# Superoxide dismutase (*EC* 1.15.1.1), zinc status and ethanol consumption in maternal and foetal rat livers

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1. The activity of superoxide dismutase (*EC* 1.15.1.1) was not greatly affected by zinc deficiency in maternal or foetal (20 d) rat livers, although in the latter tissue it did appear to be slightly raised by Zn depletion when compared with pair-fed control animals.

2. Enzyme activity was significantly higher in livers from all foetuses after administration of aqueous ethanol at 100 or 200 ml/l to the dams during pregnancy.

3. Plasma Zn levels were significantly increased in Zn-deficient dams after ingestion of alcohol during pregnancy.

Mammalian cytosolic superoxide dismutase (*EC* 1.15.1.1) consists of two identical subunits which each contain one copper and one zinc atom per monomer (Fridovich, 1975). Evidence suggests that the enzyme plays an important role in protecting biomembranes against oxidative damage by naturally-occurring superoxide radicals (Fridovich, 1975).

Recently, the activity of superoxide dismutase has been reported to be lowered by approximately 20% in regenerating livers from Zn-deficient rats (Dreosti & Record, 1978) and, although it was questioned whether a decrease of this magnitude was of physiological significance, the possibility exists that a similar or greater fall may occur in the developing foetus where the biochemical consequences of a dietary Zn deficiency are more serious (Hurley, 1977).

Accordingly, in the present study an investigation was made into the effect of Zn restriction on the activity of superoxide dismutase in foetal and maternal rat livers. In addition some dams received aqueous ethanol at 100 and 200 ml/l during gestation in order to test whether the reported lowering of plasma and liver Zn levels by alcohol (Wang & Pierson, 1975) might amplify any effect of Zn restriction on the activity of superoxide dismutase.

## METHODS

Six groups of four to eight female rats of the Hooded Wistar strain weighing approximately 220 g were mated and housed individually in stainless-steel and plastic cages throughout pregnancy. The appearance of sperm in the daily vaginal smears was taken to represent day 0 of gestation. Animals were fed either a Zn-deficient or Zn-supplemented diet from conception.

The Zn-deficient diet (Wilkins *et al.* 1972) was prepared from EDTA-extracted soya-bean flour (Davis *et al.* 1962) and contained on assay less than 0·5 mg Zn/kg diet. Zn-supplemented rats received the same diet containing an additional 100 mg Zn as zinc sulphate/kg. Vitamins were supplied separately in sucrose to all animals three times per week (Hurley & Swenerton, 1966).

Some animals received in addition aqueous ethanol at 100 or 200 ml/l as their only drinking-fluid during the course of the experiment. Pair-fed rats were apportioned daily the same amount of Zn-supplemented diet as had been consumed by their Zn-deficient

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counterparts during the previous 24 h. Food intake of the Zn-deficient animals fell from approximately the seventh day of treatment to a level approximately half of that consumed by the *ad lib.* Zn-supplemented group. All animals receiving alcohol at 200 ml/l exhibited an initial reduction (30–40%) in food intake which recovered to approximately 90% of their original consumption within 5 d.

Foetuses were delivered by Caesarean section on day 20 of pregnancy and the livers from each litter were pooled for subsequent analyses.

Superoxide dismutase was assayed by the photochemical augmentation method described by Misra & Fridovich (1977), which is based on the increased photo-oxidation of dianisidine by riboflavin in the presence of the enzyme.

Protein was assayed by the method of Lowry *et al.* (1951). Zn analyses were performed on plasma samples taken from dams at the time of killing using an atomic absorption spectrophotometer (Varian 1200; Varian Techtron Pty Ltd, Melbourne). All results were evaluated using the analysis of variance procedure described by Nie *et al.* (1975).

