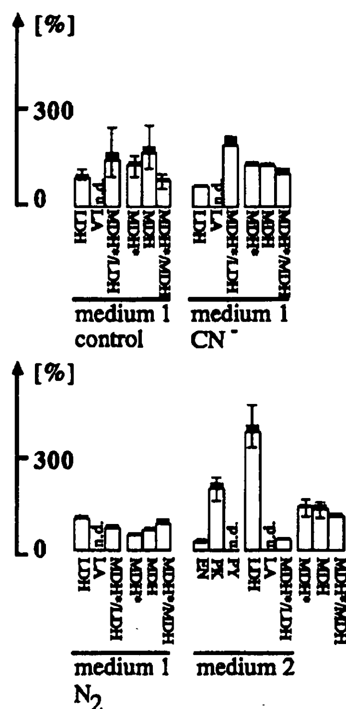

Fig. 7. Effect of oxygen on enzyme activities from confluent A2 cells, expressed in percentages relative to the corresponding values from normal adult skin (Table 1). ■, S.D.; I, extremest deviations; n.d., not done. KCN- and N_2-treatment was carried out only one time.

MDH*- and MDH-activities were found to be stable, while EN- and LDH-activities are 3-fold increased (Fig. 8). After the cells have grown up to 1.5×10^6 cells/ml (superconfluent cells), EN- and LDH-activities are 4-fold increased and PK- and MDH-activities are 3-fold elevated. MDH*-activity was found to be stable (Fig. 8). Cells grown up to a density of 2.3×10^6 cells/ml (superconfluent, focus formation) show 5- and 12-fold enhanced EN- and PK-activity, respectively. The activities of the other enzymes and the ratios of MDH*/MDH and MDH*/LDH was found to be unchanged (Fig. 8). These results suggest that the intermediary metab-

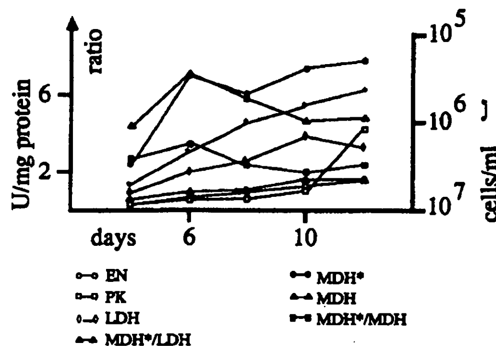

Fig. 8. Representative data of enzyme activities from PSM cells cultured in medium 2 and harvested at different degrees of cell density. Each experiment was reproduced three times and no significant deviations from the above values were observed.

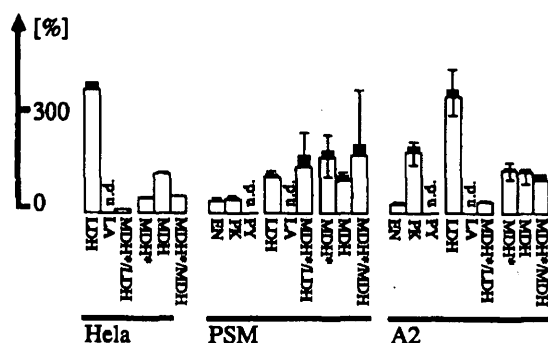

Fig. 9. Enzyme activities from confluent Hela, PSM and A2 cells cultured in medium 2, expressed in percentages relative to the corresponding values from normal adult skin (Table 1). ■, S.D.; I, extremest deviations. Most of the experiments were triplicates.

olism of PSM cells is influenced by the actual cell density in the culture.

In order to analyze the general significance of the data obtained we compared the intermediary metabolism of the fish cell lines A2 and PSM with the human tumor cell line Hela (Fig. 9). A2 cells show similar high LDH- and MDH-activities, 2.5-fold elevated ratios of MDH*/LDH and MDH*/MDH and 2.5-fold increased MDH*-activity compared to Hela cells. PSM cells display 3.5-fold lower LDH-, 4-fold higher MDH*-activities, a 4.5-fold higher ratio of MDH*/MDH and a 13-fold higher ratio of MDH*/LDH than Hela cells. MDH-activity was found to be unchanged. Compared to A2 cells, PSM cells exhibit a 5-fold elevated ratio of MDH*/LDH, a 2-fold elevated ratio of MDH*/MDH and 1.5-fold higher MDH*-activity, a 4-fold decreased PK- and a 3-fold decreased LDH-activity. Activities of EN and MDH were found to be similar in both cell lines (see Fig. 9). Intermediary metabolism of all cell lines tested differ from that of any normal tissue (Fig. 4, Fig. 9, Table 1) and in addition the intermediary metabolism of PSM cells differ from that of any melanoma analyzed (Fig. 6, Fig. 9). These data indicate that intermediary metabolism of *in vitro* cultured cells may be cell lineage specific and independent from tissue of origin.

