In vitro and *in vivo* Activity on Catalase of Electrophoretically Pure Human Serum Albumin

It has been demonstrated[1] that protein fractions, isolated from tumoral and non-tumoral ascitic fluids and consisting for the most part of albumin, decrease the liver catalase activity in mice. Therefore we tested whether normal human serum albumin, electrophoretically pure, would show a corresponding inhibiting action.

Human albumin Behringwerke (Marburg-Lahn) 'reinst' Op. Nr. 1186 II was used. The material was dissolved in physiological saline and 0.3 ml were injected subcutaneously, at concentrations varying from 5 to 100 mg/ml. Male albino mice of the inbred strain IVB (originally from Institut Curie, Paris) weighing 20–24 g, were used. Ten mice were injected for each dose and at the same time five control animals of the same strain, sex and weight were sacrificed. The % inhibition was calculated on the basis of the values found for the controls.

The catalase activity was determined, as previously described[2], 24 h after the injection by the method of VON EULER and JOSEPHSON[3], using a liver homogenate with a concentration of 1 mg of fresh liver/ml. The *in vitro* activity on crystalline catalase was also tested. Beef crystalline catalase possessing a Kf of 30000, prepared according to a method already described[4], was dissolved as a concentrated solution in 1/15 *M* phosphate buffer at pH 7.4, and then diluted to a Ko of about 1000×10^{-4} with phosphate buffer 1/150 *M* at pH 6.8.

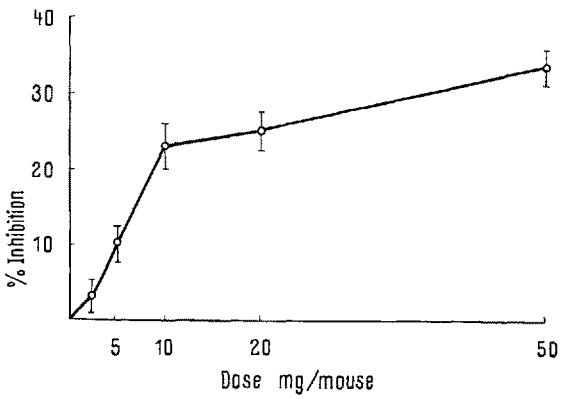

Fig. 1. *In vivo* inhibition of liver catalase by human albumin. Normal, undenatured, electrophoretically pure human albumin was injected at various dose levels to groups of 10 mice and the liver catalase activity determined 24 h after treatment. Brackets indicate the standard error.

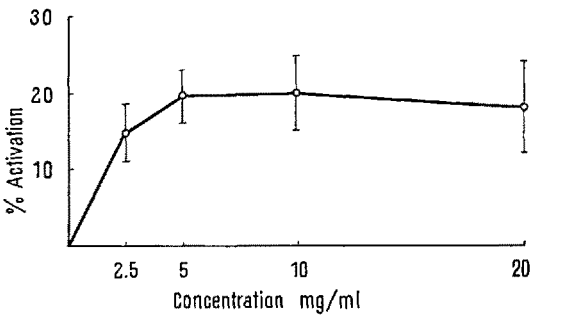

Fig. 2. *In vitro* enhancement of catalase activity by human albumin. Brackets indicate the standard error.

For the test, to 1 ml of catalase, 1 ml of albumin solution at concentrations varying from 2.5 to 40 mg/ml was added and the mixture incubated at + 2°C in a refrigerator for 1 h. After this time, the catalase activity was determined according to the method of VON EULER and JOSEPHSON[3]. Owing to the instability of catalase in dilute solutions, several controls stored under the same conditions were interposed between the single tests. No relevant variations were observed among the different controls at various times.

As shown in Figure 1, there is a decrease of liver catalase activity *in vivo*, which is proportional to the amount of albumin injected. The inhibition is already present; although not significant at 5 mg dosis, it is highly significant with 10 mg of albumin. The effect is comparable to that of potent 'toxohormone' preparations.

In vitro (Fig. 2) no inhibition of crystalline catalase was observed; indeed, on the contrary, a moderate but constant activation of the enzyme activity was always noticed. The significance of this behaviour, which was shown also by some 'toxohormone' preparations, is obscure at present. The most important fact is that a pure human albumin preparation shows towards liver catalase a behaviour indistinguishable from that of a tumor extract like 'toxohormone'.

Riassunto. Albumine umane purissime dimostrano *in vivo* sulla catalasi epatica dei topi, una inibizione molto significativa fino alla dose di 10 mg/topo; *in vitro*, sulla catalasi cristallizzata, determinano un certo aumento dell'attività di difficile interpretazione.

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Insensitivity of Erythrocyte Catalase to Substances that Depress Liver Catalase

Tumors are known to depress liver catalase activity without affecting erythrocyte catalase[1,2]. According to THEORELL et al.[3], this differential action is due to the different site of synthesis for the two catalases, namely the liver and the bone marrow. The tumor extract 'toxohormone' has been said to behave in a similar manner, and therefore it has been thought to represent or to contain the active principle of tumors[4,5]. As other substances are able to depress liver catalase *in vivo*, with or without a corresponding activity *in vitro*, we decided to investigate the action of some of these substances also on erythrocyte catalase.

