that intestinal al-Pase activity play a functional role in the active transport of thiamine. On the other hand, we failed to detect any effect of ethanol administration on ($Na^+ + K^+$)-ATPase activity. Our results are therefore in conflict with the suggestion by Hoyumpa et al.[16].

Now, it is possible to speculate from our findings that the active transport of thiamine in the intestine is decreased by thiamine deficiency. However, we previously observed that glucose-intolerance in thiamine-deficient rats was restored by thiamine at higher dose than 0.05 mg/kg, irrespectively of whether it was given orally or s.c., suggesting that the absorption of thiamine in thiamine-deficient rats was the same in the control[25]. This discrepancy may be explained by an adaptative mechanism to facilitate absorption of meager supples of thiamine in thiamine-deficient rats as suggested by Hoyumpa et al.[26].

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Serum tyrosinase in human lung carcinoma[1]

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Summary. Compared to normal humans, lung carcinoma patients show increased tyrosinase activity. 7 serum enzymic fractions or carriers were present in the diseased state. Further, serum tyrosinase inhibitory factors generally were decreased in lung carcinoma patients compared to normal individuals.

Serum tyrosinase activity appears associated with some malignant diseases[3]. Serum factors affecting tyrosinase activity have been demonstrated in melanoma patients and may be markers for the degree of malignancy[4]. The present report deals with these parameters in patients with lung carcinoma.

Materials and methods. Sera of 11 normal humans and 41 lung carcinoma patients prior to treatment were obtained (table 1). In addition, blood bank plasma also was used. The sera and plasma were fractionated with ammonium sulfate. The proteins were salted out at 60% saturation from the 50% saturation supernatant. These proteins were assayed for tyrosinase activity and for electrophoretic separations producing dopa-melanin bands[3,5]. The 60% saturation supernatant was utilized for separation of serum or plasma fractions affecting tyrosinase activity. These supernatants were desalted by dialysis and ultrafiltrated (Amicon) at 4 mol. wt ranges. The effects of these fractions on the melanogenic activity of mushroom tyrosinase were evaluated[6].

Results and discussion. Sera from normal individuals and lung carcinoma patients did not show statistically significant sex differences in serum tyrosinase activity. The data of each group, therefore, were consolidated (table 1). The tyrosinase activity present in the sera of patients with lung carcinoma was 202% of the normal. However,

Table 1. Serum tyrosinase activity in lung carcinoma.

Category	Age (years)[a] Male	Female	Tyrosinase activity[b] pmole/ml	% normal
Normal	30–50 (6)	25–46 (5)	501 ± 61[c]	–
Lung carcinoma	31–79 (31)	48–81 (10)	1011 ± 87	202

[a]Number of individuals in parenthesis. [b]L-[U-^{14}C] tyrosine conversion to melanin (30 °C, 16 h). [c]Mean ± SEM.

Table 2. R_f-values of narrow dopa-melanin electrophoretic band in sera of patients with lung carcinoma

Band	R_f-values ($\times 10^{-2}$)
1	2.53 ± 0.12[a]
2	3.46 ± 0.15
3	6.00 ± 0.17
4	10.23 ± 0.19
5	11.32 ± 0.11
6	12.99 ± 0.13
7	14.70 ± 0.23

[a]Mean ± SEM.

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Table 3. Serum inhibitory factors[a] of tyrosinase activity in lung carcinoma

Category	Tyrosinase activity (% control)[b] I_{300}	I_{100}	II	III
Normal[c]	10.93 ± 0.12[d]	8.95 ± 0.11	24.83 ± 0.34	83.01 ± 1.29
Lung carcinoma	6.99 ± 0.06	10.97 ± 0.11	61.70 ± 2.87	98.10 ± 1.83

[a]Mol.wt of fractions: I_{300}, greater than 300,000; I_{100}, 100,000–300,000; II, 50,000–100,000; III, 30,000–50,000. Each fraction added was equal to the amount obtained from 0.2 ml serum or plasma. [b]Mushroom tyrosinase (20 µg) was incubated with L-[U-^{14}C]tyrosinase with and without serum fractions at 30 °C for 1 h. L-tyrosine conversion for control (no serum factor) was calculated as 100%. [c]Blood bank plasma. [d]Mean ± SEM.

the serum tyrosinase activity in lung carcinoma was approximately one-third of that in melanoma and breast carcinoma[3].

