AN ABSTRACT OF THE THESIS OF

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Title: Selenium-75 Incorporation in Semen and Reproductive Organs of the Bull and Ram

Abstract approved: Redacted for privacy

Philip D. Whanger

Following initial intramuscular injections of 500 μCi ^{75}Se, semen was collected periodically by electroejaculation over a 63 day period from a selenium-deficient and a selenium-adequate ram. Ten and three days prior to necropsy, second and third injections of 500 μCi were given. Testes, accessory glands, and semen were obtained from a bull injected with ^{75}Se through the courtesy of the Animal Science Department of Washington State University, Pullman, Washington.

Gel filtration of ram testis cytosol resulted in four ^{75}Se peaks (Ve/Vo ratios of 1.09, 1.52, 2.30, and 2.85). In the selenium-adequate ram the GSH-Px peak (Ve/Vo 1.52) predominated, but in the selenium-deficient ram, radioactivity of the GSH-Px peak was less than that of the higher molecular weight peak (Ve/Vo 1.09). Gel

filtration chromatograms of bull testis cytosol showed five ^{75}Se peaks (Ve/Vo ratios 1.11, 1.48, 1.90, 2.41, and 2.78). In chromatograms of ram seminal plasma there were two major ^{75}Se peaks (Ve/Vo ratios of 1.06 and 1.40), and two minor peaks (Ve/Vo ratios 1.73 and 2.42). Percent ^{75}Se associated with the peaks changed with time after injection. ^{75}Se-labeled bull seminal plasma showed two ^{75}Se peaks (Ve/Vo ratios of 1.09 and 1.42) which corresponded to the major peaks of ram seminal plasma.

Bull and ram seminal plasma glutathione peroxidase activities per mg protein were comparable (27.73 and 28.97 nmoles $NADPH_{ox}$, respectively), but when expressed per ml seminal plasma, activity of the bull was greater than seven times the highest activity of ram seminal plasma (2908 and 385 nmoles $NADPH_{ox}$, respectively). Seminal vesicles of bull and ram, and the bull prostate gland possessed high glutathione peroxidase activity. The Cowper's glands of bull and ram had low activity of this selenoenzyme.

Incorporation of ^{75}Se into ram sperm occurred prior to and during the early spermatid stage. Electron autoradiography of ram sperm indicated an affinity of membranes for selenium. The nucleus also demonstrated an incorporation of selenium. The implications of these findings are discussed.

Selenium-75 Incorporation in Semen and Reproductive Organs of the Bull and Ram

by

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Redacted for privacy

Chairman Department of General Science

Redacted for privacy

Dean of Graduate School

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Typed by Jean L. Kreitlow for Finn Richard Pond

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Selenium-75 Incorporation in Semen and Reproductive Organs of the Bull and Ram

I. INTRODUCTION

Considerable evidence has accumululated for the involvement of selenium in mammalian reproductive systems. Underwood (1977) stated that selenium was implicated in reproductive processes of all animals studied. Specific biochemical functions have not been elucidated, however, nor is it clear to what extent qualitative or quantitative differences exist between species relative to their requirements for selenium in reproduction.

Testes, in contrast to ovaries, have been shown to accumulate relatively high levels of selenium. Rosenfeld (1964) reported that of all tissues examined, with exception of the kidneys, testes had the highest concentration of selenium in rats administered daily ^{75}Se tracer doses over a 27-day period. Following a single intravenous injection of either ^{75}Se-methionine or ^{75}Se-cystine, Anghileri and Marques (1965) found that mice progressively concentrated ^{75}Se in testes over a seven day period. In other tissues, peak ^{75}Se concentrations were reached within the first day, after which levels began to decline. This pattern in mice was confirmed by Gunn et al. (1967). In addition, they showed that the subsequent loss of ^{75}Se from the testis was accompanied by a marked rise in ^{75}Se content of the epididymis, indicative of the metabolic incorporation of selenium

into developing spermatozoa. Previously, Hansson and Jacobsson (1966) had demonstrated, by use of whole-body autoradiography, the presence of selenium at high concentrations in the seminal vesicles and, to a lesser extent, the epididymides of mice. In the rat, ^{75}Se accumulated in the testis for one to two weeks following administration of a tracer dose (Gunn and Gould, 1970). As radioactivity fell in the testis, the ^{75}Se pulse progressed from the caput epididymis through the corpus into the cauda edpididymis. Furthermore, surgical severance of the vasa efferentia, disrupting the sperm-transport pathway, resulted in a low concentration of ^{75}Se in the epididymis. Whitfield (1978) found that testes of voles have a high affinity for selenium relative to other tissues. Also noted was a pattern of delayed incorporation of selenium into testes similar to that seen in mice and rats.

McCoy and Weswig (1969) presented further evidence for the involvement of selenium in reproduction. They reported that rats fed low selenium diets grew and reproduced normally but their offspring exhibited reduced growth, alopecia, and reproductive failure. These conditions were corrected by a dietary supplement of 0.1 ppm selenium. These findings were supported by those of Halverson (1974). Wu et al. (1969) found decreased epididymal concentration of sperm in second generation rats fed a selenium-deficient diet. The majority of sperm showed impaired motility and breakage near the midpiece of the tail. Also, deficient animals had smaller testes. These conditions were unresponsive to a variety of intracellular antioxidants, including

vitamin E (Wu et al., 1973). More recently, electron microscopy revealed damage in selenium-deficient rats in the membrane system near the sperm midpiece (Wu et al., 1979). Brown and Burk (1973) demonstrated selenium concentration in the midpiece of rat sperm using autoradiographic techniques. No pathological changes in testes or semen quality, as assessed by percent dead or abnormal sperm, were detected in rams fed low selenium diets for 140 days (Buchanan-Smith et al., 1969). However, the duration of selenium deprivation in their study may have been insufficient for testicular impairment to be manifested.

