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# BLOOD LEVELS OF CALCITONIN IN BULLS OF VARYING AGES*

By

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FORSLUND, K., L.-E. EDQVIST, K. LUNDSTRÖM and P.-O. NILSSON: *Blood levels of calcitonin in bulls of varying ages.* Acta vet. scand. 1980, *21*, 185—196. — Blood levels of calcitonin (CT), calcium (Ca), inorg. phosphate (P) and alkaline phosphatase (ALP) in 27 bulls of Swedish Red and White Breed (SRB) and 21 Swedish Friesian Breed (SLB) were studied. There was a significant lowering of Ca, P, ALP and CT with increasing age. The fact that the osteopetrotic syndrome is increasing with age in bulls and that the CT level in blood is decreasing with age does not support the idea that an increased CT discharge could be responsible for the osteopetrotic syndrome in bulls.

calcitonin, bulls, osteopetrosis.

Stiff back and diseases of the joints are common problems in bulls used as semen donors for artificial insemination. Necropsy of these bulls often reveals several cases of spondylosis, spondyloarthrosis and arthrosis in various joints of the legs. These changes are usually referred to as osteopetrosis. The frequency of such osteopetrotic changes has been shown to increase with age (*Bane & Hansen* 1962) and was in 1 study reported to occur in all investigated bulls over 7 years of age (*Krook et al.* 1971). The last authors suggested that this syndrome is due to an excessive dietary calcium intake followed by hyperplasia or neoplasia of the parafollicular cells (C-cells) of the thyroid gland

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leading to an increased calcitonin discharge. Furthermore, *Hansen* (1975) reported a positive correlation between the occurrence of osteopetrosis and C-cell tumors in bulls.

Although the hypothesis proposed so far is in favour of a nutritional hypercalcitoninism the possibility of a certain degree of heritability cannot be excluded. In man medullary carcinoma (C-cell tumor) is often found in association with phaechromocytomas and is known to occur in certain families. *Tashjian & Melvin* (1968) describe the first case of hypercalcitoninism with very high levels of plasma calcitonin due to medullary carcinomas. It was also associated with hypocalcemia, hypophosphatemia, the occurrence of tetany, high levels of circulating parathyroid hormone and a low rate of bone turnover. No changes in bone density could be demonstrated, however.

Since the frequency of osteopetrotic changes in bulls increases with age, and because an increased calcitonin secretion has hypothetically been suggested to play an important role in the syndrome, the present study investigates the blood plasma calcitonin levels in bulls of different ages.

## MATERIALS AND METHODS

Twenty-seven bulls of the Swedish Red and White Breed (SRB) and 21 bulls of the Swedish Friesian Breed (SLB) were studied. Their ages varied from 1 to 9 years. The SRB bulls were housed at 2 A. I. stations, while the SLB bulls were all housed in 1 A. I. station. The animals were fed 8 kg of hay and 4 kg of concentrate which yields a daily Ca intake of about 40 g/day. No extra mineral supplement was given. This investigation was carried out in September 1978. Before the blood samples were collected the animals were examined clinically with special emphasis on lameness and mounting behavior. In the SRB, blood samples were drawn from the jugular vein using a heparinized vacutainer tube and in the SLB bulls from the plexus of vessels underneath the second vertebra of the tail. Blood drawn from these vessels is in most cases arterial. The differences in sampling techniques were necessary because of the means available for restraining the animals at the 2 A. I. stations. To check if the CT level differs between arterial tail blood and venous jugular blood, 6 bulls were sampled from both sites.

The blood samples were immediately placed in ice water and

centrifuged within 10 min. The plasma was removed and immediately frozen on dry ice and kept frozen until analyzed.

The plasma calcium (Ca) was determined using an atomic absorption spectrophotometer. The plasma inorg. phosphate (P) values were determined according to a method described by *Itaya & Ui* (1966). The plasma alkaline phosphatase (ALP) was determined by a kinetic technique on an LKB Reaction Rute Analyzer according to the recommendations of the *Scandinavian Committee of Enzymes* (1974). The calcitonin (CT) levels in plasma were determined by radioimmunoassay (*Forslund & Stridsberg* 1980).

