In vivo inhibition of the tyrosinase of hamster melanoma with sodium diethyldithiocarbamate

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Summary. In vivo inhibition of the DOPA-oxydase activity of the soluble tyrosinase fraction of melanotic hamster melanoma is found after i.p. administration of diethyldithiocarbamate, with considerable increase in the content of SH- groups which probably play the role of free radical scavengers.

The metalloenzymes are studied through their inhibition with metal-ion-chelating agents[1]. Diethyldithiocarbamate (DDC) is used as a ligand for the copper ions in in vitro experiments with tyrosinase (1.10.3.1), resulting in loss of activity of the purified enzyme preparations and inhibition of the DOPA-oxidizing action of homogenates and subcellular fractions of melanin-synthesizing tissues[2-4]. DDC contains free SH- groups and it has been proved in vitro that thiol compounds inhibit tyrosinase[5,6]. This study is an attempt at establishing the possible in vivo inhibitory action of DDC on the activity of the soluble tyrosinase fraction of experimental hamster melanoma.

Materials and methods. The experiments were carried out on 24 2-month-old male hamsters, average weight about 60 g. On the same animals consecutive subcutaneous transplantation was performed of a melanotic tumor on one side of the back, and of an amelanotic tumor on the other side of the back[7]. In the optimum term for the growth of the 2 melanoma tumors, 30–32 days for melanotic and 8–9 days for amelanotic tumor after the transplantation, the animals were divided in 2 groups. The experimental animals were injected i.p. with aqueous solution of DDC 40 mg/100 g weight. The hamsters in both groups were sacrificed 60 min later. At 4 °C a 10% homogenate was made from the tumor tissue in 0.25 M sucrose using the Potter-Elvehjem homogenizer. A 'soluble tyrosinase fraction' was obtained as the supernatant from centrifugation at 105,000 × g for 60 min.

Tyrosinase (DOPA-oxidase) activity was determined spectrophotometrically with l-DOPA as substrate 0.76 M in 0.1 M phosphate buffer, pH 6.8, after incubation at 37 °C[8]. The results were estimated in $\Delta E^{60\,min}_{475\,nm}$/mg protein. The free SH- groups were determined by Ellman's reagent containing 5,5-dithiobis (nitrobenzoic acid) (DTNB), pH 8.0[9]. The results were estimated with respect to l-cysteine standards. The content of unstable SH- groups was determined after Beutler[10]. Copper content in the materials was determined spectrophotometrically with oxalyldihidrazide and acetaldehyde[11]. Protein content was determined after Lowry et al.[12] with standards of calf serum albumin.

All measurements were made in triple parallel samples, taking into account interference by the reagents and DOPA-autooxidation.

Results and discussion. DOPA-oxidase activity of the soluble tyrosinase fraction of the melanotic melanoma (table) is close to previously established values[13]. Such enzymatic activity is completely lacking in the cytoplasm of the amelanotic tumor of the same animals.

The content of free SH- groups in the cytoplasm of the melanotic tumors in twice as low as that in the amelanotic tumors. In the melanotic tumors half of the total number of SH- groups are unstable, readily reacting. Such groups are not found in the samples of amelanotic melanoma of the same animals.

Treatment of the experimental animals with DDC under the conditions described results in approximately twice as high a content of the free SH- groups in the cytoplasm of both types of tumors ($p < 0.05$ for melanotic tumor), with an increase in the content of unstable groups in the melanotic melanoma and their appearance in the cytoplasm of the amelanotic tumor as well ($p < 0.05$). DOPA-oxidase activity in the cytoplasm of the melanotic melanoma of the DDC-treated animals is considerably reduced (by 44%) compared with the control animals (table).

Copper content in the cytoplasm of both tumors does not change in the experimental animals compared with the DDC-untreated ones, and it is approximately the same. Other authors have also found insignificant differences in the copper content of melanotic and amelanotic hamster melanomas[14].

In vivo inhibition of the DOPA-oxidase activity in the cytoplasm of the melanotic melanoma and the considerably increased content of free SH- groups after DDC administration in the experimental animals are probably causally linked phenomena. DDC is thought to be metabolized in mammalian organisms to CS_2 or active thiol compounds[15], which may have interferred with the determination of SH-groups with DTNB and may be related to the tyrosinase activity, owing to the great affinity between enzymatic copper and sulphur-containing substances[16].

It is known that SH- groups play a definite role as melanogenesis inhibitors in vivo. In normal and tumor melanin-synthesizing tissues the opposite dependence of the content of SH- groups is found with respect to the amount of melanin synthesized in the cells, on the one hand, and the tyrosinase activity established there, on the other[4,5,17].

The increased content of free SH- groups and especially of free readily reacting groups in the cytoplasm of both types of hamster melanoma, transplanted in the same animals after DDC treatment, suggests changes in the oxidation-reduction potential of the tumor tissue. The inhibition of the DOPA-oxidase activity of the soluble tyrosinase fraction of the melanotic melanoma in this case is probably only one manifestation of this change and may represent an expression of free radical scavengers when it is known that the free radicals play an activating role in melanogenesis[18].