Whether selenium functions in an enzymatic or structural role in the maintenance of sperm integrity are questions as yet unanswered. Selenium has been documented as a component of glutathione peroxidase (GSH-Px; glutathione:H_2O_2 oxidoreductase, E.C. 1.11.1.9), an enzyme that breaks down not only hydrogen peroxide but also lipid hydroperoxides. This action against lipid hydroperoxides protects cellular and subcellular membranes from oxidative damage (Hoekstra, 1975). Testes, having an active oxidative metabolism, are expected to be prone to oxidative stress. Hydrogen peroxide is produced in seminal plasma and testes of various species by spermine oxidase activity (Mann, 1964). Also, Tabor and Rosenthal (1956) found that reduced glutathione, but not catalase, prevented immobilization of sperm by spermine oxidase. This suggests a possible role for GSH-Px in male reproduction.

In contrast to other selected tissues, in rats fed a low-

selenium diet for 17 days, GSH-Px activity of testes failed to decrease but instead increased about 87% above the original activity (Chow and Tappel, 1974). In testes of rats injected with a tracer dose of ^{75}Se, Prohaska et al. (1977) found four ^{75}Se containing peaks in gel filtration chromatographs of the cytosol. Two peaks with peroxidase activity were observed, only one of which was associated with a selenium containing peak. McConnell et al. (1979) showed a shift with time after injection in the molecular weight components of rat testis cytosol to which selenium is predominantly associated. Li (1975) found activity of GSH-Px in semen of dog, goat, ram, and human, but detected no activity in semen of the boar or rabbit. High GSH-Px activity has been reported in bull seminal plasma (Brown et al., 1977; Smith et al., 1979).

The major proportion of GSH-Px in cells has been reported to be in the cytosolic fraction (Green and O'Brien, 1970; Noguchi et al., 1973; Godwin et al., 1975). Mitochondrial and nuclear fractions possess most of the noncytosolic GSH-Px (Noguchi et al., 1973). Most of the selenium in rat liver mitochondria was reported to be in the form of GSH-Px and was released from the mitochondria during swelling induced by reduced glutathione (Neubert et al., 1962). Pallini and Bacci (1979) reported that selenium was selectively localized in the mitochondria of bull sperm. The selenium was bound to a structural polypeptide of about 20,000 daltons (This value is in concordance with the molecular weight of a single subunit of GSH-Px) which was released from the sperm mitochondria upon mercaptoethanol-induced swelling.

Calvin (1978) found that, following an intratesticular injection of ^{75}Se, the label was localized primarily in tail keratin of the rat spermatozoon. He identified a 17,000 dalton polypeptide to which selenium was associated. Calvin and Cooper (1979) proposed that the ^{75}Se-labeled polypeptide was associated with the outer mitochondrial membranes and in some way essential for assembly of the mitochondrial sheath. They doubted this was a subunit of GSH-Px since (1) no GSH-Px has been detected in ^{75}Se-labeled sperm extracts under conditions in which activity is normally measured; (2) the selenopolypeptide was bound to fibrous material, whereas GSH-Px is in the mitochondrial matrix of other cells: (3) ^{75}Se accumulation in testes is progressive and appears to be organ-specific, while GSH-Px is distributed throughout the body and is not especially high in testes; and (4) ^{75}Se in testes cytosol is not exclusively or even principally associated with GSH-Px.

Selenoproteins other than GSH-Px have been found. Two microbial seleno-enzyme systems, glycine reductase of Clostridia and bacterial formate dehydrogenase, have been described (Stadtman, 1974). Whanger *et al.* (1973) isolated selenium containing protein from heart and semitendenosus muscle of lambs; possibly a selenium containing cytochrome which may participate in oxidation-reduction reactions, though its catalytic functions are not yet determined. Selenium is also associated with pyruvate oxidation in liver preparations, which suggests that it may function in oxidative processes of the tricarboxylic acid cycle *in vivo* (Bull and Oldfield, 1967).

Godwin et al. (1974) demonstrated lower respiratory rates with fatty acid substrates in muscle mitochondria from selenium deficient lambs suffering from white muscle disease, though no change was found with pyruvate or succinate as substrate. Levander et al. (1973a, 1973b, 1974) have presented evidence and developed the hypothesis that selenium is involved in electron transport. The existence of a nonheme-iron-selenide protein having a role in the electron transport function of mitochondria and smooth endoplasmic reticulum has been postulated (Diplock et al., 1971; Diplock and Lucy, 1973; Diplock, 1974).

It remains unresolved whether selenium in mammalian spermatozoa is a component of GSH-Px, serves a structural role, or functions in some other capacity. Also uncertain is the extent of species differences with respect to selenium utilization in male reproductive processes. In this study, ^{75}Se was used as a radioactive label to investigate the extent of selenium's involvement in reproductive processes of the ram and bull. This was done by using gel filtration and electron autoradiography.

II. ^{75}SE INCORPORATION IN REPRODUCTIVE ORGANS OF RAM AND BULL

Introduction

Gel filtration using Sephadex G-150 showed selenium to be associated with at least four proteins in rat testis cytosol, two of which possessed peroxidase activity (Prohaska et al., 1977). One of these appeared to be selenium dependent and the other non-selenium dependent. McConnell et al. (1978) demonstrated strong selenium binding to three cytosol proteins in rat testis with Ultragel ACA-22 chromatography. However, to date no work has been reported with testis cytosols of other species. Experimental findings of the present study demonstrated the incorporation of ^{75}Se in ram and bull testis cytosols.

Methods and Materials

Two nine-month old, predominantly Suffolk rams, one selenium-deficient (< 0.01 ppm whole blood Se) and one selenium-adequate (0.14 ppm whole blood Se), weighing approximately 53 kg and 68 kg respectively, were injected intramuscularly in the neck with 500 µCi carrier-free ^{75}Se as sodium selenite. Second and third injections of 500 µCi were given ten and three days prior to necropsy, which was 73 days following the initial injection. Selected tissues were removed at necropsy, portions dissected for counting and the remainder immediately frozen on dry ice and stored at -70° C for later assay. ^{75}Se-labeled bull tissues were obtained from a bull injected with

^{75}Se-selenite (Smith et al., 1979) through the courtesy of Dr. P. L. Senger (Dept. Anim. Sci., Washington State University, Pullman, WA). They arrived at our laboratory packed in dry ice, and were stored at -70° C until assayed. Cytosols were prepared by homogenization in 5 vol 10% sucrose -- 0.1M phosphate buffer, pH 6.3, with a Potter-Elvehjem homogenizer followed by centrifugation at 161,000 x g at 4° C for 90 min.

