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## THE RELATIONSHIP OF ERYTHROCYTE GLUTATHIONE PEROXIDASE TO BLOOD SELENIUM IN SWINE \*

By

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SIVERTSEN, TORE, JAN T. KARLSEN and ARNE FRØSLIE: *The relationship of erythrocyte glutathione peroxidase to blood selenium in swine.* Acta vet. scand. 1977, 18, 494—500. — The erythrocyte glutathione peroxidase activity and blood selenium have been investigated in swine fed a Se deficient diet with, and without, selenium supplementation. A highly significant correlation ( $r = 0.90$ ) between erythrocyte glutathione peroxidase and blood selenium was found.

glutathione peroxidase; selenium; swine blood.

Since the discovery that glutathione peroxidase (Glutathione  $H_2O_2$  oxido-reductase EC 1.11.1.9 — GSH-Px) is a seleno-enzyme (Rotruck *et al.* 1973, Flohe *et al.* 1973), much literature has been published on the GSH-Px—selenium relationship in organs of various species. Thus, a linear correlation between erythrocyte or whole blood GSH-Px activity and blood selenium has been demonstrated in the blood of sheep and cattle (Allen *et al.* 1975, Oh *et al.* 1976b, Thompson *et al.* 1976, Wilson & Judson 1976). In swine blood, however, Thompson *et al.* found no such correlation.

The aim of the present investigation was to study the GSH-Px—selenium relationship in the blood of swine fed a Se deficient diet with, and without, selenium supplementation.

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## MATERIALS AND METHODS

*Experimental animals*

The data are derived from 3 experiments on vit. E-selenium deficiency in pigs. All pigs were fed at vit. E-selenium deficient diet from the age of 6 weeks. This diet, based mainly on Se poor barley and Toprina®\* single-cell protein, is described in detail by *Teige jr. et al.* (1977).

Expt. 1 included 24 animals, in 4 groups. Groups 2 and 4 were given a supplement of 200 mg  $\alpha$ -tocopherol and 0.2 mg selenium\*\* per pig daily, while Groups 1 and 3 were unsupplemented. In addition, the diets of Groups 1 and 2 included 3 % tocopherol-stripped\*\*\* cod liver oil. The pigs were bled after 10 weeks of feeding.

Expt. 2 included 6 animals, fed the basic diet with an addition of 5 % tocopherol-stripped cod liver oil. Two of the animals received weekly injections of vit. E and selenium\*\*\*\* equivalent to approx. 0.15 mg Se and 50 mg  $\alpha$ -tocopherol per kg feed, while the rest were not treated. The pigs were bled every 2 weeks for a period of 2 months.

Expt. 3 included 24 animals, arranged in 4 groups. They were fed the basic diet with no cod liver oil, and given the following supplements: Group 1: None. Group 2: 200 mg  $\alpha$ -tocopherol per pig per day. Group 3: 0.2 mg selenium per pig per day. Group 4: 200 mg  $\alpha$ -tocopherol and 0.2 mg selenium per pig per day. The animals were bled 3 times, at 2-weekly intervals.

*Analytical procedures*

Erythrocyte glutathione peroxidase was determined according to *Beutler* (1975). A Beckman DB-G spectrophotometer with a temperature controlled sample compartment set at 37°C was used. The activity was calculated from the slope of the reaction curve during the first 30 sec. of the reaction, and expressed in i.u. per g Hb according to *Beutler*.

Blood selenium was determined by a fluorometric method

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\* Produced by British Petroleum.

\*\* As sodium hydroselenite.

\*\*\* Subjected to a silica earth absorption treatment, which reduces the tocopherol content (*Teige jr. et al.* 1977).

\*\*\*\* E-vimin® (Ferrosan) and Tokosel® (Agrivet).

(Ihnat 1974). The samples (1—2 ml) were digested by a wet-digestion procedure described by Beal (1975).

Serum  $\alpha$ -tocopherol was determined by a spectrophotometric method (Hashim & Schuttringer 1966)\*.

### RESULTS AND DISCUSSION

A highly significant correlation between erythrocyte glutathione peroxidase and blood selenium in swine blood was found (Fig 1). As seen in Fig. 2, the 2 variables almost parallel each other from week to week in the individual animals.

