

From the Department of Clinical Chemistry, College of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

## EFFECTS OF SELENIUM ADMINISTRATION ON ERYTHROCYTE AND BLOOD PLASMA GLUTATHIONE PEROXIDASE ACTIVITY IN GOATS

By

*Kamal S. M. Hussein and Bernt-E. V. Jones*

HUSSEIN, KAMAL S. M. and BERNT-E. V. JONES: *Effects of selenium administration on erythrocyte and blood plasma glutathione peroxidase activity in goats.* Acta vet. scand. 1982, 23, 559—564. — The activity of glutathione peroxidase (GSH-Px, E.C. 1.11.1.9.) was determined in heparinized whole blood, blood plasma and washed erythrocytes from goats before and up to 4 weeks after the administration of selenium (0.4 mg/10 kg BW) and vitamin E (20 mg/10 kg BW) or only vit. E (20 mg/10 kg BW). It was found that Se administration caused a significant increase in enzyme activity in whole blood and washed erythrocytes first detected 2 weeks after the intramuscular injection of Se. No changes were observed in plasma from the treated animals. Minor and insignificant changes were seen in the vit. E treated control animals. It is concluded that GSH-Px activity in blood plasma or serum is of no value as a short-term indicator of the selenium status of goats but whole blood is a good indicator of the long-term status.

goat; glutathione peroxidase; selenium;  
vitamin E.

One specific biochemical role of selenium has been established, i.e. as a component of the enzyme glutathione peroxidase (GSH-Px, E.C. 1.11.1.9.). This enzyme catalyzes the reduction of  $H_2O_2$  and various hydroperoxides with reduced glutathione (GSH) as a cofactor.

In the blood, GSH-Px is present mainly in the erythrocytes where it acts as an integral part of the antioxidative system protecting hemoglobin from oxidation to methemoglobin by hydroperoxide (*Mill & Randall 1958*).

As there are 4 moles of selenium to 1 mole of enzyme a close correlation between selenium concentration in blood and GSH-Px activity in erythrocytes has been established for cattle (*Wilson & Judson 1976*), sheep, pigs (*Jørgensen et al. 1977*), horses (*Caple et al. 1978*) and humans (*Thompson et al. 1977*). Consequently, the activity of the enzyme, which is more easily determined than elemental selenium, is used as an indicator of the selenium status in animals.

High blood plasma GSH-Px activity was found in pigs by *Jørgensen et al. (1977)*. Later *Hakkarainen et al. (1978)* demonstrated a close correlation between blood selenium and serum GSH-Px in pigs. These authors preferred determination of serum GSH-Px to erythrocyte enzyme activity determination in evaluating selenium status in the pig, as the response to changes was faster in serum.

The present study was undertaken to investigate the possibility to use plasma GSH-Px activity in goats as a short-term indicator of selenium status in this species.

#### MATERIAL AND METHODS

Nine adult female goats (Swedish Land Breed) were used in this experiment. They were kept together in boxes and had free access to feed and water. Blood samples were obtained from the jugular vein into heparinized Vacutainers® (~ 15 I.U./ml, Becton Dickinson, cat. no. 606480).

The GSH-Px activity was determined according to *Paglia & Valentine (1967)* as previously described (*Hussein & Jones 1981*) in whole blood, washed erythrocytes and blood plasma. Cumenehydroperoxide was used as substrate in the analyses.

Six of the animals were given an intramuscular injection of a selenium, vitamin E preparation (Tokosel vet, Agrivet AB, Uppsala, Sweden) at a dose of 0.4 mg Se and 20 mg vit. E per 10 kg body weight (BW). Three animals were injected with the same amount of vit. E (Ido-E Aquosum vet., AB Ferrosan, Malmö, Sweden) as a control group. Blood samples were taken from all animals 5 h after the injection and once every week thereafter for 4 weeks. The blood samples were divided into 2 portions, one portion for the determination of the enzyme activity in whole blood hemolysate, the second portion was centrifuged and the plasma removed for enzyme determination. The sedi-

mented erythrocytes were washed 4 times with phosphate buffer, pH 7.5 (74.8 g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  and 10.88 g  $\text{KH}_2\text{PO}_4$  in 1 l of dist. water) and resuspended in this buffer to retain the original blood volume before determination of the enzyme activity.

