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Selenium and Glutathione Peroxidase Distribution in Bovine Semen and Selenium-75 Retention by the Tissues of the Reproductive Tract in the Bull[1,2,3]

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ABSTRACT

In order to compare the distribution of selenium and glutathione peroxidase (GSH-Px) in bovine semen and to determine the stage at which selenium is incorporated into bovine spermatozoa, 3 bulls were each injected with a single dose of ^{75}Se-selenite. After injection, radioactivity and GSH-Px activity in blood and semen were monitored for a period of 75 days. For all bulls ^{75}Se was detected in seminal plasma 6 h postinjection and levels in the seminal plasma exceeded blood levels by 24 h postinjection. Radioactivity increased rapidly in the seminal plasma for the first 10 days following injection and then decreased slowly for the remainder of the collection period. Between Days 15 and 20 postinjection, ^{75}Se increased in the spermatozoa. GSH-Px activity was present only in blood and seminal plasma.

In a second experiment, the relative tissue retention of ^{75}Se by the reproductive tract and other body tissues was examined in 4 bulls. Each bull received a second injection of ^{75}Se-selenite. Bulls were slaughtered at 23 days postinjection when levels of ^{75}Se in the semen were approximately 16 times greater than blood levels and both spermatozoa and seminal plasma were labeled. Selenium-75 retention expressed as cpm/g of tissue was determined for a total of 14 tissues. Epididymis (67,078 ± 4,787 cpm/g) and testis (33,459 ± 783 cpm/g retained the greatest amount of ^{75}Se with the exception of the kidney (105,439 ± 5,936 cpm/g). Among the accessory glands, the prostate (15,927 ± 1,356 cpm/g) and the seminal vesicles (12,491 ± 986 cpm/g) contained the highest levels of ^{75}Se. Sperm concentration and ^{75}Se were determined in each of 20 serial samples of the epididymis from the proximal caput through the distal cauda in 3 bulls. Both sperm concentration and ^{75}Se retention changed dramatically within the caput and cauda regions and for all samples (n=60) epididymal ^{75}Se was correlated with sperm concentration ($r=0.92$, $P<0.01$).

INTRODUCTION

Recent evidence indicates that selenium may be important for normal reproductive function in the male. Reports by Brown and Burk (1972) and Burk et al. (1972) described tissue retention of ^{75}Se in selenium deficient rats. They found that the testicles accumulated and retained a large portion of an intravenous dose of ^{75}Se-selenite. They further demonstrated that peak radioactivity followed the normal progression of spermatozoa from the testis through the epididymis. A similar pattern was noted by Gunn et al. (1967) for mice fed a selenium adequate diet. Autoradiography of spermatozoa recovered from the epididymis indicated that the isotope was associated with the midpiece (Brown and Burk, 1972). Although the exact role of selenium in testicular function or in the spermatozoa has not been defined, studies with selenium deficient rats suggest a possible involvement in sperm maturation and structural integrity (Wu et al., 1973).

Selenium is an essential component of the enzyme glutathione peroxidase (EC 1.11.1.9) (GSH-Px) (Rotruck et al., 1973; Flohé et al., 1973; Oh et al., 1974; Nakamura et al., 1974). This selenoenzyme has been found in most mammalian tissues where it functions in the reduction of endogenous peroxides (Flohé, 1976). GSH-Px activity has been found in the semen of several species including ram, dog, human, goat, (Li, 1975) and bull (Brown et al., 1977). In the bull GSH-Px activity is associated with the seminal plasma and not spermatozoa (Brown et al., 1977; Brown and Senger, 1977).

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[3] Bulls were provided by Allwest Breeders, Burlington, WA and Select Sires, Plain City, Ohio.

Both selenium and GSH-Px appear to be involved in the male reproductive system yet little information is available regarding selenium distribution in bovine semen. The objectives of this study were: 1) to describe the distribution of selenium in bovine semen over time using an isotopic tracer (^{75}Se) and 2) to compare directly the distribution of ^{75}Se with that of GSH-Px in various bovine seminal components. In addition, retention of ^{75}Se by the tissues of the reproductive tract of the bull was examined.