The melanin bands obtained after electrophoresis and incubation with L-dopa were variable in number but as many 7 or more frequently were present. In contrast to melanoma and breast carcinoma sera[3], the intensities of all dopa-melanin bands formed were decreased. Also, their R_f-values (table 2) differed from those derived from sera of patients with melanoma. In melanoma[4], the dopa-melanin bands varied from 0.0013 (band 1) to 0.080 (band 4) and then from 0.138 (band 5) to 0.188 (band 7). It is possible that all the serum tyrosinase carriers are not all the same in lung carcinoma and melanoma. The characteristic melanin bands in different diseases may identify the specific serum immunoglobulins carrying tyrosinase in each disease.

In addition to the narrow melanin bands, 1 wide (1 cm) melanin band (R_f: 0.254 ± 0.004) occurred in some patients of the study. This R_f-value was greater than those of the narrow melanin bands. The function represented by this wide band is not clear at present.

Coincident with the demonstration of the above electrophoretic patterns, inhibitory sites of dopa oxidation in lung carcinoma were observed as 3 colorless bands (R_f: 0.1740 ± 0.0049; 0.3084 ± 0.0047; 0.5649 ± 0.0046), width 2–3 mm. These may represent tyrosinase inhibitors in the enzyme preparation and differ in mobilities from enzyme-carrier complexes. When eluted these inhibitors depress both enzymic and autoxidative melanin formation from dopa[4]. The colorless bands, therefore, result from inhibition of L-dopa autoxidation on the gels.

4 fractions inhibiting tyrosinase activity have been separated by ultrafiltration of the 60% saturation (ammonium sulfate) supernatant. The inhibitory potencies of these fractions from both lung carcinoma patients and normal individuals on the activity of mushroom tyrosinase differ (table 3). The normal material was blood bank plasma. The inhibitory potencies in fractions I_{300} and I_{100} showed small differences between the normal individuals and patients with lung carcinoma. However, the inhibitory potencies in fractions II and III were much lower in patients (fraction II, 38%; fraction III, negligible) than in normal individuals (fraction II, 78%; fraction III, 17%). Further, tyrosinase activating fractions IV and V observed in melanoma[4] were absent in both normal and lung carcinoma preparations.

In vitro effects of melanocytolytic agents and other compounds upon dominant human melanoma tyrosinase activity[1]

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Summary. The activity of dominant human melanoma tyrosinase isozyme was greatly decreased by certain reducing agents. The number and geometry of phenolic groups as well as free –SH group appear important for enzymic inhibition.

The in vitro effects of a number of melanocytolytic agents and related compounds upon tyrosinase activity in human melanoma homogenates have been reported[3]. To more precisely define the effects of these agents, it is necessary to test their effects upon purified human melanoma tyrosinase. Three isozymes of human melanoma tyrosinase have been reported[4–11]. As the dominant human melanoma tyrosinase may be prepared with large yield[6,9,10], this isozyme was used in the present study.

Materials and methods. Highly melanized human melanomas obtained at surgery were processed to obtain a lipase solubilized fraction derived from the particulate fraction as described previously[6,9,10]. The dominant human melanoma tyrosinase was isolated from this fraction by 50–70% ammonium sulfate fractionation and purified further by sephadex G-100 chromatography[12]. Tyrosinase was assayed radiometrically[13,14] using L-

In vitro effects of melanocytolytic agents on the activity of dominant human melanoma tyrosinase

Melanocytolytic agent	Tyrosinase activity (percent control)		
	9×10^{-5} M	9×10^{-4} M	9×10^{-3} M
Hydroquinone	51.2	13.5	11.1
Catechol	92.7	85.8	18.2
Resorcinol	81.6	22.8	1.7
MEA HCl[a]	34.4	15.5	0.5
Cystamine HCl	90.5	70.8	50.9
L-Cysteine HCl	81.7	58.0	1.0
Glutathione	17.9	13.3	1.2
Ascorbic acid	88.5	2.0	0.5
L-Cystine	90.0	89.6	87.4
DL-Methionine[b]	94.7	90.5	67.9
Pyrogallol	102.3	5.9	1.0
Phloroglucinol	98.9	35.4	3.7
DDC ($3H_2O$)[c]	3.5	0	0
EDTA[d]	80.4	73.1	51.2

[a]MEA, β-mercaptoethylamine. [b]Concentration represented L-form only. [c]DDC, diethyldithiocarbamate. [d]EDTA, ethylenediaminetetraacetic acid.

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