The experimental group was compared to the control by the t-test.

## RESULTS

The activity of GSH-Px in whole blood is presented in Table 1. A significant increase in the enzyme activity ( $P < 0.001$ ) was seen in the 2, 3 and 4 week samples after selenium administration compared to the pre-treatment values. The control animals showed a slight and non-significant increase in the enzyme activity during the experimental period. Since the enzyme activity in the washed erythrocytes was almost identical to the whole blood values the erythrocyte values will not be presented here.

Table 1. The activity of GSH-Px in whole blood of goats injected intramuscularly with Se (0.4 mg/10 kg BW) and vit. E (20 mg/10 kg BW) compared during a 4-week period to control animals given only vit. E (20 mg/10 kg BW).

		Pretreatment	5 h	1 w	2 w	3 w	4 w
Exp. Animals n=6	Mean $\mu\text{kat/l}$	444 A,B,C	555	497	545 A	634 B	892 C
	Range	371—531	449—688	434—688	484—658	593—667	686—1231
Control Animals n=3	Mean $\mu\text{kat/l}$	335	452	359	352	468	530
	Range	248—485	409—477	230—466	250—505	373—604	373—702

Figures followed by the same upper case letter are significantly different at  $P < 0.001$ .

Table 2 shows the GSH-Px activity in plasma for the treated and the control animals. The plasma enzyme activity was low throughout the experiment both in experimental and control animals.

Table 2. The activity of GSH-Px in blood plasma from goats injected intramuscularly with Se (0.4 mg/10 kg BW) and vit. E (20 mg/10 kg BW) compared during a 4-week period to control animals given only vit. E (20 mg/10 kg BW).

		Pretreatment	5 h	1 w	2 w	3 w	4 w
Exp. Animals n=6	Mean μkat/l	2.0	2.1	2.5	2.6	2.1	1.6
	Range	1.6—2.6	1.6—2.4	2.0—3.7	2.2—2.8	1.8—2.7	1.2—1.8
Control Animals n=3	Mean μkat/l	2.0	2.0	2.0	2.0	1.9	1.6
	Range	1.9—2.1	1.9—2.1	2.0—2.0	1.9—2.2	1.6—2.1	1.2—1.9

## DISCUSSION

The present results show that GSH-Px activity in goat blood increases from 2 weeks after administration of selenium. A similar delay in the increase of enzyme activity was found in horses (*Caple et al.* 1978), cattle (*Thompson et al.* 1981), and rats (*Hafeman et al.* 1974). Since mature erythrocytes are incapable of protein synthesis the response in GSH-Px activity will be related to the turnover time for the circulating erythrocytes. The administered selenium must be incorporated with the synthesised GSH-Px into the cell during the hematopoiesis. According to *Schalm et al.* (1975) the erythrocyte life span in goats is 125 days. The present results confirm the suggestion by *Hafeman et al.* (1974) that erythrocyte GSH-Px activity is a long-term measure of the selenium intake of an animal.

The low blood plasma enzyme activity shown in this experiment is in agreement with previous studies in ruminants, e.g. by *Richard et al.* (1981) and *Thompson et al.* (1981). *Hakkarainen et al.* (1978) on the other hand found high enzyme activities in blood serum from pigs. These authors used serum instead of plasma as they did not find any difference between these two materials. However, in many cases it is an advantage to use plasma as the risk of hemolysis in serum is greater. The low enzyme activity in plasma from goats will not, as demonstrated in this study, influence the determination of the enzyme in whole blood.