## RESULTS AND DISCUSSION

The activity of superoxide dismutase was not greatly affected by Zn deficiency in maternal or foetal rat livers, although in the latter tissue it did appear to be raised by Zn depletion when compared with pair-fed control animals (Table 1). The findings are contrary to expectation as they indicate a somewhat increased activity of a Zn-metalloenzyme during a state of Zn deficiency. Possibly they arise as a consequence of other metabolic changes induced by the deficiency in the foetuses which in turn lead to higher levels of cytosolic superoxide radicals. By contrast Cu deficiency has been shown to lead to a severe reduction (85%) in the activity of superoxide dismutase in swine erythrocytes (Williams *et al.* 1975). Possibly the reason for this difference lies in the fact that Cu is the metal catalytically active in the enzyme while the role of Zn is probably more that of a stabilizing ion. In vitro evidence in fact suggests that active Cu-Zn superoxide dismutase can be obtained in the absence of Zn or when Zn is replaced by other divalent cations (Fridovich, 1975).

The activity of the enzyme in the maternal livers was considerably higher than in foetal tissues which confirms a similar observation by Utsumi *et al.* (1977). Plasma Zn levels were reduced by approximately 67% in the Zn-deficient dams and foetuses from these animals were severely stunted.

Ethanol administered concurrently to the Zn-deficient dams and to animals receiving 100 mg Zn/kg in their diet significantly enhanced the activity of the enzyme in foetal livers. The increase was mirrored, but to a lesser extent, in adult tissues. The effect possibly reflects increased enzyme synthesis in response to raised levels of superoxide radicals which may arise from the activity of the microsomal ethanol-oxidizing system described by Cederbaum *et al.* (1977). It would be anticipated that this system would be more active in animals with low levels of alcohol dehydrogenase (*EC* 1.1.1.1) (Bode, 1978), and it is known this enzyme occurs at reduced levels in foetal livers (Greengard, 1977). The influence of ethanol on superoxide dismutase is a new observation and one which indirectly lends support to the proposed role of the NADPH-dependent microsomal oxidizing system as an alternate pathway for ethanol metabolism (Cederbaum *et al.* 1977), especially in foetal livers.

Plasma Zn levels rose by approximately 60–70% in the Zn-deficient group receiving ethanol which is contrary to the findings of Wang & Pierson (1975) and possibly represents the initial redistribution of Zn in the body after the ingestion of ethanol reported by Dreosti (1978). In addition, foetal growth was slightly improved (17%) in the 20%

Table 1. *Superoxide dismutase* (EC 1.15.1.1; *SOD*) *in maternal and foetal rat livers of differing zinc and ethanol status**

(Mean values with their standard errors)

| Dietary regimen | Maternal plasma Zn (μg/ml) | | Foetal weight (g) | | Activity of SOD (units† SOD activity/mg protein) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Foetal liver | | Maternal liver | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Zn-deficient | 0·49 | 0·05 | 1·97 | 0·16 | 1·91 | $0·05^{a,b,e}$ | 11·1 | 0·23 |
| Zn-supplemented (100 mg/kg): Pair-fed | 1·27 | 0·25 | 2·50 | 0·31 | 1·21 | $0·09^{a,f,h,i}$ | 11·5 | 2·43 |
| Zn-supplemented (100 mg/kg): *ad lib.*-fed | 1·25 | 0·86 | 3·13 | 0·03 | 1·43 | $0·29^{c,g,j}$ | 10·0 | 0·36 |
| Zn-deficient + 100 ml ethanol/l | 0·76 | 0·11 | 1·76 | 0·22 | 2·93 | $0·17^{d,e,h,j}$ | 12·8 | 2·53 |
| Zn-deficient + 200 ml ethanol/l | 0·87 | 0·18 | 2·30 | 0·35 | 2·20 | $0·36^{c,d,f}$ | 11·7 | 2·70 |
| Zn-supplemented (100 mg/kg) + 200 ml ethanol/l: *ad lib.*-fed | 1·26 | 0·27 | 2·47 | 0·13 | 2·76 | $0·53^{b,g,i}$ | — | — |

a–j, Mean values with the same superscript letter were significantly different (a–d, $P < 0·05$; e–g, $P < 0·005$; h–j, $P < 0·001$).