Samples of cytosol were applied to Sephadex G-150 columns (2 x 115 cms), equilibrated with 0.1M phosphate buffer containing 0.03% EDTA and 0.01% NaN_3. Columns were standardized with Blue Dextran, bovine serum albumin, γ-globulin, ovalbumin, soybean trypsin inhibitor, and riboflavin. Effluent fractions were monitored at 280 nm and selected fractions assayed for protein content by the method of Lowry et al. (1951) using bovine serum albumin as standards. In addition, testis cytosols were applied to columns after refrigeration for 24 hours at 4° C, or following dialysis at 4° C for 24 hours against either 0.1M phosphate, pH 6.3, or 0.1M carbonate-bicarbonate, pH 11.0.

Radioactivity was measured with a Beckman 8000 Gamma Counter at an efficiency of 42%. Glutathione peroxidase activity was determined by the coupled enzyme procedure of Paglia and Valentine (1969) as modified by Whanger et al. (1977).

Results

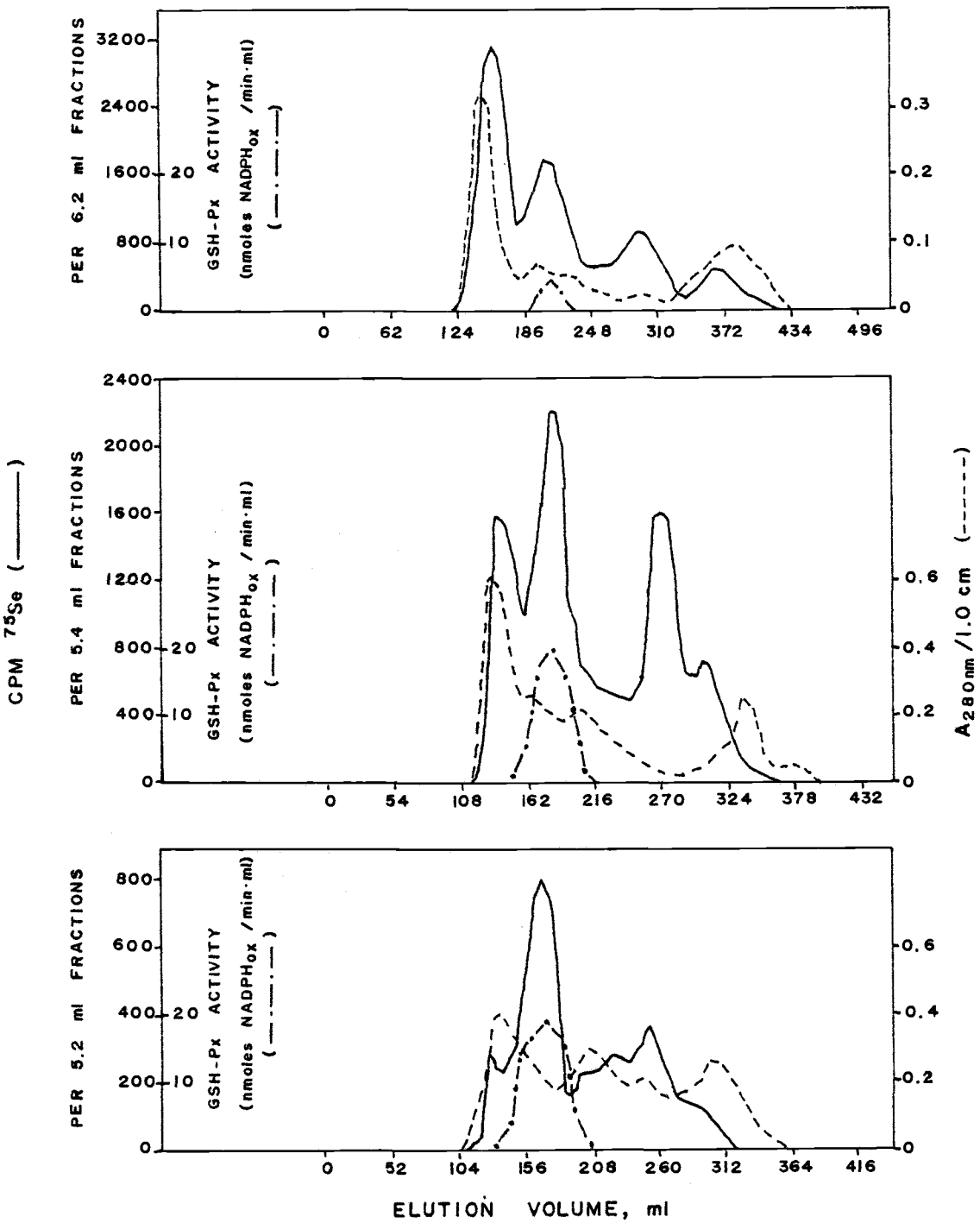
Four ^{75}Se-labeled peaks were obtained from gel filtration of

ram testis cytosol (Figure 1). The Ve/Vo ratios* were approximately 1.09, 1.52, 2.30, and 2.85. Gel filtration of bull testis cytosol resulted in five peaks with Ve/Vo ratios of approximately 1.11, 1.48, 1.90, 2.41, and 2.78 (Figure 1). Glutathione peroxidase activity was detected in the elution peaks having Ve/Vo ratios of 1.52 in the ram and 1.48 in the bull.