Thus, when using GSH-Px activity to monitor the selenium status of the animal, the erythrocyte life span of the species

concerned must be borne in mind. Whole blood GSH-Px activity is a good indicator of the long-term selenium status of goats and significant changes will not be observed until about 2 weeks after the administration of therapeutic doses of selenium. Determination of GSH-Px activity in blood plasma or serum from goats is of less value and could not be used as a short-term indicator of the selenium status.

## REFERENCES

- Caple, I. W., S. J. A. Edwards, W. M. Forsyth, P. Whiteley, R. H. Selth & L. J. Fulton*: Blood glutathione peroxidase activity in horses in relation to muscular dystrophy and selenium nutrition. *Aust. vet. J.* 1978, *54*, 57—60.
- Hakkarainen, J., P. Lindberg, G. Bengtsson & L. Jönsson*: Serum glutathione peroxidase activity and blood selenium in pigs. *Acta vet. scand.* 1978, *19*, 269—289.
- 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.
- Hussein, Kamal S. M. & Bernt-E. V. Jones*: Effects of different anticoagulants on determination of erythrocyte glutathione peroxidase. *Acta vet. scand.* 1981, *22*, 472—479.
- Jørgensen, P. F., J. Hyldgaard-Jensen & J. Moustgaard*: Glutathione peroxidase activity in porcine blood. *Acta vet. scand.* 1977, *18*, 323—334.
- Mills, G. C. & H. P. Randall*: The protection of hemoglobin from oxidative breakdown in the intact erythrocyte. *J. biol. Chem.* 1958, *232*, 589—598.
- Paglia, E. D. & W. N. Valentine*: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. clin. Med.* 1967, *70*, 158—169.
- Richard, W. S., D. A. Todhunter & L. S. Cook*: Selenium content and glutathione peroxidase in tissues of young cattle fed supplemented whole milk diets. *Amer. J. vet. Res.* 1981, *42*, 1718—1723.
- Schalm, O. W., N. C. Jain & E. J. Carrol*: *Veterinary Hematology*. 3rd Ed., Lea & Febiger, Philadelphia 1975.
- Thompson, C. D., H. M. Rea, V. M. Doesburg & M. F. Robinson*: Selenium concentration and glutathione peroxidase in whole blood of New Zealand residents. *Brit. J. Nutr.* 1977, *37*, 457—460.
- Thompson, K. G., A. J. Fraser, B. M. Harrop, J. A. Kirk, J. Bullans & D. O. Cordes*: Glutathione peroxidase activity and selenium concentration in bovine blood and liver as indicators of dietary selenium intake. *N.Z. vet. J.* 1981, *29*, 3—6.
- 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.

## SAMMANFATTNING

*Effekter av selen tillförsel på aktiviteten av glutathionperoxidase i blodplasma och röda blodkroppar hos get.*

Aktiviteten av enzymet glutathionperoxidase (GSH-Px, E.C. 1.11.1.9.) bestämdes i hepariniserat helblod, plasma och tvättade blodkroppar från getter före och efter intramuskulär tillförsel av selen (0.4 mg/10 kg kroppsvikt) och vitamin E (20 mg/10 kg kroppsvikt) eller enbart vit. E (20 mg/10 kg kroppsvikt). Tillförsel av Se gav en signifikant ökning av enzymaktiviteten i helblod och tvättade blodkroppar mätbar från 2 veckor efter tillförseln av Se. Plasmaproven uppvisade inga förändringar. Proven från de getter som enbart fått vit. E visade mindre och icke signifikanta ändringar av GSH-Px aktiviteten. Slutsatsen av experimentet blir att enzymaktiviteten i blodplasma eller serum inte kan användas som en indikator på getens kortsiktiga selenstatus men att bestämning av GSH-Px i helblod eller röda blodkroppar är en god indikator på djurets långsiktiga selenstatus.

*(Received September 13, 1982).*

Reprints may be requested from: Bernt-E. V. Jones, the Department of Clinical Chemistry, College of Veterinary Medicine, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.