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Comparison between liver and blood catalase inhibition, in tumor-bearing and in tyrosine and denatured albumin injected mice, 24 h after treatment

Number of mice	Treatment	% inhibition of hepatic catalase activity	Optical density of hemolysates at 575 mμ E	Catalase activity of hemolysates Ko 0.10^4	Ko/E
20	none	—	0.487 ± 0.024	507 ± 27	0.104
10	S. 180 of 10 days	30	0.465 ± 0.011	475 ± 47	0.103
16	S. 180 of 17 days	49	0.512 ± 0.004	550 ± 42	0.107
10	Tyrosine 5 mg	32	0.520 ± 0.015	517 ± 63	0.099
15	Tyrosine 10 mg	40	0.502 ± 0.006	555 ± 57	0.111
10	Denatured albumin 25 mg	37	0.570 ± 0.010	615 ± 31	0.120

The substances tested were: tyrosine which is active both *in vitro*[6] and *in vivo*[7] and a denatured albumin fraction from human neoplastic ascitic fluid, which is active only *in vivo*[8]. The latter material, before denaturation, is active also *in vitro*, but it loses this property when denatured by treatment with acetic acid at pH 4 for 1 h and is subsequently precipitated with acetone.

Tyrosine was injected subcutaneously in 10 mg doses, in 1 ml, as a fine suspension, obtained by neutralizing its acid solution.

The denatured albumin was injected subcutaneously in doses of 25 mg, suspended in 1 ml of physiological saline.

Male albino mice of the strain IVB (Institut Curie, Paris) of 18–22 g of weight were used. They were killed 24 h after the injection by decapitation, and the blood collected in citrated physiological saline. The erythrocytes were washed twice with the same solution and twice with plain saline. They were then lysed with 4 ml of distilled water, by repeated freezing and thawing. After centrifugation, the solutions were diluted to an optical density of approximately 0.500, when read on a Beckman DU spectrophotometer at 575 mμ. 1 ml of this solution was poured into 50 ml of 0.01 N H_2O_2 and catalase activity determined by the method of VON EULER and JOSEPHSON[9]. The catalase activity was referred to the hemoglobin content, by the ratio Ko to the optical density at 575 mμ. The activity of liver catalase was also determined using 1 ml of a homogenate diluted with phosphate buffer 1/150 M, pH 6.8, to have 1 mg of fresh liver/ml.

As control, normal healthy mice, and mice bearing Sarcoma 180, 10 and 17 days after implantation, were used.

As appears from the Table, the erythrocyte catalase is not affected either by tyrosine or by denatured albumin; on the contrary, there is a marked decrease of liver catalase activity for both treatments. The picture is therefore superimposable to that given by tumors.

Apparently, 'toxohormone' is not the only material acting like tumors, and it is not sufficient to observe a depression of liver catalase, without a corresponding decrease of erythrocyte catalase, to correlate the activity found with that of living tumors.

Riassunto. La tirosina che inibisce *in vivo* l'attività della catalasi epatica dei topi ed *in vitro* l'attività della catalasi cristallizzata, ed una frazione di albumine denaturate attiva sulla catalasi solo *in vivo*, si sono dimostrate inattive sulla catalasi eritrocitaria.

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Étude électrophorétique concernant les protéines et les acides nucléiques tissulaires a propos de l'inhibition de la cancérogenèse

L'inhibition de la cancérogenèse par administration d'implants cancéreux passés 24 h sous la peau du lapin, a donné des résultats très encourageants soit pour le cancer mammaire des souris R III, soit pour les souris AkR leucémiques[1], enfin ces mêmes techniques furent appliquées au cancer chez l'homme.

Nous avons analysé avant et après passage sur lapin les protéines et les acides nucléiques de divers tissus de souris et humains, normaux et cancéreux, pour mieux connaître nos implants néoplastiques. L'importance des acides nucléiques dans la cancérogenèse est généralement admise; notamment LATARJET et al.[2] sont parvenus à produire des cancers multiples chez la souris par administration d'acides nucléiques extraits de tissus leucémiques.

Matériel et méthodes. Les analyses électrophorétiques[3] et immunoélectrophorétiques[4] ont été effectuées sur lames de verre gélosées en milieu tampon véronal (pH = 8,6; $\mu = 0{,}06$; 6 volts/cm). La durée a été de 2 h pour les protéines (1 h pour l'immunoélectrophorèse) et de 30 min pour les acides nucléiques.

Les analyses électrophorétiques des protéines ont été effectuées soit directement sur le tissu (0,5 à 2 mg), soit après extraction à + 4° (5 à 40 mg) par un tampon phosphate 0,02 M, pH = 7,3 (coloration: Amidosschwarz).

Les acides nucléiques[5] examinés également après électrophorèse, ont été extraits selon la méthode de SCHUSTER et al.[6] sur 5 à 20 mg de tissu frais (coloration: vert de methyl-pyronine). Leur concentration a été appréciée d'après une échelle étalon effectuée avec un acide nucléique

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