Treatment of testis cytosols prior to gel filtration affected the distribution of ^{75}Se in the elution peaks (Figures 2 and 3). Figure 4 shows the actual percent distribution of ram testis cytosol radioactivity among the gel filtration peaks following the different treatments. Dialysis against pH 6.3 and pH 11.0 buffers resulted in 19% and 18% losses of total radioactivity, respectively. The relative change in radioactivity associated with specific peaks after treatment is indicated in Figure 5. The values for peaks from the untreated samples were set at 100 and the corresponding peaks of treated cytosol expressed as a percent of this value. Alkaline dialysis of ram testis cytosol resulted in substantial loss of radioactivity (88%) from the fourth peak (Ve/Vo 2.85), and also a loss from the first peak (Ve/Vo 1.08) (49%). Substantial losses occurred in the lower molecular peak (Ve/Vo 2.15) with other treatments; 87% when dialyzed against pH 6.3 buffer and 72% when stored for 24 hours before chromatography. The radioactivity lost from this peak during storage was apparently redistributed among the other

* Ve = elution volume of the peak and Vo = void volume

Figure 1. Sephadex G-150 gel filtration of selenium-deficient ram, selenium-adequate ram, and bull testis cytosols (2 x 115 cms columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.01% NaN_3, pH 6.3).



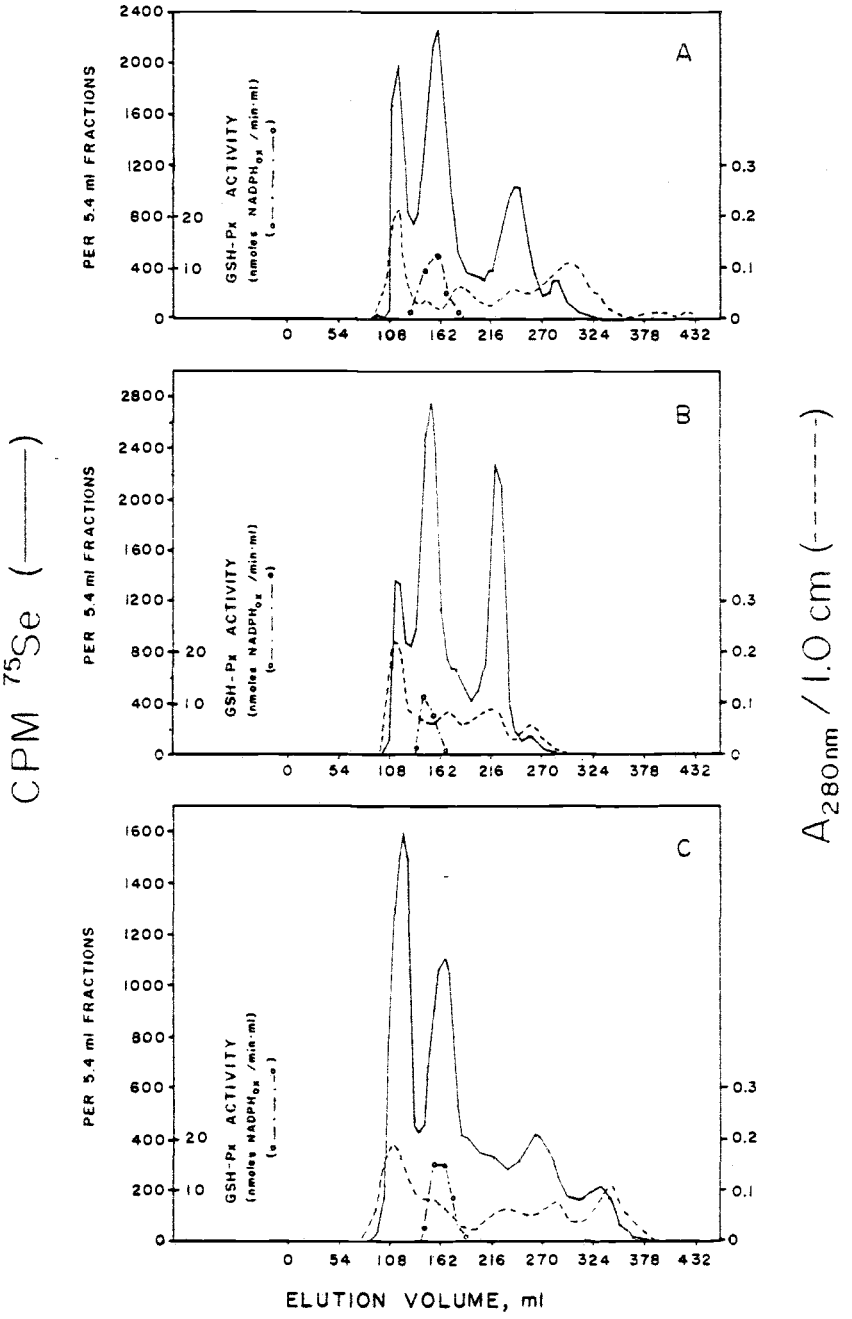
A selenium-deficient ram testis cytosol

B selenium-adequate ram testis cytosol

C bull testis cytosol

Figure 1

Figure 2. Sephadex G-150 gel filtration of selenium-adequate ram testis cytosols following 24 hour dialysis or storage (2 x 115 cms columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.1% NaN_3, pH 6.3).