DISCUSSION

Determination of enzyme activities and substrate levels of different non-transformed tissue from adult fish revealed different metabolic states, which are specific for each tissue and correspond to their normal function. Data obtained from brain, heart and muscle of *Xiphophorus* show comparable values in enzyme activities and substrate levels to that reported for the same tissue from other fish species (Bilinski, 1974; Knox *et al.*, 1980; Pasdair *et al.*, 1984) and even to chicken and mammals (Asaga and Konno, 1975; Farina *et al.*, 1974; Tolle *et al.*, 1976). These data suggest that intermediary metabolism of non-transformed tissues of *Xiphophorus* is comparable to that detected in the corresponding homologous tissues of other vertebrates.

No genotype specificity of intermediary metabolism in normal organs was detected. This was partly unexpected since it was reported earlier that the tyrosine specific protein kinase activity of pp60$^{c\text{-}src}$ varied in a genotype-dependent manner (Schartl *et al.*, 1982, 1985). An effect on LDH-activity in fish brain might be expected, since LDH has been detected as a potential substrate for the viral counterpart of pp60$^{c\text{-}src}$ (Cooper *et al.*, 1983). However this was not the case. We suppose that either LDH is not a substrate for the cellular pp60^{src} kinase in fish cells, or that tyrosine phosphorylation of LDH has no effect on the LDH-activity. The latter possibility was found to be the case for LDH phosphorylated through pp60$^{v\text{-}src}$ (Hunter and Cooper, 1985).

Only minor changes in enzyme activities during late embryogenesis and postnatal development was observed. Since the embryos of *Xiphophorus* are very small and only whole individuals could be analyzed, antagonistic differences in enzyme activities between the different developing and differentiating organs could be responsible for the minor values of changes detected. Most of the enzyme activities and the derived ratios show an age dependent oscillation, which could be caused by small changes in enzyme activities during late differentation and growth of the different fish tissues. Another possible explanation are periodic changes of enzyme activities in the fish. Circadian rhythms in enzyme activities are known for various mammalian organs (Vermouth *et al.*, 1984).

Data obtained from liver of fish bearing tumors of different etiology and histopathology revealed that in several instances the intermediary metabolism may be influenced as a consequence of the pathological situation. EN-, PK- and LDH-activities were found to be altered in livers of some melanoma and fibrosarcoma bearing fish. We were not able to detect any dependency on tumor histiotype or tumor malignancy. It is possible, that changes in intermediary metabolism in livers of several tumorous fish are caused by cachexia and/or metastasis of the primary neoplasm. Changes in ornithine aminotransferase-, malic enzyme-, alanine aminotransferase- and glucokinase-activities in livers of rats, bearing different implanted neoplasia are known (Cayanis and Greengard, 1983).

All neoplasia of *Xiphophorus* and transformed fish cell lines tested so far show specific characteristics of intermediary metabolism. They differ from their normal corresponding tissue—skin as compared to melanomas and fibrosarcomas—skeletal muscle as compared to rhabdomyosarcomas. We could not define a melanoma specific intermediary metabolism, so a correlation with the expression of *Tu* and/or c-*src* was not detectable. However we found tumor compartment specific changes in EN-, LDH- and MDH* activities, which may be caused by higher proliferative rates in the invasive tumors or tumor compartments. It is known that slowly growing, highly differentiated tumors show low glycolytic rates (Elwood *et al.*, 1963) and that fast growing rat hepatomas exhibit elevated activities of hexokinase, phosphofructokinase and pyruvate kinase, compared to slow growing hepatomas (Weber *et al.*, 1971; Balinsky *et al.*, 1973). Our data may also indicate tumor histiotype- and etiology-specific differences between the different fish neoplasia in LDH-, MDH*- and MDH-activities, perhaps similar to that found in many human neoplasia (Herzfeld *et al.*, 1978; Weber *et al.*, 1976, 1980). However more data are needed for

a biochemical characterization of the different fish neoplasia.