MATERIALS AND METHODS

Three mature dairy bulls (2 Holstein, 1 Jersey) and 1 10-month-old Holstein bull were used in this study. Bulls were maintained on a selenium adequate diet both prior to and during the course of these experiments. Mean blood Se was 0.17 ppm for the 3 bulls as determined by fluorometric analysis (Hoffman et al., 1968). Ejaculates were collected with an artificial vagina and blood was collected via jugular puncture. Thirty to 60 min after collection, ejaculates were evaluated for motility and sperm concentration was determined turbidimetrically.

Experiment 1

In this experiment, 3 bulls each received a single i.v. injection (1 mCi/550 kg body weight) of ^{75}Se-selenite in 0.9% saline. The specific activity was 162.6 mCi/mg and the total selenium injected did not exceed 12.3 μg/animal. Following injection, blood and semen collections were made at 6 h intervals for 48 h and 3 times weekly thereafter for a period of 28 days. Collections were continued at weekly intervals for an additional 4 weeks and a final sample from each bull was collected at 75 days postinjection.

In order to determine critically the distribution of ^{75}Se and GSH-Px within semen, all ejaculates were treated in the following manner: 1) whole semen was centrifuged to separate spermatozoa from seminal plasma; 2) the spermatozoa were then washed in 2.9% sodium citrate and centrifuged to remove residual seminal plasma; 3) the washed spermatozoa were resuspended in distilled H_2O, frozen (0°C) and thawed to rupture sperm membranes and again centrifuged; 4) the pellet which comprised primarily the more insoluble structural components of the heads and tails was resuspended in distilled H_2O. Spermatozoa in the final suspension were characterized by head-tail fragmentation and complete absence of the acrosome as determined by differential interference contrast microscopy. All centrifugations were at 27,000 × g for 20 min.

Levels of radioactivity in 0.5 or 1 ml aliquots of blood, whole semen, seminal plasma, sodium citrate supernatant (S1), distilled H_2O supernatant (S2) and final sperm suspension were determined in a Nuclear Chicago 1185 series automatic gamma spectrophotometer. Three 0.5 ml counting standards were prepared at the outset and were included with each group of samples to correct for physical decay of the isotope. The counting efficiency for ^{75}Se was 80%. One ml aliquots of each of the samples were packaged in glass ampules, frozen in liquid N_2 vapor and stored in liquid nitrogen for later GSH-Px assay.

Glutathione peroxidase activity was determined by a procedure adapted from Paglia and Valentine (1967). Glutathione peroxidase was coupled to NADPH via glutathione reductase (GSSG-R). The rate of NADPH oxidation was measured spectrophotometrically at 340 nm. The reaction mixture consisted of 2 mM reduced glutathione (GSH), 0.12 mM hydrogen peroxide (H_2O_2), 0.1 mM NADPH, 1 unit GSSG-R (1 unit oxidized 1 mmol NADPH per min), 100 mM phosphate buffer pH 7.0, 3 mM EDTA and 1 mM sodium azide (NaN_3). All reactions were carried out at 25°C. Enzyme activity (eu/ml) was expressed as μmoles NADPH oxidized/min/ml of original sample after subtraction of nonenzymatic rate. All samples were assayed in duplicate. The coefficient of variation between duplicates was 4.2%. Interassay variation, determined by multiple measurements of a single sample of seminal plasma, was 4.7%.

Experiment 2

Five months after the first injection, each of the 3 bulls from experiment 1 and a 4th bull used in a pilot study received a second i.v. injection of ^{75}Se-selenite (1 mCi/550 kg body weight) with a specific activity of 71 mCi/mg. Residual levels of ^{75}Se in blood and semen were determined for each bull immediately prior to injection. Approximately 10% of the initial blood ^{75}Se was still present. Levels in the semen were about 20% of peak levels. Following injection blood and semen were collected twice weekly and radioactivity was monitored as described in experiment 1. Bulls were slaughtered at 23 days postinjection when levels of ^{75}Se in the semen were approximately 16 times greater than blood levels and both spermatozoa and seminal plasma were labeled. At slaughter, the reproductive tract was removed and the accessory glands, testes and epididymides were dissected and weighed. In addition, liver, kidney, heart and lung were removed and samples of brain, hide, muscle and fat were obtained. Radioactivity in 3 0.5–1 g (wet weight) samples from each type of tissue was determined and ^{75}Se retention was expressed as cpm/g of tissue.