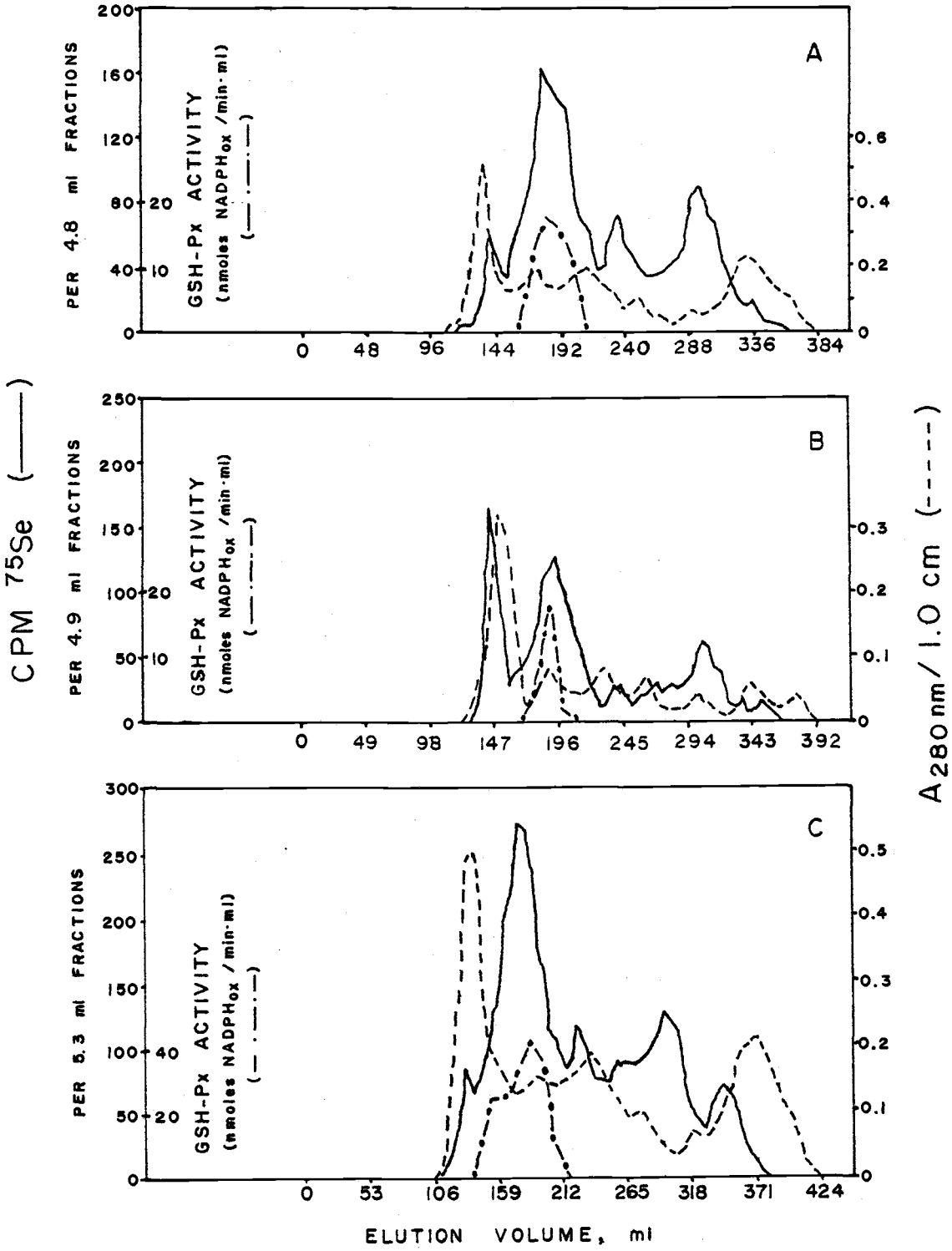
A 24 hour dialysis at 4° C against pH 6.3.

B 24 hour dialysis at 4° C against pH 11.0.

C 24 hour storage at 4° C.

Figure 2

Figure 3. Sephadex G-150 gel filtration of bull testis cytosols following 24 hour dialysis or storage (2 x 115 cms columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.01% NaN_3, pH 6.3).

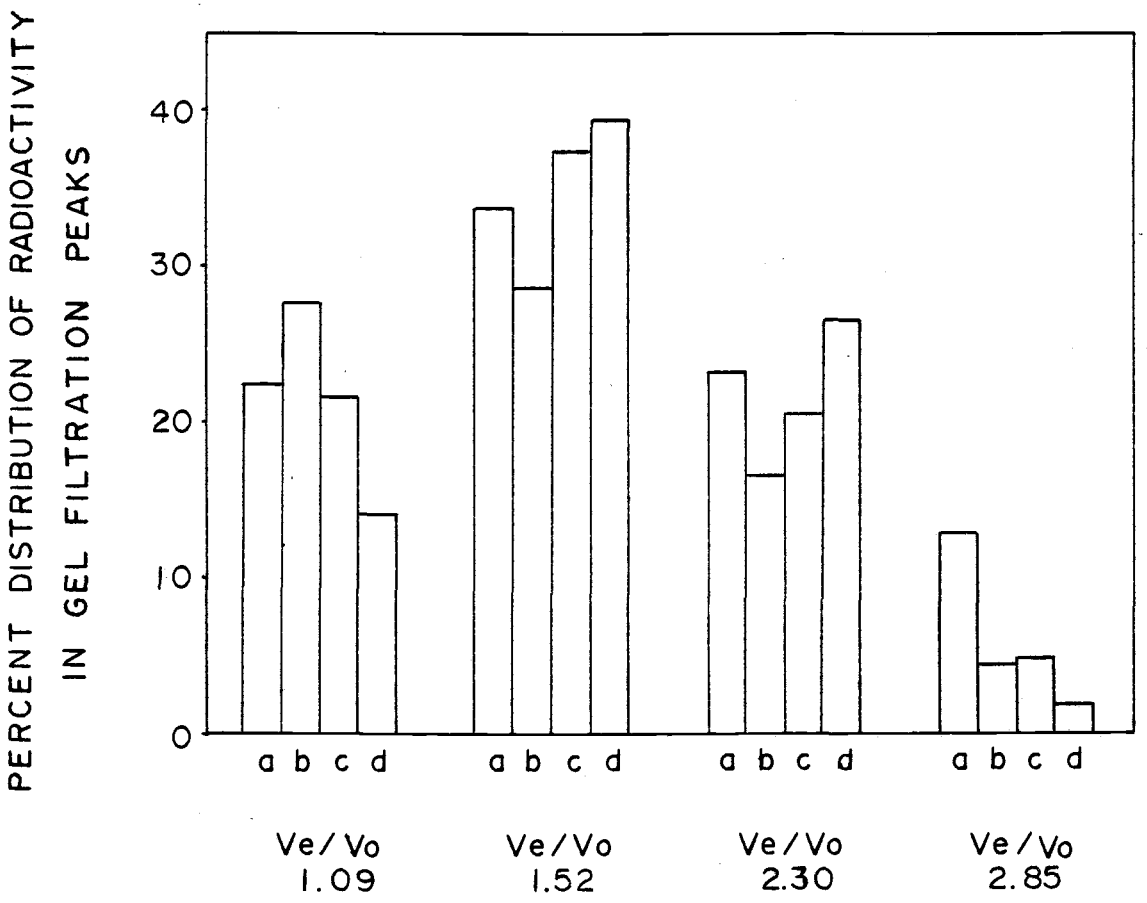


A 24 hour dialysis at 4° C against pH 6.3.