Tyrosinase activity, content of free and free unstable SH – groups and of copper in cytosol fraction of melanotic and amelanotic hamster melanoma in untreated (C) and treated (DDC) experimental animals

Melanoma	Tyrosinase activity in $\Delta E_{60\,min}$/mg protein	SH – groups content in $\times 5.10^{-6}$ M/mg protein	Unstable SH – groups	Copper content in $\times 10^{-3}$ µg/mg protein
Melanotic (C)	0.390	45.00	20.00	8.6
Melanotic (DDC)	0.175	95.00	42.00	8.5
Amelanotic (C)	0.000	92.00	00.00	9.1
Amelanotic (DDC)	0.000	118.00	28.00	9.2

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The pattern of endothelial cell boundaries in regenerated aortae

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Summary. The pattern of endothelial cell boundaries in the regenerated aorta was shown to differ greatly from that of control specimens as seen in silver stained preparations under the scanning electron microscope.

The use of silver stains to demarcate the intercellular boundaries between adjacent endothelial cells has been a relatively common practice since the description of the technique by Florey et al. in 1959[2]. Endothelial cells were described as being elongated with centrally-located ovoid nuclei. More recently silver stains have been employed in scanning electron microscopy[3]. This study utilized a silver salt which demarcates intercellular boundaries as zigzag lines between each elongated cell whose long axis is parallel to the long axis of the blood vessel. Other studies agree with these findings[4,5]. The present study was undertaken to determine the difference between the appearance of the normal endothelial cell pattern and the appearance of area where an injury caused removal of the endothelial covering and subsequent regeneration of the endothelium.

24 male rats of the Sprague-Dawdley strain, weighing about 200–250 g were used in this study. The rats were opened by a midline abdominal incision and their aortae were exposed. In half of the rats a stainless steel probe previously cooled in liquid nitrogen, was brought into contact with the aorta for 1 min. This had the effect of freezing the entire thickness of the aorta. This site was

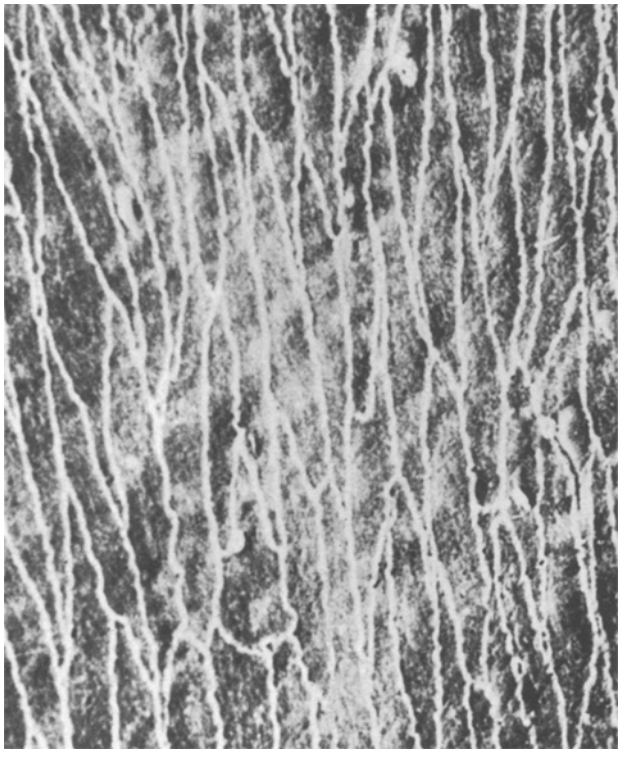

Fig. 1. The pattern of intercellular boundaries in control specimens.

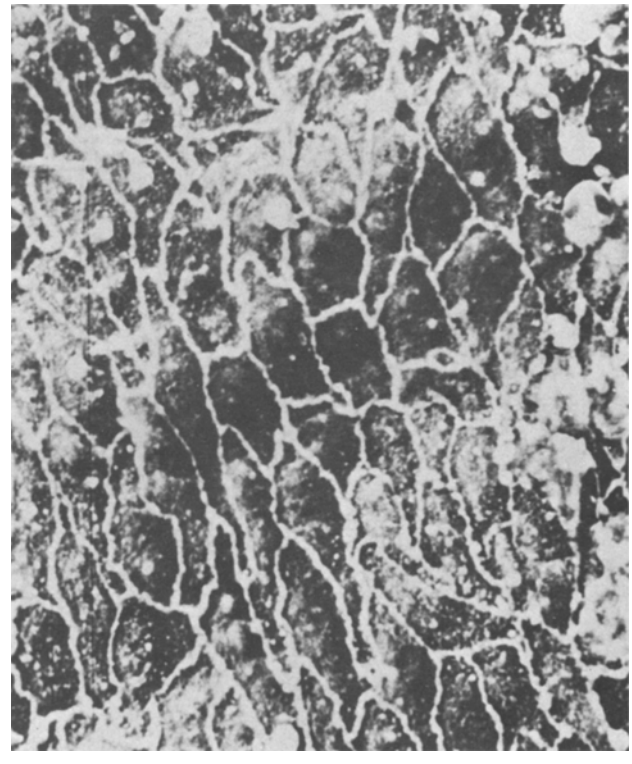

Fig. 2. The irregular pattern of endothelial cells over the regenerated area. × 1300.