In order to determine if ^{75}Se in the epididymis was associated with spermatozoa, epididymal tissue or both, the left epididymis from each bull was divided into 20 serial sections from the proximal caput through the distal cauda (Fig. 1). Selenium-75 retention and sperm concentration were determined for each section. Sperm concentration was determined by a method adapted from Amann (1969). A known weight of tissue (0.5–1 g wet weight) was homogenized for 2 min with a Polytron tissue homogenizer in 20 ml of a solution containing 0.9% saline (w/v), 0.05% Triton-X 100 (v/v) and 0.01 methiolate (w/v). Sperm nuclei in an aliquot of the homogenate were counted using a hemocytometer. The mean of 10 separate counts was used to calculate the sperm concentration per gram of tissue for each epididymal section.

Statistical Analysis

Differences in GSH-Px activity in blood and semen between bulls and between days of collection were analyzed by analysis of variance according to the model:

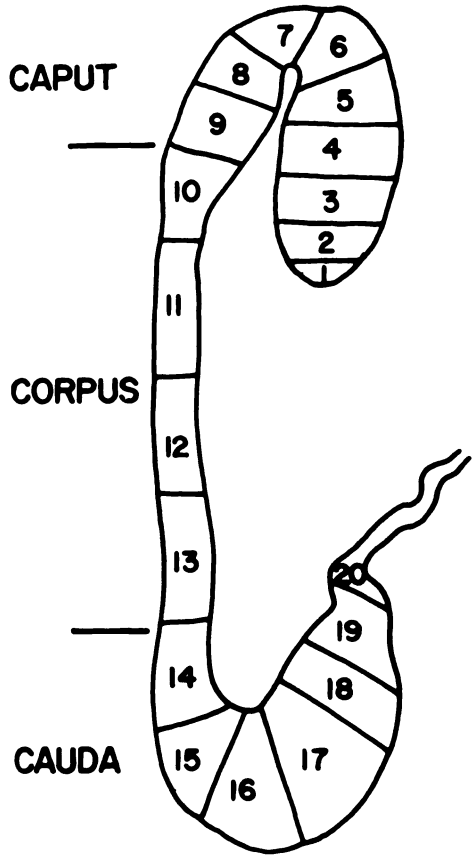

FIG. 1. The left epididymis of each bull was divided into sections as shown above. Divisions were based on the distinct lobulations present in the epididymis. ^{75}Se retention and sperm concentration were determined for each section.

$$y_{ij} = \mu + b_i + d_j + e_{ij}$$

where b_i is the effect due to bulls and d_j is effect of collection day.

The correlation between ^{75}Se retention and sperm concentration was computed over all bulls and all epididymal sections.

RESULTS

Experiment 1

Changes in ^{75}Se in blood, whole semen, seminal plasma and spermatozoa are presented in Fig 2. Blood levels of ^{75}Se declined rapidly during the first 48 h postinjection and declined slowly thereafter. In contrast, ^{75}Se in whole semen increased steadily until 5 days postinjection and continued to increase at a slower rate until 38 days postinjection. At this time radioactivity in the semen was approximately 19 times greater than in the blood (68,832 cpm/ml and 3,607 cpm/ml, respectively). The pattern of ^{75}Se incorporation and the distribution of ^{75}Se in the semen was similar for all bulls studied.

Initially all of the ^{75}Se in the semen was present in the seminal plasma (Fig. 2). Peak radioactivity in the seminal plasma occurred at 10 days postinjection (40,212 cpm/ml). Levels of ^{75}Se in seminal plasma, although declining thereafter remained elevated when compared to blood levels at all subsequent collection times.

Between Days 15 and 20 postinjection increased radioactivity was detected in the final suspension of spermatozoa (Fig. 2). Levels associated with the spermatozoa increased until 24 days postinjection then appeared to stabilize for the next 3 weeks at approximately 20,000 cpm/10^9 sperm.