The bulls which were slaughtered for various reasons were examined for the presence of osteopetrotic changes. This examination was performed by the slaughter-house veterinarian. In some cases the thyroid glands were histologically examined at the National Veterinary Institute. Sections of the thyroid gland were fixed in 10 % aqueous solution of formaldehyd. After paraffin embedding and sectioning, the sections were stained with hematoxylin and eosin.

*Statistical methods*

All calculations were done using the Statistical Analysis System (*Barr et al.* 1976). To test the effect of the sampling site (blood drawn from the jugular vein versus blood from the tail) the following model was used:

$$Yi_{jk} = \mu + a_i + s_j + (as)_{ij} + e_{ijk}$$

where

$Y_{ijk}$ = the $ijk$th observation

$\mu$ = general mean

$a_i$ = effect of the $i$th animal ($i = 1, 2, \ldots 6$)

$s_j$ = effect of the $j$th sampling site ($j = 1, 2$)

$as_{ij}$ = effect of the interaction between the $i$th animal and the $j$th sampling site

$e_{ijk}$ = residual random term with variance $\sigma^2_e$

The effect of the animal was regarded as random and the effect of the sampling site as fixed. The restriction $\Sigma_j s_j = 0$ was imposed on the model.

The effect of breed was tested according to the following model:

$$Y_{ij} = \mu + r_i + b_1 x_{ij} + b_2 x_{ij}^2 + e_{ij}$$

where

$Y_{ij}$ = the $ij$th observation

$\mu$ = general mean

$r_i$ = effect of the $i$th breed ($i = 1, 2$)

$b_1, b_2$ = linear and quadratic regression on age at sampling

$x_{ij}$ = age at sampling in years of the $ij$th individual

$e_{ij}$ = residual random term with variance $\sigma^2_e$

The effect of breed was regarded as fixed and the restriction $\Sigma_i r_i = 0$ was imposed on the model. The quadratic regression was only included when significant. The interaction between breed and the linear as well as the quadratic regression on age at sampling was tested but was found to be non-significant for all substances, and only the average regressions were used. Due to confounding between the age of the bulls and the A. I. station, the effect of station could not be considered in the statistical evaluation.

## RESULTS

There were no significant differences in the CT level in the blood taken from the jugular vein as compared to the blood drawn from the plexus of vessels under the second vertebra of the tail using the statistical Model I ($P > 0.05$).

An individual presentation of the data from the bulls is given in Table 1. In Table 2 are presented the least-square means and the standard errors for the blood substances. The effect of breed was not significant ($P > 0.05$) for any of these blood compounds (Table 2). All blood parameters studied showed significant regressions on age (Table 2 and Figs. 1—2). The slopes for the regression lines for the breeds were not significantly different for any substance, as the interactions between breed and age were non-significant.

One bull (708 Ronstad) suffered from a stiff back at the time of blood sampling. None of the other bulls showed such signs on this occasion. Of the 48 bulls investigated, 24 were slaughtered approximately within 1 year after the sampling occasion. Out of these 24 bulls only 2 had skeletal pathological findings (426 Stavröd and 347 Bäcks). It should, however, be noted that the post-mortem examination was not a detailed one but a gross examination on the slaughter-house line. The thyroid glands from 12 of the bulls were submitted to histo-pathological examination. Hyperplasia of the C-cells was found in 1 of these

Table 1. Results of the blood analysis in individual bulls.

