yield of recovered enzyme is drastically reduced. This may reflect the importance of competing side reactions of the dissociated LDH monomer in the in vitro system[11].

Conclusion. The purpose of this investigation was to discriminate between specific peptide inhibitors of the reactivation process and a hypothetical proteolytic enzyme effect which could lead to a similar kinetic behavior[10]. Since coenzymes often have the ability to protect their apoenzymes against proteolytic degradation, we had to design an experiment which excludes the trivial possibility that the reduced inhibition in the presence of coenzymes is due to a coenzyme-induced folding of the monomers which could lead to resistance against proteolytic enzymes. The fact that the reactivation is induced even after prolonged exposure to the inhibiting principle (fig. 2) unambigously demonstrates that the blocked reconstitution is truly reversible and therefore cannot be due to proteolysis.

Further consideration deserves the isoenzyme specific effect of the 2 coenzymes. The occurrence of 2 discrete isoenzyme specific inhibitors[9] which are susceptible to different antagonists suggests that the biological significance of the existence of 2 LDH-types might be due to different regulatory properties rather than differing metabolic functions.

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Trace metals and melanogenesis[1]

D.P. Chakraborty[2], C. Chakraborty, M. Ganguly and A.K. Chakraborty

Bose Institute, Department of Chemistry, 93/1, Acharya Prafulla Chandra Road, Calcutta-700009 (India), May 7, 1982

Summary. Emission spectroscopic analysis of amphibian and mammalian skin ash for the study of trace metals shows that nickel, lead and tin may play some role in the pigmentation-depigmentation process.

In a previous communication Chakraborty et al.[3] showed that the activity of the melanogenic enzyme, tyrosinase (a monooxygenase) is inversely related to the one of tryptophan pyrrolase (a dioxygenase) during experimental depigmentation, and pigment regeneration by psoralen, in *Bufo melanostictus.* The role of copper is well documented in the tyrosinase action[4]. It is known that nickel in plant systems can increase the monooxygenase activity of catalase[5] while it inhibits the dioxygenase, peroxidase[6]. Lack of any report regarding the involvement of metals other than copper in depigmentation and melanogenesis prompted us to find out whether during experimental depigmentation or regeneration of pigment with pigmentogenic drugs, the normal concentration of trace metals is affected in the amphibian and mammalian skins.

In the present communication, the levels of lead, arsenic, nickel, cobalt, copper and tin in the experimental depigmentation and regeneration of pigment with psoralen (plus sunlight) in *B. melanostictus* have been investigated. Comparative studies of different trace metals in pigmented rat skin with those of albino rat skin have also been done.

Materials and methods. 90 Indian male toads *(B. melanostictus)* of 40–50 g b.wt were used for the experiment; of these, 60 were fed hydroquinone (100 µg/toad/day) for 7 days. On the 8th day 30 of the hydroquinone-treated toads and 30 normal toads were sacrificed for analysis of trace metals in the ventral skin tissues. The remaining 30 toads were fed psoralen (1 mg/toad/day) and kept under ordinary sunlight for 7 days. These toads were sacrificed on the 15th day. Ventral skins were dissected out.

6 pigmented Bandicoot rats of 200–250 g b.wt and 6 albino rats (Wistar strain) of the same b.wt, maintained for a few days on the same diet and in the same atmosphere, were sacrificed for analyses of trace metals in the skin tissues.

For dissection of the skin we used a stainless steel blade. The skins were cut into pieces, washed with deionized water and dried in an air oven. Ashing of the tissues was done by the oxidizing flame of a Bunsen burner keeping the tissues in a silica crucible. The ashes were analyzed for trace metals using a Jarrel Ash Emmission Spectrograph (JAES).

Results and discussions. Table 1 shows that during induced depigmentation, levels of lead and tin in the skin of *B. melanostictus* increase abnormally and during regeneration of pigmentation with oral administration of psoralen (plus sunlight) about normal levels have been obtained in

Table 1. Emission spectroscopic data for hydroquinone and psoralen treated toad skins

	Mean result (ppm) ± SD				
	Pb	Sn	Ni	Co	Cu
Normal	160 ± 9	250 ± 13	80 ± 3	18 ± 3	25 ± 2
Hydroquinone-fed toads (depigmentation)	250 ± 12*	430 ± 12*	80 ± 7 (NS)	15 ± 3 (NS)	22 ± 2 (NS)
Psoralen plus sunlight (after recovery)	180 ± 11*	150 ± 7*	70 ± 8 (NS)	12 ± 3 (NS)	25 ± 3 (NS)

Number of trials (n) = 6. Each trial contains ventral skin ash from 5 toads. *$p < 0.001$; NS = not significant.