B 24 hour dialysis at 4° C against pH 11.0.

C 24 hour storage at 4° C.

Figure 3



a. no treatment prior to gel filtration

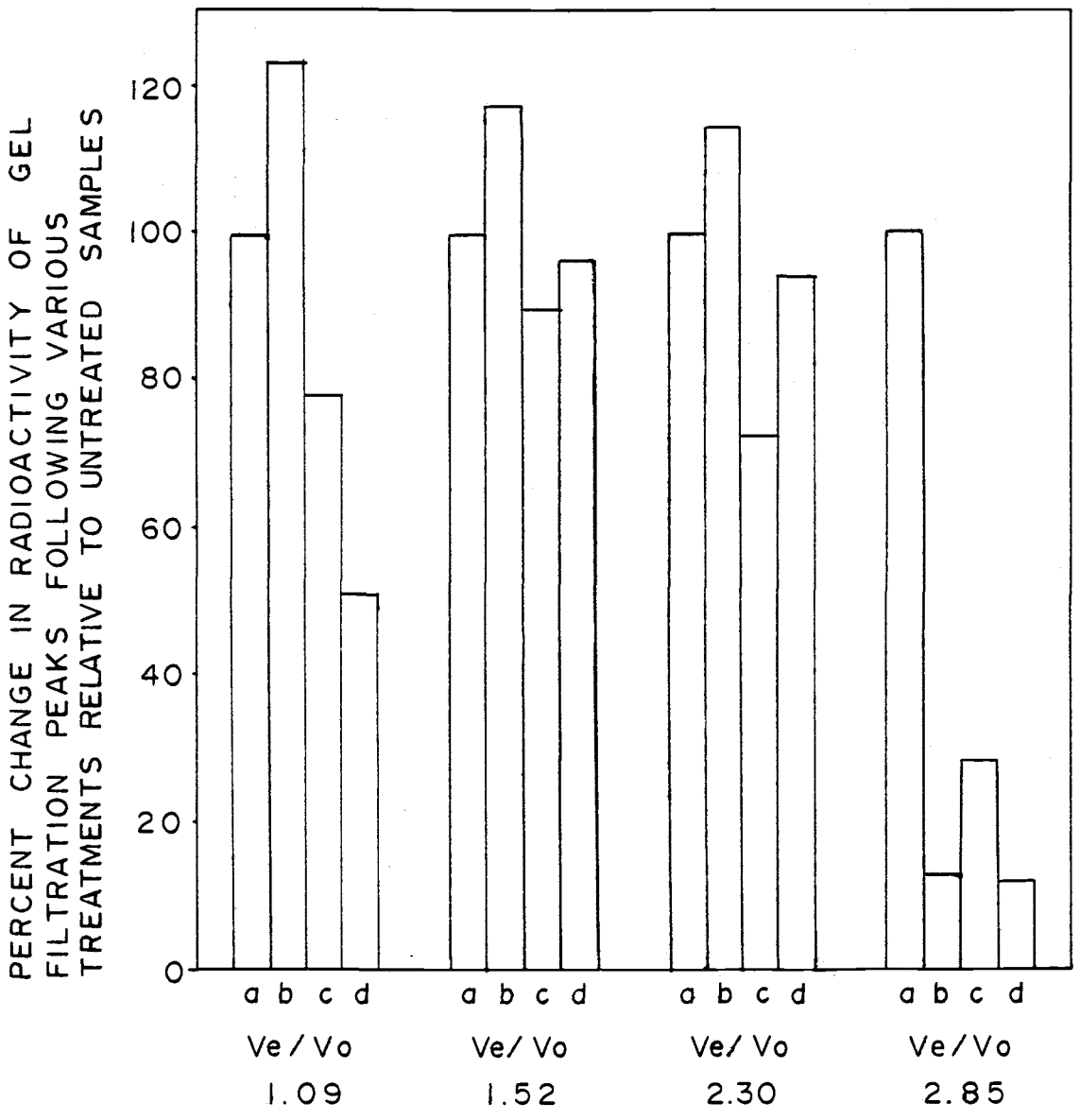
b. storage at 4° C for 24 hours prior to gel filtration

c. dialysis at 4° C for 24 hours against pH 6.3 buffer prior to gel filtration

d. dialysis at 4° C for 24 hours against pH 11.0 buffer prior to gel filtration

Figure 4. Actual percent distribution of testis cytosol radioactivity in Sephadex G-150 filtration peaks from a selenium-adequate ram, following various treatments.