| SRB Bull No. | Name | Born | CT µg/l | CT mean | Ca mmol/l | Ca mean | P mmol/l | P mean | ALP | ALP mean | Slaughtered | Pathological findings |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 717 | Åslemåla | 770404 | 2.32 | | 2.90 | | 2.4 | | 4.6 | | 790313 | Thyr. 0 |
| 719 | Ekenäs | 770428 | 0.62 | 1.16 | 2.70 | 3.08 | 2.5 | 2.6 | 3.1 | | | |
| 716 | Snäshult | 770314 | 0.82 | ±0.69 | 2.80 | ±0.69 | 2.6 | ±0.2 | 3.4 | 3.9 | | |
| 715 | Simonsgård | 770311 | 1.29 | | 2.70 | | 2.6 | | 3.2 | | 790313 | Thyr. 0 |
| 708 | Ronstad | 770213 | 0.77 | | 4.30 | | 2.8 | | 2.7 | | | |
| 659 | Stenslanda | 761023 | 0.97 | | 2.80 | | 2.3 | | 3.1 | | | |
| 656 | Systertorp | 761014 | 0.93 | 0.90 | 3.00 | 2.80 | 2.8 | 2.4 | 4.0 | 2.88 | | |
| 653 | Brände | 761008 | 1.14 | ±0.19 | 2.70 | ±0.14 | 2.3 | ±0.3 | 2.5 | | | |
| 649 | Björnås | 760915 | 0.61 | | 2.70 | | 2.6 | | 2.1 | | | |
| 643 | Gräsgärde | 760827 | 0.87 | | — | | 2.0 | | 2.7 | | 790724 | — |
| 555 | Herrgården | 751214 | 0.97 | | 2.70 | | 2.4 | | 2.3 | | | |
| 547 | Dröpstad | 751103 | 0.66 | 0.60 | 2.75 | 2.66 | 2.2 | 2.4 | 4.0 | 2.35 | | |
| 521 | Solhult | 750407 | 0.44 | ±0.28 | 2.65 | ±0.08 | 2.8 | ±0.3 | 1.6 | | | |
| 508 | Lenastorp | 750301 | 0.34 | | 2.55 | | 2.0 | | 1.5 | | | |
| 437 | Örsjö | 741019 | 0.55 | | 2.65 | | 2.3 | | 1.5 | | | |
| 441 | Basebo | 741024 | 0.34 | 0.50 | 2.65 | 2.70 | 2.1 | 2.2 | 1.4 | | | |
| 451 | Månstorp | 741208 | 0.69 | ±0.15 | — | ±0.09 | — | ±0.1 | 1.6 | 1.38 | | |
| 426 | Stavröd | 740512 | 0.43 | | 2.80 | | 2.2 | | 1.0 | | 790405 | Arthrosis in the carpal and tarsal joints. Thyr. 0 |
| 329 | Södertorp | 730907 | 2.13 | | 2.70 | | 2.2 | | 0.8 | | | |
| 314 | Magda | 730421 | 0.18 | 0.84 | 2.80h* | 2.64 | 1.9h* | 2.0 | 10.5h* | | | |
| 344 | Thorarp | 731207 | 0.63 | ±0.75 | 2.60 | ±0.11 | 2.1 | ±0.1 | 1.2 | 3.02 | 790126 | Thyr. 0 |
| 322 | Solhult | 730507 | 0.78 | | 2.60 | | 2.1 | | 1.6 | | 781123 | Thyr. 0 |
| 347 | Bäcks | 731208 | 0.52 | | 2.50 | | 1.9 | | 1.0 | | 790405 | Spondylosis def. Thyr. C-cell hyperplasia |
| 223 | Månstorp | 720608 | 0.11 | 0.34 | 2.70 | 2.78 | 1.8 | 1.9 | 1.0 | | | |
| 114 | Byabacken | 720124 | 0.35 | ±0.22 | 3.00 | ±0.18 | 2.1 | ±0.2 | 4.5 | 2.43 | | |
| 233 | Flishult | 721020 | 0.55 | | 2.65 | | 1.9 | | 1.8 | | 790928 | Thyr. 0 |
| 527 | Smedsta | 711003 | 0.86 | 0.86 | 2.70 | 2.70 | 2.0 | 2.0 | 1.3 | 1.3 | 790110 | Thyr. 0 |

* h = hemolysis.