Table 2. Emission spectroscopic data of pigmented rat skin and albino rat skin

Ash of rat skins	Mean result (ppm) ± SD				
	Pb	As	Ni	Co	Cu
Albino	150 ± 13	500 ± 17	30 ± 4	10 ± 1	600 ± 20
Pigmented	70 ± 11*	500 ± 13 (NS)	10 ± 1*	10 ± 1 (NS)	120 ± 12*

n = 6; *p < 0.001; NS = not significant.

case of these 2 metals only. Nickel, cobalt and copper which have also been detected in toad's skin show an insignificant change after depigmentation as well as after recovery. Considering the intermediate status of the mobility of skin lead, as compared with those of bones and soft tissues[7], the variation of lead concentration under different experimental conditions could be expected. The higher concentration of lead found in albino rat skin (table 2) is parallel with our observations during experimental depigmentation (table 1). The observation of the larger amount of copper in albino rat skin (table 2) is analogous to the observations of Genov et al.[8]., regarding a 30% higher total copper level with the concomitant higher oxidase activity of ceruloplasmin (130%) as compared with normal subjects. Albino rat skin also contains more nickel than the pigmented skin. Both the varieties of rat contain a significant amount of arsenic in their skins, although the difference between the two is not significant. This arsenic which could normally be present in rat hair might come from endogenous sources, or from exogenous sources by adsorption in the hair. In our experiments, arsenic was not detected in toad skin.

It is well documented that lead inhibits tyrosine hydroxylase activity by reducing the tetrahydrobiopterin level in serum[7]. Lead has also been suggested to alter the metabolism of tryptophan[9]. Kurbanov et al.[10] as well as Roy Chowdhury et al.[11] have shown that abnormal tryptophan metabolism may be involved in vitiligo. Again, schizophrenia, which has been related to abnormal tryptophan metabolism also produces elevated lead levels in hair[12]. The increased concentration of skin lead in experimental depigmentation are therefore interesting, and calls for further investigations. From our results it appears that apart from copper, other trace metals may be involved in melanogenesis affecting the activity of the oxygenase enzymes involved in it.

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Lactate dehydrogenase activity in cell subpopulations of 7,12-dimethylbenz(a)anthracene-induced mammary tumors[1]

A.R. Safa and M.T. Tseng[2]

Department of Anatomy, and Regional Cancer Center, University of Louisville, Health Sciences Center, Louisville (Kentucky 40292, USA), July 19, 1982

Summary. Heterogeneity of cells in a solid tumor prevents direct assessment of enzyme activity of cell subpopulations by conventional homogenization techniques. Lactate dehydrogenase (LDH) activity before and after ovariectomy and following estrogen supplementation in density-defined cell subpopulations from DMBA-induced mammary tumors was determined. Different levels of LDH activity were found in different cell subpopulations. After ovariectomy the level of LDH activity declined. Restoration of the circulatory estrogen level resulted in increased enzyme activity. The highest level of LDH was found in a band which consisted mainly of poorly differentiated cells. This cell subpopulation also tended to be more responsive to endocrine manipulation.

Mammary tumors induced by 7,12-dimethylbenz(a)anthracene contain extremely high lactate dehydrogenase (LDH) activity, specifically LDH isozyme 5, and ovariectomy of tumor-bearing rats causes tumor regression and a decrease in LDH isozyme[5]. The estrogen dependency of LDH has also been shown in the MCF-7 human breast cancer cell line[4]. Solid tumors such as breast neoplasms consist of multiple epithelial and stromal cell types[5]. The heterogeneity of the tumor cells within a given tumor makes it impossible to pinpoint the cellular source of LDH activity after tissue homogenization. A higher content of estrogen receptors in better differentiated cell subpopulations of the DMBA-induced mammary tumor has been reported[6]. Similarly, the existence within a single solid tumor of multiple