Separate vit. E supplementation had no effect on erythrocyte GSH-Px activity. On the other hand, the results indicate an elevating effect of selenium/GSH-Px on vit. E in plasma in the groups not supplemented with vit. E (Table 1).

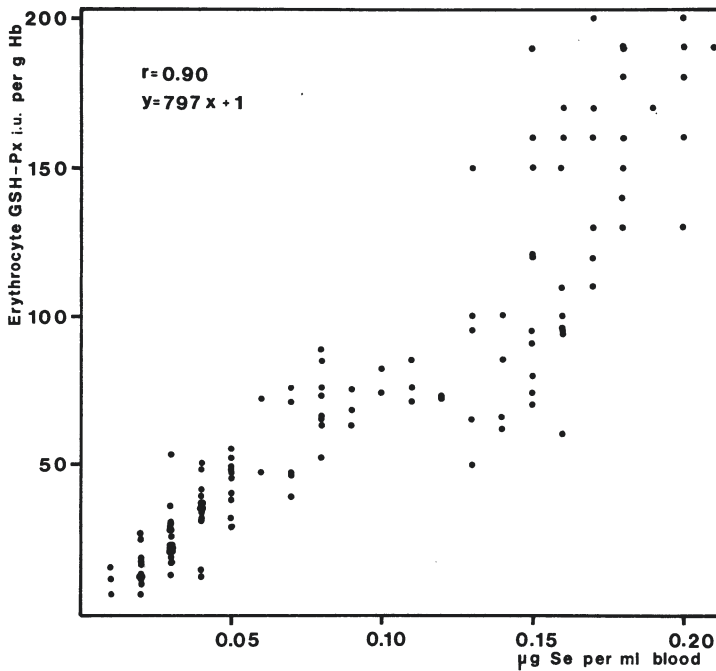


Figure 1. Relationship of erythrocyte GSH-Px activity to blood selenium in swine. The figure shows results of 127 blood samples from 3 experiments on Se-vit. E deficiency, comprising 54 animals.

\* Analyses performed by J. U. Skåre, Veterinary College of Norway, Oslo.

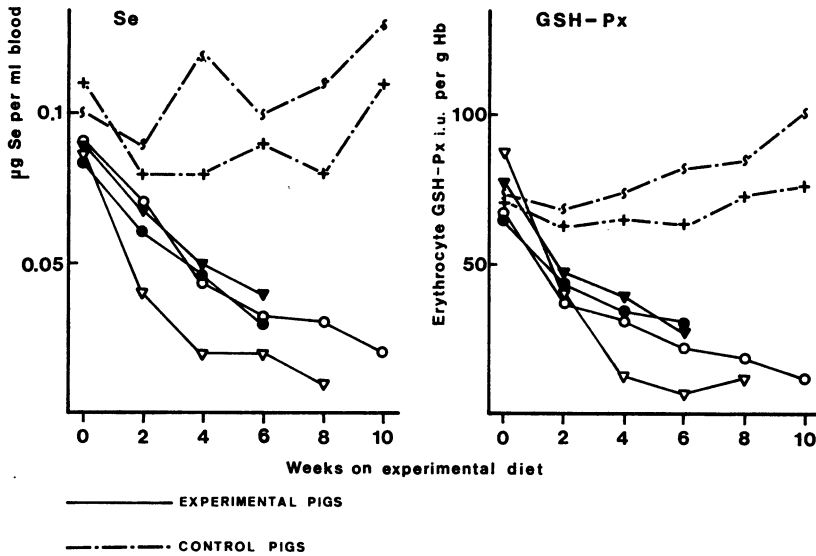


Figure 2. Levels of blood selenium (left) and erythrocyte GSH-Px activity (right) in 4 pigs fed a Se-vit. E deficient diet with 5% tocopherol-stripped cod liver oil (Expt. 2). The 2 control pigs were given weekly injections of vit. E and selenium. Three of the experimental pigs died during the experiment, after 7, 8 and 9 weeks. The post-mortem findings were typical of Se-vit. E deficiency.