Table 1 (continued).

| SLB Bull No. | Name | Born | CT µg/l | CT mean | Ca mmol/l | Ca mean | P mmol/l | P mean | ALP | ALP mean | Slaughtered | Pathological findings |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 341 | Lufs | 770812 | 0.64 | | 2.90 | | 2.8 | | 4.5 | | 790103 | Thyr. not examined |
| 64 | Slöjd | 770719 | 0.72 | 0.71 | 3.30 | 2.90 | 3.0 | 2.8 | 3.8 | | 780928 | Thyr. 0 |
| 220 | Bonus | 770125 | 0.64 | ±0.10 | 2.80 | ±0.29 | 2.9 | ±0.2 | 3.1 | 3.55 | 780928 | Thyr. 0 |
| 477 | Betyg | 770531 | 0.85 | | 2.65 | | 2.5 | | 2.8 | | 780928 | Thyr. 0 |
| 520 | Sibb | 760908 | 1.09 | | 2.55 | | 2.6 | | 2.9 | | 791205 | Thyr. 0 |
| 225 | Sloan | 760820 | 0.87 | 0.89 | 2.70 | 2.71 | 3.1 | 2.8 | 3.3 | | | |
| 598 | Samos | 760902 | 0.59 | ±0.22 | 2.70 | ±0.14 | 2.5 | ±0.3 | 1.8 | 2.43 | | |
| 601 | Sirap | 760930 | 1.01 | | 2.90 | | 3.0 | | 1.7 | | | |
| 89 | Skytt | 750430 | 0.63 | | 2.60 | | 2.2 | | 2.0 | | 790629 | Thyr. not examined |
| 914 | Sarri | 750710 | 0.65 | 0.61 | 2.50 | 2.58 | 2.4 | 2.3 | 1.3 | | 790418 | „ |
| 613 | Ledig | 750103 | 0.59 | ±0.04 | 2.50 | ±0.09 | 2.2 | ±0.1 | 1.3 | 1.6 | 790423 | „ |
| 1087 | Lod | 750915 | 0.58 | | 2.70 | | 2.3 | | 1.8 | | 790516 | „ |
| 532 | Legg | 740601 | 0.63 | | 2.80 | | 2.5 | | 2.1 | | 790114 | „ |
| 345 | Liqör | 741029 | 0.15 | 0.48 | 2.50 | 2.63 | 2.2 | 2.0 | 1.5 | | 790427 | „ |
| 83 | Barre | 740523 | 0.56 | ±0.22 | 2.70 | ±0.15 | 1.9 | ±0.4 | 0.8 | 1.4 | 790417 | „ |
| 26 | Snygg | 740211 | 0.59 | | 2.50 | | 1.5 | | 1.2 | | | |
| 313 | Sesam | 730301 | 0.34 | 0.32 | 2.90 | 2.78 | 1.4 | 1.75 | 4.3 | | | |
| 351 | Söder | 731115 | 0.31 | ±0.03 | 2.65 | ±0.18 | 2.1 | ±0.5 | 1.4 | 2.55 | 790104 | „ |
| 557 | Andi | 720222 | 0.77 | 0.77 | 2.50 | 2.50 | 1.9 | 1.9 | 0.9 | 0.9 | 790323 | „ |
| 95 | Stekel | 701230 | 0.25 | 0.25 | 2.70 | 2.70 | 1.3 | 1.7 | 1.5 | | 791009 | „ |
| 158 | Hakan | 691220 | 0.25 | ±0 | 2.70 | ±0 | 2.0 | ±0.5 | 0.8 | 1.15 | | |

