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.

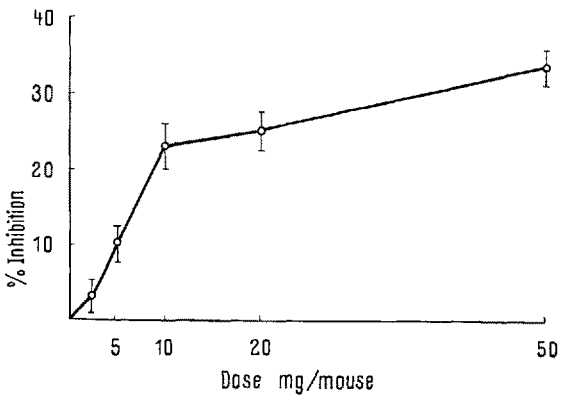

Fig. 1. *In vivo* inhibition of liver catalase by human albumin. Normal, undenatured, electrophoretically pure human albumin was injected at various dose levels to groups of 10 mice and the liver catalase activity determined 24 h after treatment. Brackets indicate the standard error.

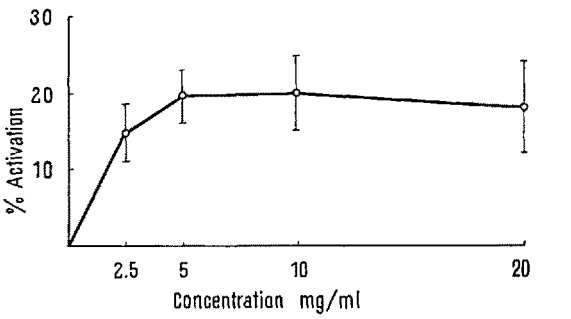

Fig. 2. *In vitro* enhancement of catalase activity by human albumin. Brackets indicate the standard error.

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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