Figure 5. Relative percent change with respect to untreated cytosol in radioactivity of Sephadex G-150 gel filtration peaks of ram testis cytosol following various treatments.



a. no treatment prior to gel filtration

b. storage at 4° C for 24 hours prior to gel filtration

c. dialysis at 4° C for 24 hours against pH 6.3 buffer prior to gel filtration

d. dialysis at 4° C for 24 hours against pH 11.0 buffer prior to gel filtration

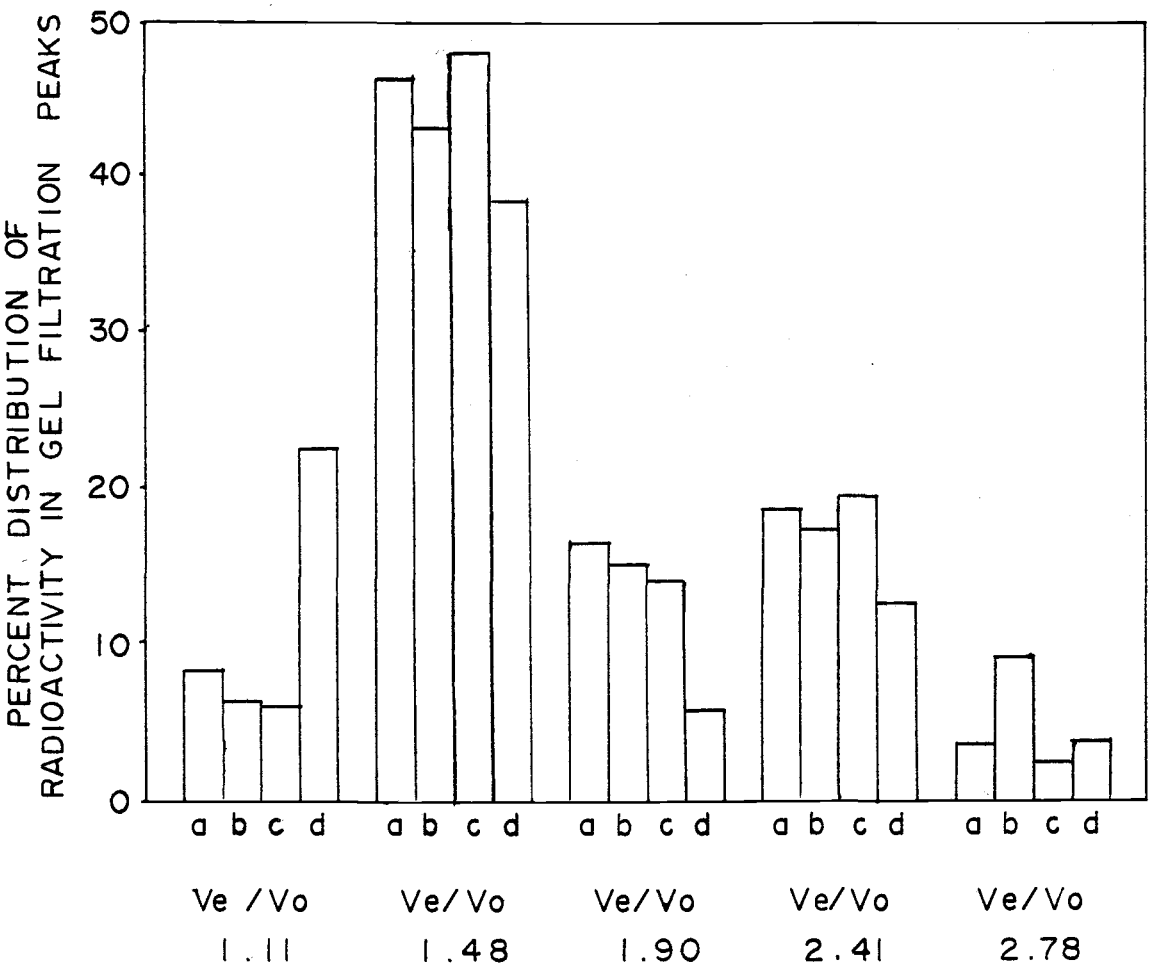
Figure 5

peaks.

The actual percentage distribution of ^{75}Se in bull testis cysotol gel filtration peaks (Figure 6) and the pattern of ^{75}Se loss from treated cytosol, relative to untreated cytosol (Figure 7), differed considerably from that for the ram. Dialysis against pH 6.3 buffer resulted in a 42% loss of total radioactivity, while 50% was lost when dialysed against pH 11.0 buffer. ^{75}Se label was lost from all peaks when dialysed at pH 6.3, but at pH 11.0 the first peak gained radioactivity while radioactivity was lost in other peaks. Also in contrast to the ram, 24 hour storage resulted in a loss from the first peak (Ve/Vo 1.11) and a gain of ^{75}Se in the low molecular weight peak (Ve/Vo 2.78).

Mammalian testes are known to progressively accumulate ^{75}Se and seminal vesicles in rats have been reported as having high ^{75}Se concentrations (Anghileri and Marques, 1965); but levels of ^{75}Se in accessory glands relative to the concentration in testes have not been reported. Seminal vesicles of the ram and bull, and the bull prostate gland accumulated higher levels of ^{75}Se, and showed greater total GSH-Px activity than did Cowper's glands (Table 1). Levels of GSH-Px and ^{75}Se radioactivity in testes exceeded those of accessory glands. The ratio of cpm ^{75}Se to GSH-Px activity was fairly constant among bovine glands, but in the ram this ratio appeared to be higher for the testis than for other tissues.

Gel filtration elution profiles of seminal vesicle and Cowper's gland cytosols from ram and bull, and prostate gland cytosol from