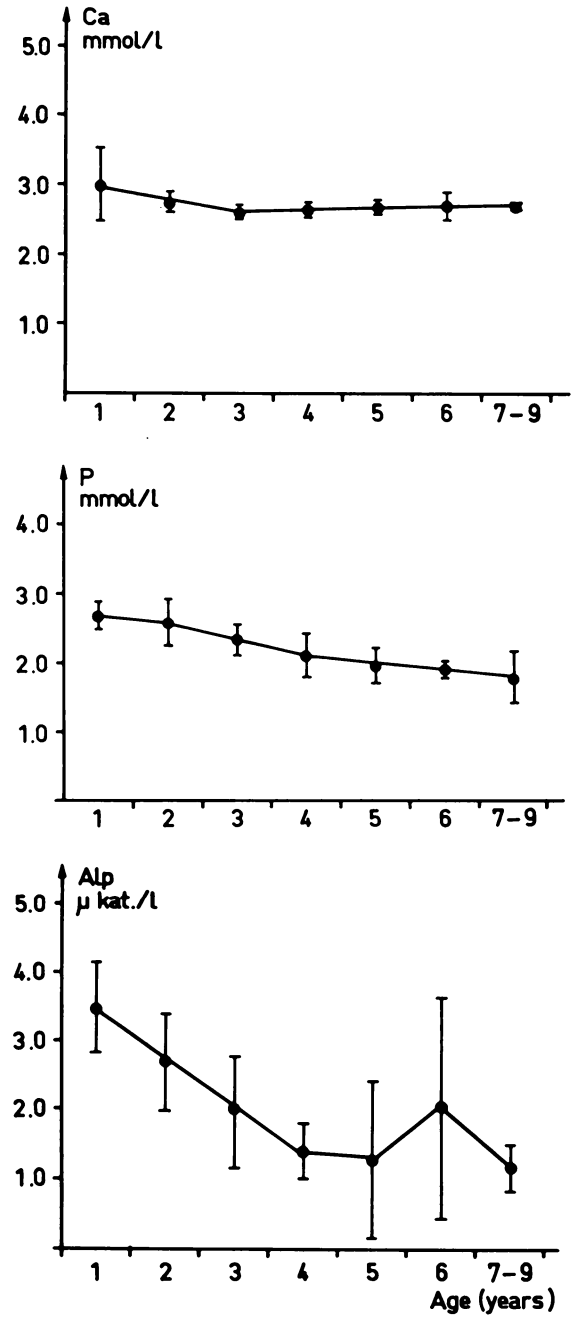


Figure 1. Mean blood levels in Ca (above), P (middle) and ALP (below) in all bulls at different ages.

Table 2. Least-square means ± standard error of the mean (s.e.m.) and levels of significance for the blood substances.

| Substance | Least-square means ± s.e.m. | | Level of significance | | |
|---|---|---|---|---|---|
| | | | | regression on age | |
| | SRB (n=27) | SLB (n=21) | breed | linear | quadratic |
| Ca | 2.79±0.05 | 2.69±0.06 | n.s. | ** | * |
| P | 2.26±0.06 | 2.30±0.06 | n.s. | *** | n.s. |
| ALP | 2.31±0.18 | 2.10±0.20 | n.s. | *** | * |
| CT | 0.77±0.07 | 0.61±0.08 | n.s. | ** | n.s. |

Levels of significance: n.s. = not significant ($P > 0.05$);
* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

12 bulls (347 Bäcks). The other 11 bulls were considered to have normal C-cells, which means that the thyroid glands from 717 Åslemåla, with high blood level of CT at the sampling occasion, showed no pathological changes.

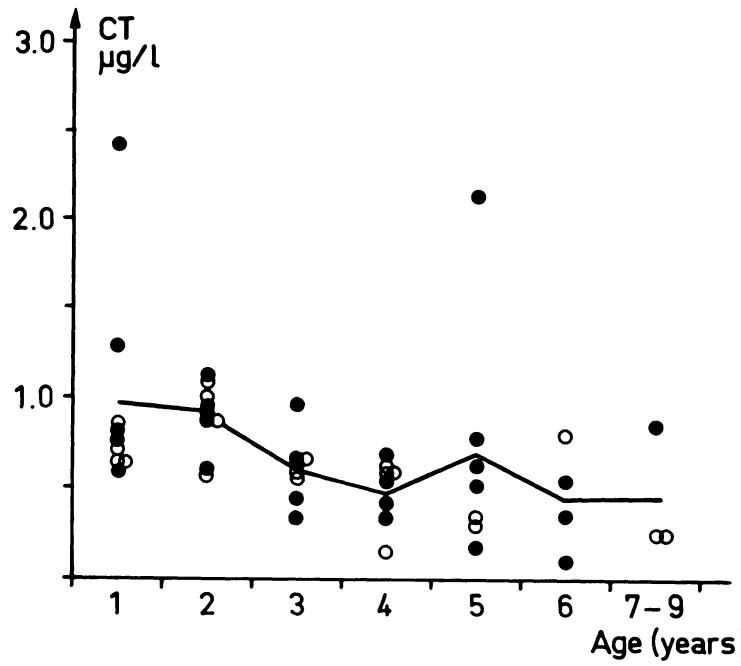


Figure 2. Individual CT level of the SRB (●) and SLB (○) bulls. Mean CT levels (———) for all bulls at different ages.