* For details of diet and experimental procedures, see p. 399.

† One unit of SOD activity is defined as the amount of enzyme required to inhibit the rate of reduction of cytochrome *c* by 50% under certain defined conditions (McCord & Fridovich, 1969).

ethanol-treated Zn-deficient animals, possibly due to the increased availability of Zn to the foetuses in this group, although the response must certainly have been attenuated by the growth-depressing effect of ethanol *per se* on the foetus (Henderson & Schenker, 1977).

The results presented in this paper together with earlier results obtained on regenerating rat livers (Dreosti & Record, 1978) strongly suggest that Zn deficiency has little or no significant effect on the activity of superoxide dismutase in adult and foetal rat livers. In addition, Bettger *et al.* (1978) have reported no effect of Zn status on superoxide dismutase in rat erythrocytes. It seems unlikely, therefore, that diminished activity of superoxide dismutase is responsible for the severe developmental defects associated with Zn deficiency in rats.

Consumption of ethanol together with the Zn-deficient diet did not exacerbate the nutritional Zn deficiency as expected. On the contrary the results tend to support the view that in the initial stages, ingestion of ethanol may lead to some release of Zn from otherwise inert deposits of the element within the animal body (Dreosti, 1978). Ethanol did, however, lead to increased levels of superoxide dismutase in the livers of all animals, especially in the foetuses, which suggests that the microsomal ethanol oxidizing system may be of particular importance for the metabolism of ethanol by foetal liver.

## REFERENCES

Bettger, W. J., Fish, T. J. & O'Dell, B. L. (1978). *Proc. Soc. exp. Biol. Med.* **158**, 279.
Bode, J. Ch. (1978). *J. R. Coll. Phyn.* **12**, 122.
Cederbaum, A. I., Dicker, E., Rubin, E. & Cohen, G. (1977). *Biochem. Biophys. Res. Comm.* **78**, 1254.
Davis, P. N., Norris, L. C. & Kratzer, F. H. (1962). *J. Nutr.* **78**, 445.
Dreosti, I. E. (1978). *Proc. Nutr. Soc. Aust.* **3**, 25.
Dreosti, I. E. & Record, I. R. (1978). *Br. J. Nutr.* **40**, 133.
Fridovich, I. (1975). *A. Rev. Biochem.* **44**, 147.
Greengard, O. (1977). *Pediat. Res.* **11**, 669.
Henderson, G. I. & Schenker, S. (1977). *Res. Comm. chim. Path. Pharmac.* **16**, 15.
Hurley, L. S. (1977). *Progress in Clinical and Biological Research.* vol. 14, p. 47 [G. J. Brewer and A. D. Prasad, editors]. New York: Alan R. Liss Inc.
Hurley, L. S. & Swenerton, H. (1966). *Proc. Soc. exp. Biol. Med.* **123**, 692.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
McCord, J. M. & Fridovich, I. (1969). *J. biol. Chem.* **244**, 6056.
Misra, H. P. & Fridovich, I. (1977). *Archs Biochem. Biophys.* **181**, 308.
Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K. & Bent, D. H. (1975). *Statistical Package for Social Sciences*, 2nd ed. New York: McGraw-Hill.
Utsumi, K., Yoshioka, T., Yamonaka, N. & Nakazawa, T. (1977). *FEBS Lett.* **79**, 1.
Wang, J. & Pierson, R. N. (1975). *J. Lab. clin. Med.* **85**, 50.
Wilkins, P. J., Grey, P. C. & Dreosti, I. E. (1972). *Br. J. Nutr.* **27**, 113.
Williams, D. M., Lynch, R. E., Lee, G. R. & Cartwright, G. E. (1975). *Proc. Soc. exp. Biol. Med.* **149**, 534.

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