Low levels of ^{75}Se were present in the sodium citrate supernatant for all ejaculates due to residual seminal plasma in this wash fraction (10–1877 cpm/ml). Low levels were also present in the distilled H_2O supernatant (0–805 cpm/ml) at some collection times but there was no consistent pattern across bulls.

Glutathione peroxidase activity in blood, whole semen and the fractions studied is presented in Fig. 3. There was significant variation in enzyme activity in blood and semen among bulls and among samples on different days ($P<0.01$), however, the relative distribution of GSH-Px within the seminal components was the same for all bulls and all ejacu-

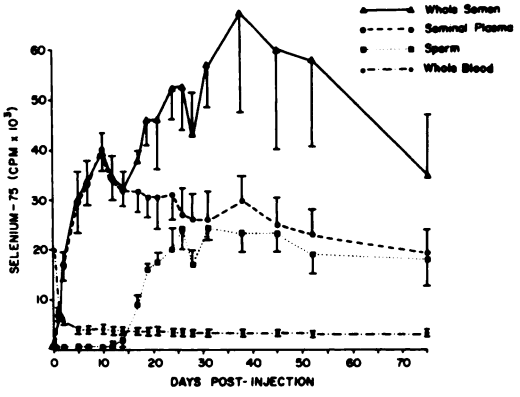

FIG. 2. Selenium-75 in whole blood (cpm/ml), sperm (cpm/10^9 sperm), seminal plasma (cpm/ml) and whole semen (cpm/ml) following a single intravenous injection of ^{75}Se-selenite. Each point represents the mean ± SEM for 3 bulls.

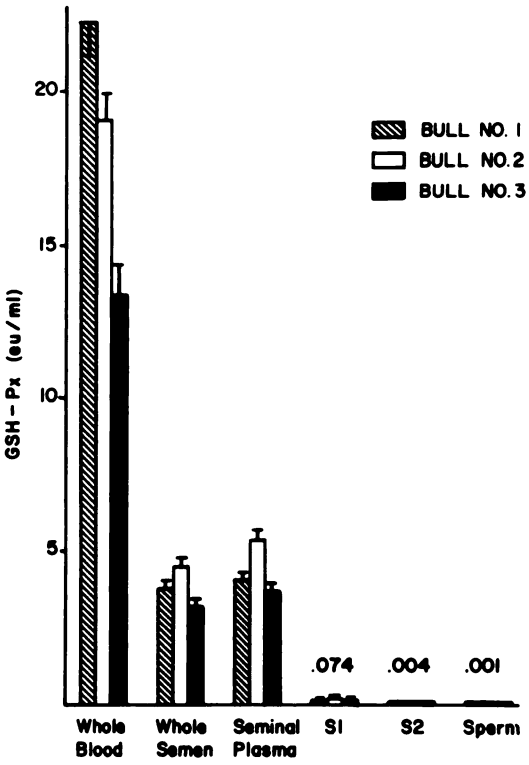

FIG. 3. GSH-Px activity in blood, whole semen, seminal plasma, S1, S2 and sperm suspension for 3 bulls. Each bar represents the mean ± SEM for 3 preinjection and all postinjection samples through Day 20 (n=18). S1 - sodium citrate supernatant used for the initial wash. S2 - distilled H_2O supernatant used to lyse the spermatozoa.