## DISCUSSION

A significant lowering of the Ca, P and ALP in the blood with increasing age has been described previously for bulls (*Krook et al.* 1971). The quadratic regression for Ca and ALP indicates the decrease as being less pronounced with increasing age. Similar to the situation in man (*Samaan et al.* 1975), a significant decrease of CT with increasing age was found in the animals of this study. Furthermore, *Deftos et al.* (1979) found CT levels to be significantly higher in bulls younger than 6 years of age as compared to the older bulls.

The incidence of osteopetrosis in the present material is considerably lower than reported by *Krook et al.* This difference might be due to the fact that the animals in the present study were not submitted to complete post-mortem examinations. It might also be explained by the dietary Ca intake in the investigation by *Krook et al.* varying from 67 to 88 g Ca/day. The bulls in the present study received about 40 g/day.

One bull (347 Bäcks) had a normal CT level for his age but showed an unclassified interfollicular hyperplasia in the thyroid gland and spondylosis deformans. Humans suffering from C-cell carcinoma do not always have elevated CT levels (*Trump et al.* 1979). On the other hand, if stimulated by Ca infusion or alcohol (*Telenius-Berg et al.* 1975, *Dymling et al.* 1976) they have elevated CT levels in the blood. Such stimulating tests could not be done in these "priceless" A.I. bulls.

Elevated CT levels were recorded in 1 young bull (717 Åslemåla). Whether or not such an elevation present at a young age will induce skeletal changes at a later age is not known. When compared to man this seems less likely since persons with elevated CT levels do not suffer from osteopetrosis but from gastrointestinal symptoms (*Otto Ljungberg,* pers. com.).

*Kent et al.* (1979) reported a decreased response to calcitonin in osteopetrotic mouse bone compared to normal mouse bone in vitro. They found no difference in the number of osteoclasts in their experimental condition. Instead they suggested a difference in the number or nature of the calcitonin receptors in the osteoclasts or an alteration in the mechanism for transmitting the calcitonin signal at the membrane level as an explanation for the decreased response. The specific cause for such differences at the receptor level might be genetically based according to these authors. This is also supported by *Heersche et al.* (1978)

who suggested that bone tissue has a cellular mechanism to compensate for overexposure to e.g. calcitonin and parathyroid hormone. Furthermore, some authors are of the opinion that CT cannot stimulate the apposition of bone to an osteoporotic skeleton (*Sørensen et al.* 1972, *Melick et al.* 1973, *Rebel et al.* 1977, *Kanis et al.* 1977). On the other hand, *Milhaud et al.* (1978) found a deficiency of CT in age related osteoporosis.

The acute Ca-lowering effect of CT might more be due to an increased Ca excretion through the kidney than to a decreased bone resorption (*Simonnet et al.* 1978). This is supported by the findings of elevated CT levels in renal failure in man (*Ardaillou et al.* 1975, *Silva et al.* 1977) and the accumulation of radiolabelled CT in the intact rat kidney (*Forslund et al.* 1980).

As pointed out in the introduction, it has been suggested that an increased CT discharge could be responsible for the osteopetrotic syndrome in bulls. Furthermore, the frequency of the syndrome increases with age (*Krook et al.*). The continuous lowering of the CT levels with advancing age in the bulls of this study, in combination with the questionable effect of CT on Ca apposition to bone as the major Ca-lowering effect of CT, does not support such a suggestion.

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SAMMANFATTNING

*Blodnivåer av calcitonin hos tjurar av olika åldrar.*

Nivåerna av calcitonin (CT), kalcium (Ca), oorganiskt fosfor (P) och alkaliskt fosfatas (ALP) i blod undersöktes på 48 semintjurar (27 SRB- och 21 SLB-tjurar). Blodnivåerna av samtliga undersökta parametrar sjönk med stigande ålder. Det faktum, att de osteopetrotiska lidandena ökar med stigande ålder och att CT-nivåerna i blodet sjunker med åldern, stöder inte idén om att en ökad CT-insöndring skulle orsaka osteopetros hos tjurar.

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