Table 1. Erythrocyte GSH-Px, blood selenium and plasma  $\alpha$ -tocopherol in growing swine after 6 weeks on a Se-vit. E. deficient diet with, and without, Se/vit. E supplementation (Expt. 3). Group mean  $\pm$  s.

	Group 1 No supplements	Group 2 200 mg $\alpha$ -tocopherol per pig daily	Group 3 0.2 mg Se per pig daily	Group 4 200 mg $\alpha$ -toco- pherol + 0.2 mg Se per pig daily
Erythrocyte GSH-Px, i.u. per g Hb	28 $\pm$ 7	29 $\pm$ 8	150 $\pm$ 40	120 $\pm$ 40
Blood Se, $\mu$ g per ml	0.04 $\pm$ 0.01	0.03 $\pm$ 0.01	0.19 $\pm$ 0.02	0.16 $\pm$ 0.02
Plasma $\alpha$ -tocopherol*, $\mu$ g per ml	0.7 $\pm$ 0.4	2.8 $\pm$ 0.3	1.2 $\pm$ 0.2	3.1 $\pm$ 1.0

\* These values will also be published elsewhere (Teige jr. et al. in press).

The inclusion of 3 % cod liver oil in the diet (Expt. 1) did not influence GSH-Px activity.

The erythrocyte GSH-Px activity has previously been shown to be a useful indicator of the Se balance in rats (*Hafeman et al.* 1974, *Reddy & Tappel* 1974, *Smith et al.* 1974), chickens (*Omaye & Tappel* 1974) and sheep (*Oh et al.* 1976 a, b). As mentioned, several authors have reported a linear correlation between erythrocyte or whole blood GSH-Px and blood Se in sheep and cattle (*Allen et al.* 1975, *Oh et al.* 1976 b, *Thompson et al.* 1976, *Wilson & Judson* 1976). This indicates that GSH-Px activity can be used as a direct parameter for blood Se status. Based on these results, a simplified GSH-Px method has been developed for field tests for selenium deficiency in sheep (*Board & Peter* 1976).

Our results indicate that erythrocyte GSH-Px activity may be used as a direct parameter of blood Se status also in swine. This is in contrast to the findings of *Thompson et al.*

Recently, *Bengtsson et al.* (1976) reported a close correlation between serum GSH-Px activity and blood selenium in swine.

Selenium levels in the present material range from normal to extreme deficiency. This also applies to the other reports mentioned.

In a study in man, no correlation was found between GSH-Px and total Se in erythrocytes (*Schmidt & Heller* 1976).

The finding that dietary vit. E has no influence on pig erythrocyte GSH-Px activity corresponds with the results of *Fukuzawa & Tokumura* (1976) in the mouse, but not with those of *Scott et al.* (1976) in the rat.

The lack of influence of unsaturated lipids in the diet on erythrocyte GSH-Px activity does not correspond with the results of *Reddy & Tappel* in the rat. However, their experimental diet included as much as 15 % tocopherol-stripped corn oil.

#### REFERENCES

- Allen, W. M., W. H. Parr, P. H. Anderson, S. Berrett, R. Bradley & D. S. P. Patterson*: Selenium and the activity of glutathione peroxidase in bovine erythrocytes. *Vet. Rec.* 1975, 96, 360—361.
- Beal, A. R.*: Selenium determination on fish tissue. *J. Fish. Res. Board Can.* 1975, 32, 249—252.