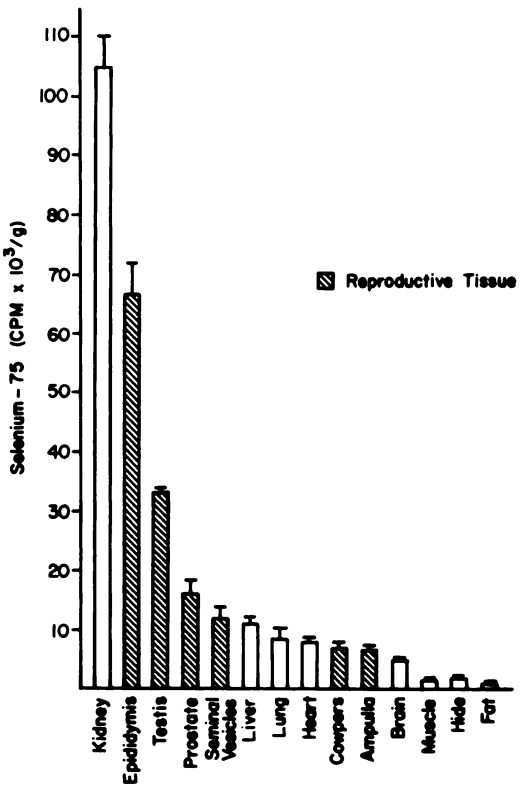

FIG. 4. Tissue retention of ^{75}Se 23 days after an i.v. injection of ^{75}Se-selenite (1 mCi/550 kg body weight). Bars represent the mean ± SEM for 4 bulls.

lates. Blood GSH-Px was approximately 4 times greater than that of whole semen. All GSH-Px activity in the semen was associated with the seminal plasma.

Experiment 2

Tissue retention of ^{75}Se expressed as cpm/g of tissue is presented in Fig. 4. Epididymis (67,078 ± 4,787 cpm/g) and testes (33,459 ± 783 cpm/g) retained the greatest amount of ^{75}Se with the exception of the kidney (105,439 ± 5,936 cpm/g). Among the accessory glands the tissue of the disseminate prostate (15,927 ± 1,356 cpm/g) and seminal vesicles (12,491 ± 986 cpm/g) contained the highest levels of ^{75}Se.

Selenium retention and sperm concentration in the epididymis from the proximal caput through the distal cauda (vas deferens) are shown in Fig. 5. Both ^{75}Se and sperm/g of tissue changed dramatically within the caput and cauda regions of the epididymis. No changes occurred in the corpus region. For all epididymal samples ^{75}Se retention was highly

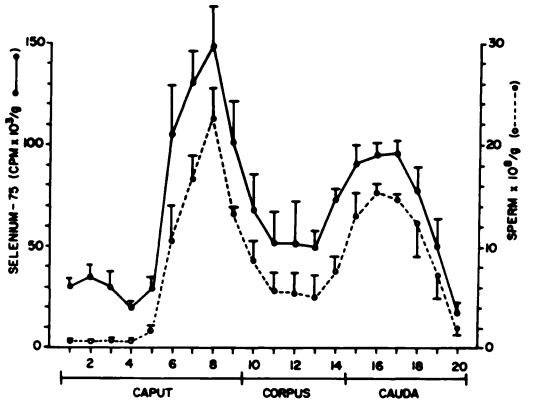

FIG. 5. Selenium-75 retention and sperm concentration in 20 serial samples of the epididymis. Each point represents the mean ± SEM for 3 bulls.

correlated with sperm concentration (r=0.92, P<0.01) (Fig. 6).

DISCUSSION

This study demonstrates that ^{75}Se is concentrated in bovine semen following a single i.v. dose and that this isotope is incorporated in both spermatozoa and seminal plasma. In addition, the data suggest that GSH-Px found in seminal plasma may, in part, account for the presence of ^{75}Se in this component of bull semen, but the incorporation of ^{75}Se into bovine spermatozoa is not due to this selenoenzyme.

The presence of ^{75}Se in seminal plasma at levels greatly exceeding blood suggests accumulation and secretion by one or more of the accessory glands. Furthermore, the presence of elevated ^{75}Se over an extended period of time may indicate a possible storage of selenium by these accessory glands. Tissue retention data indicated that the prostate and seminal vesicles contained levels of ^{75}Se exceeded only by the testis, epididymis and kidney, therefore it is possible that these glands are responsible for the majority of the ^{75}Se in the seminal plasma. Considerably higher levels of ^{75}Se might have been found in the accessory glands if samples were taken nearer the time of peak ^{75}Se in the seminal plasma (i.e., 5–10 days rather than 23 days postinjection). Hannson (1966), using whole body autoradiography in mice, found that the seminal vesicles showed the highest concentration of ^{75}Se 4 days after injection.