In order to get a better insight into how intermediary metabolism of transformed fish cells *in vivo* may be dependent upon oxygen, cell proliferation or different cell types, we studied *in vitro* cultured transformed fish cells and Hela cells, since it was easier to maintain these cells under defined conditions. We could demonstrate that the cells reduce MDH*- and MDH-activities when they were maintained under N_2 and enhanced drastically their LDH-activity when they were degased with air containing 10% CO_2. In addition, no changes in enzyme activities after KCN treatment was observed. These data suggest that the cells possess a broad adaptation capability of intermediary metabolism for oxygen, which allows the cells to survive extreme supply situations. Adaptation of intermediary metabolism of *in vitro* cultured mammalian tumor cells to extremely poor supply situations for oxygen, glucose, glutamine etc. are known (for review see Eigenbrodt *et al.*, 1985; McKeehan, 1982). We also were able to show that a fish melanoma cell line exhibited enhanced activities of EN, PK, LDH, MDH* and MDH directly dependent upon the cell density. Such a dependency has also been observed for glycolytic enzymes in murine sarcoma virus transformed but not in normal rat kidney cells (Gregory and Bose, 1977) and is believed to be a feature of transformed cells. If transformed cells change their energy production from glycolysis to glutaminolysis (an important metabolic pathway for many mammalian tumor cells), the activities of several enzymes are enhanced. For example, not only glutamateoxalacetate transaminase and malic enzyme but also that of the glycolytic enzymes EN, PK and LDH are elevated in activity (see Eigenbrodt *et al.*, 1985; McKeehan, 1982; Sauer and Dauchy, 1978). Since we observed the most dramatic elevations of enzyme activities in EN, PK and LDH, we assume that the melanoma cells possibly change their ATP-production from glycolysis to glutaminolysis when they reach higher cell densities. Comparison of the enzyme activities of the two transformed fish cell lines with that of the human tumor cell line Hela revealed specific pattern for each cell line. We suppose that these patterns are a consequence of the different tissues of origin from which the cell lines were derived.

We still do not understand the molecular mechanism of neoplastic transformation in the *Xiphophorus* melanoma system. We could not detect any correlation between our data and the expression of *Tu* or $pp60^{c\text{-}src}$ activity, which has been shown to be high in a variety of neoplasia (Schartl *et al.*, 1985). It is possible that the potential substrates for $pp60^{v\text{-}src}$ (EN, LDH) are not phosphorylated through $pp60^{c\text{-}src}$ in neoplastic cells of *Xiphophorus*, as we proposed for the fish brain, or that a tyrosine-specific phosphorylation of these enzymes has no effect on the enzyme activities. Therefore the function of $pp60^{c\text{-}src}$ in neoplastic transformation and its possible influence on intermediary metabolism of transformed fish cells remains unclear. On the other hand, our data on intermediary metabolism of spontaneously occurring or induced neoplasms or *in vitro* cultured transformed fish cell lines lead us to assume that the observed features are not the primary result of the neoplastic transformation. Instead our results seem to reflect features which may be more involved with the maintenance of the neoplastic phenotype of the transformed fish cells. Interestingly, most of the differences in enzyme activities between the different tumors and transformed cells concern EN-, PK- and LDH-activities. Possibly these differences could be due to a changed pattern of isoenzymes of EN, PK and LDH. If this is the case, it should be possible to find marker isoenzymes for distinct tumor types of the fish. Evidence for this assumption comes from Ahuja *et al.* (1975) and Schwab *et al.* (1976), who found elevated expression of the B_4-isoenzyme (heart type) of LDH in spontaneously developing melanoma of *Xiphophorus* hybrids. Distinct isoenzymes of PK, LDH, MDH and EN seem to be the most promising candidates for marker enzymes in diagnosis and prognosis of different human cancer (Balinsky *et al.*, 1983; von Eyben, 1983; Esscher *et al.*, 1985; Ibsen *et al.*, 1982; Ishiguro *et al.*, 1984; Polonis *et al.*, 1984).

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