- Bengtsson, G., J. Hakkarainen, L. Jönsson, P. Lindberg & N. Lannek:* The requirement for selenium and vitamin E in weaned pigs. Int. Pig Vet. Soc., 4th Int. Congr., Ames 1976.
- Beutler, E.:* Red Cell Metabolism. A Manual of Biochemical Methods. 2nd Ed. Grune & Stratton, New York 1975, 160 pp.
- Board, P. G. & D. W. Peter:* A simple test for glutathione peroxidase and selenium deficiency. Vet. Rec. 1976, 99, 144—145.
- Flohe, L., W. A. Günzler & H. H. Schock:* Glutathione peroxidase: A selenoenzyme. FEBS Lett. 1973, 32, 132—134.
- Fukuzawa, K. & A. Tokumura:* Glutathione peroxidase activity in tissues of vit. E-deficient mice. J. Nutr. Sci. Vitaminol. 1976, 22, 405—407.
- Hafeman, D. G., R. A. Sunde & W. G. Hoekstra:* Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr. 1974, 104, 580—587.
- Hashim, S. A. & G. R. Schuttringer:* Rapid determination of tocopherol in macro- and microquantities of plasma. Amer. J. clin. Nutr. 1966, 19, 137—145.
- Ihnat, M.:* Fluorometric determination of selenium in foods. J. Ass. Off. Anal. Chem. 1974, 57, 368—372.
- Oh, S-H., R. A. Sunde, A. L. Pope & W. G. Hoekstra:* Glutathione peroxidase response to selenium intake in lambs fed a torula yeast based, artificial milk. J. Anim. Sci. 1976a, 42, 977—983.
- Oh, S-H., A. L. Pope & W. G. Hoekstra:* Dietary selenium requirement of sheep fed a practical-type diet as assessed by tissue glutathione peroxidase and other criteria. J. Anim. Sci. 1976b, 42, 984—992.
- Omaye, S. T. & A. L. Tappel:* Effect of dietary selenium on glutathione peroxidase in the chick. J. Nutr. 1974, 104, 747—753.
- Reddy, K. & A. L. Tappel:* Effect of dietary selenium and autoxidized lipids on the glutathione peroxidase system of gastrointestinal tract and other tissues in the rat. J. Nutr. 1974, 104, 1069—1078.
- Rotruck, J. T., A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman & W. G. Hoekstra:* Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973, 179, 588—590.
- Schmidt, K. & W. Heller:* Selenkonzentration und Aktivität der Glutathion-peroxydase im Lysat menschlicher Erythrozyten. (Selenium concentration and activity of glutathione peroxidase in lysate of human erythrocytes). Blut 1976, 33, 247—251.
- Scott, D. L., J. Kelleher & M. S. Losowsky:* The effect of dietary selenium and vitamin E on glutathione peroxidase and glutathione in the rat. Biochem. Soc. Trans. 1976, 4, 295—296.
- Smith, P. J., A. L. Tappel & C. K. Chow:* Glutathione peroxidase activity as a function of dietary selenomethionine. Nature (Lond.) 1974, 247, 392—393.

- Teige jr., J., K. Nordstoga & J. Aursjø*: Influence of diet on experimental swine dysentery. 1. Effects of a vitamin E and selenium deficient diet supplemented with 6.8 % cod liver oil. *Acta vet. scand.* 1977, 18, 384—396.
- Teige jr., J., F. Saxegaard & A. Frøslie*: Influence of diet on experimental swine dysentery. 2. Effects of a vitamin E and selenium deficient diet supplemented with 3 % cod liver oil, vitamin E or selenium. In press.
- Thompson, R. H., C. H. McMurray & W. J. Blanchflower*: The levels of selenium and glutathione peroxidase activity in blood of sheep, cows and pigs. *Res. vet. Sci.* 1976, 20, 229—231.
- Wilson, P. S. & G. J. Judson*: Glutathione peroxidase activity in bovine and ovine erythrocytes in relation to blood selenium concentration. *Brit. vet. J.* 1976, 132, 428—434.

## SAMMENDRAG

*Forholdet mellom glutathion peroksydaseaktivitet i røde blodlegemer og selenkonsentrasjon i blod hos svin.*

Aktiviteten av glutathion peroksydase (GSH-Px) i røde blodlegemer og konsentrasjonen av selen i blod er målt hos griser som ble satt på selen/E-vitaminfattig fôr fra 6-ukers alder. Grisene fikk dels tilskudd av selen, dels av E-vitamin, dels både selen og E-vitamin, i tillegg til at noen griser fikk tilskudd av E-vitaminfattig tran.

Det ble funnet en positiv, lineær korrelasjon mellom blodkonsentrasjonen av selen og GSH-Px aktiviteten i blodlegemene ( $r = 0,90$ ;  $y = 797x + 1$ ;  $n = 127$ ). Hverken tran tilskudd eller E-vitamin tilskudd alene hadde noen effekt på aktiviteten av GSH-Px i blodlegemene.

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