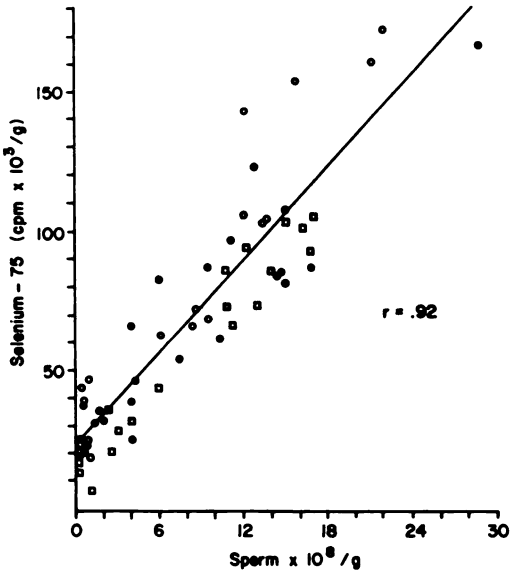

FIG. 6. The relationship between ^{75}Se retention and sperm concentration in 20 serial samples of the epididymis. Bull 1 (●), Bull 2 (○) and Bull 3 (□).

While this study has not attempted to characterize the biochemical nature of selenium in seminal plasma, preliminary data indicate that the majority of the ^{75}Se is associated with protein as determined by trichloroacetic acid precipitation. Small amounts of selenium are found in most proteins due to nonspecific binding to protein-SH groups or substitution of seleno-amino acids for their sulfur counterparts (Ganther, 1974; Martin, 1973). In addition to this nonspecific association of selenium with protein in general, a few proteins have been isolated which contain stoichiometric quantities of selenium (Stadtman, 1974; Ganther, 1975). In mammals, GSH-Px is the best documented protein of this type (Rotruck, 1973; Flohé et al., 1973; Oh et al., 1974) although other selenoproteins have been tentatively identified (Pederson et al., 1972). The presence of GSH-Px in bovine seminal plasma provides indirect evidence that ^{75}Se incorporation may be due, in part, to this selenoprotein. Recently Calvin (1978) reported that ^{75}Se was localized in keratinoid proteins of the rat sperm tail. Based on sperm fractionation and subsequent biochemical analysis he suggested that a selenopolypeptide is present and is critical for normal formation of the sperm tail in the rat.

The appearance of ^{75}Se in ejaculated spermatozoa approximately 20 days following injection indicates that selenium is incorporated by the spermatid. This is supported by evidence that the relationship between sperm concentration and ^{75}Se retention remains the same throughout the epididymis. Based on similar data in the rat, Gunn and Gould (1970) suggested that ^{75}Se is incorporated by the early spermatid or secondary spermatocyte and that ^{75}Se is not directly incorporated by spermatozoa residing in the epididymis. The incorporation of selenium by the spermatid may reflect a requirement of selenium for normal spermiogenesis.

The fluctuations in sperm concentration seen in both the caput and cauda epididymis are consistent with evidence for fluid reabsorption and secretion in these regions (Waites and Setchell, 1969). The sperm concentration in the distal cauda was further reduced due to the increase in connective tissue in this region.

Significant GSH-Px activity was not detected in the final spermatozoa suspension or in any supernatant wash. This agrees with the indirect (Brown et al., 1977) and direct evidence (Brown and Senger, 1977) that bovine spermatozoa contain little or no GSH-Px and indicates that ^{75}Se incorporation in spermatozoa is not due to GSH-Px. Spermatozoa of eutherian mammals are known to contain a high concentration of cysteine-rich structural proteins in both the nucleus and tail (Calvin, 1974) and selenium is generally found to be associated with protein sulfhydryls (Ganther, 1974). Therefore, because ^{75}Se was not released upon rupture of the sperm membranes but remained with the more insoluble components of the spermatozoa, it is possible that selenium is incorporated in the keratin like proteins of the heads and tails.

The incorporation of ^{75}Se in bovine semen and the retention of ^{75}Se by the tissues of the reproductive tract of the bull are undoubtedly due at least in part to the nonspecific association of selenium with protein. However, the maintenance of high levels of ^{75}Se when compared to blood and other tissues over an extended period suggests a more specific uptake of selenium by the male reproductive system. Because GSH-Px in bovine semen is limited to seminal plasma, ^{75}Se incorporation by bovine spermatozoa may indicate a hitherto unrecognized role of selenium which may be important for normal reproductive function in the bull.

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