a. no treatment prior to gel filtration

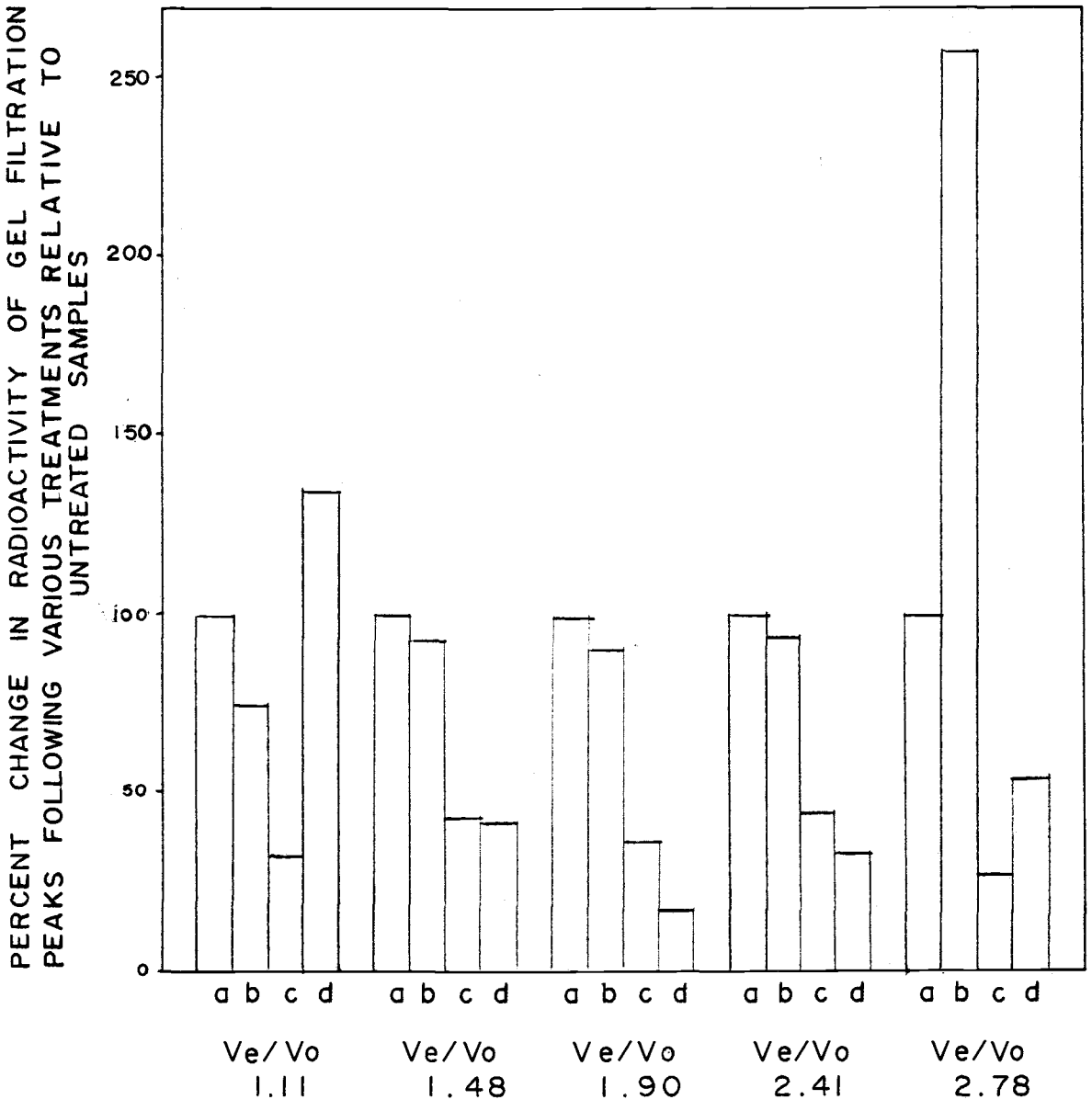
b. storage at 4° C for 24 hours prior to gel filtration

c. dialysis at 4° C for 24 hours against pH 6.3 buffer prior to gel filtration

d. dialysis at 4° C for 24 hours against pH 11.0 buffer prior to gel filtration

Figure 6. Actual percent distribution of bull testis cytosol radioactivity in Sephadex G-150 gel filtration peaks following various treatments.

Figure 7. Relative percent change with respect to untreated cytosol in radioactivity of Sephadex G-150 gel filtration peaks of bull testis cytosol following various treatments.



a. no treatment prior to gel filtration

b. storage at 4° C for 24 hours prior to gel filtration

c. dialysis at 4° C for 24 hours against pH 6.3 buffer prior to gel filtration

d. dialysis at 4° C for 24 hours against pH 11.0 buffer prior to gel filtration

Figure 7

Table I. Glutathione peroxidase activities and ^{75}Se cpm/g tissue in testis and accessory gland cytosol from ram and bull.

	(1) nmoles $NADPH_{ox}$/min·ml	(2) cpm/g	ratio $\frac{(2)}{(1)}$
RAM TISSUES			
Testis	686	40,456	59
Seminal vesicles	434	18,968	44
Cowper's glands	203	8,492	42
BULL TISSUES			
Testis	683	22,850	33
Seminal vesicles	346	12,431	36
Cowper's glands	152	4,369	29
Prostate gland	506	15,043	30

the bull are presented in Figures 8-10. Ram seminal vesicle cytosol showed two broad ^{75}Se peaks (Figure 8). The appearance of the peaks, in comparison with those of the Cowper's gland (also Figure 8) suggest that increased chromatographic resolution may reveal more peaks. There are at least two ^{75}Se peaks in the chromatogram of bull seminal vesicles (Figure 9) (Ve/Vo ratios of 1.10 and 1.45). The major peak (Ve/Vo 1.45) possessed GSH-Px activity. In contrast, at least three ^{75}Se peaks were apparent in Cowper's gland cytosol of the bull (Figure 9) (Ve/Vo ratios of approximately 1.20, 1.50, and 2.00), none of which had detectable levels of GSH-Px activity. Prostate gland cytosol from the bull (Figure 10) also possessed three ^{75}Se gel filtration peaks (with approximate Ve/Vo ratios of 1.14, 1.50, and 2.14). Considerable GSH-Px activity was seen in the predominant ^{75}Se peak (Ve/Vo 1.50) and a small amount of activity was also present in the peak at 1.14 Ve/Vo.

Elution profiles of liver cytosol from the bull and ram (Figure 11) provide a comparison to the profiles of cytosol from the testes and accessory glands of bull and ram. Ram liver cytosol chromatographs showed one major and three minor ^{75}Se peaks (Ve/Vo ratios of 1.09, 1.47, 2.47, and 3.12). Likewise, one major peak and suggestions of at least three minor peaks were found in bull liver cytosol (Ve/Vo ratios of 1.14, 1.47, 1.64, and 2.47). The position of ^{75}Se peaks in ram testis and liver cytosols were similar, though there were three major peaks in ram testis cytosol and only one in ram liver cytosol. Bull testis and liver cytosols had similar

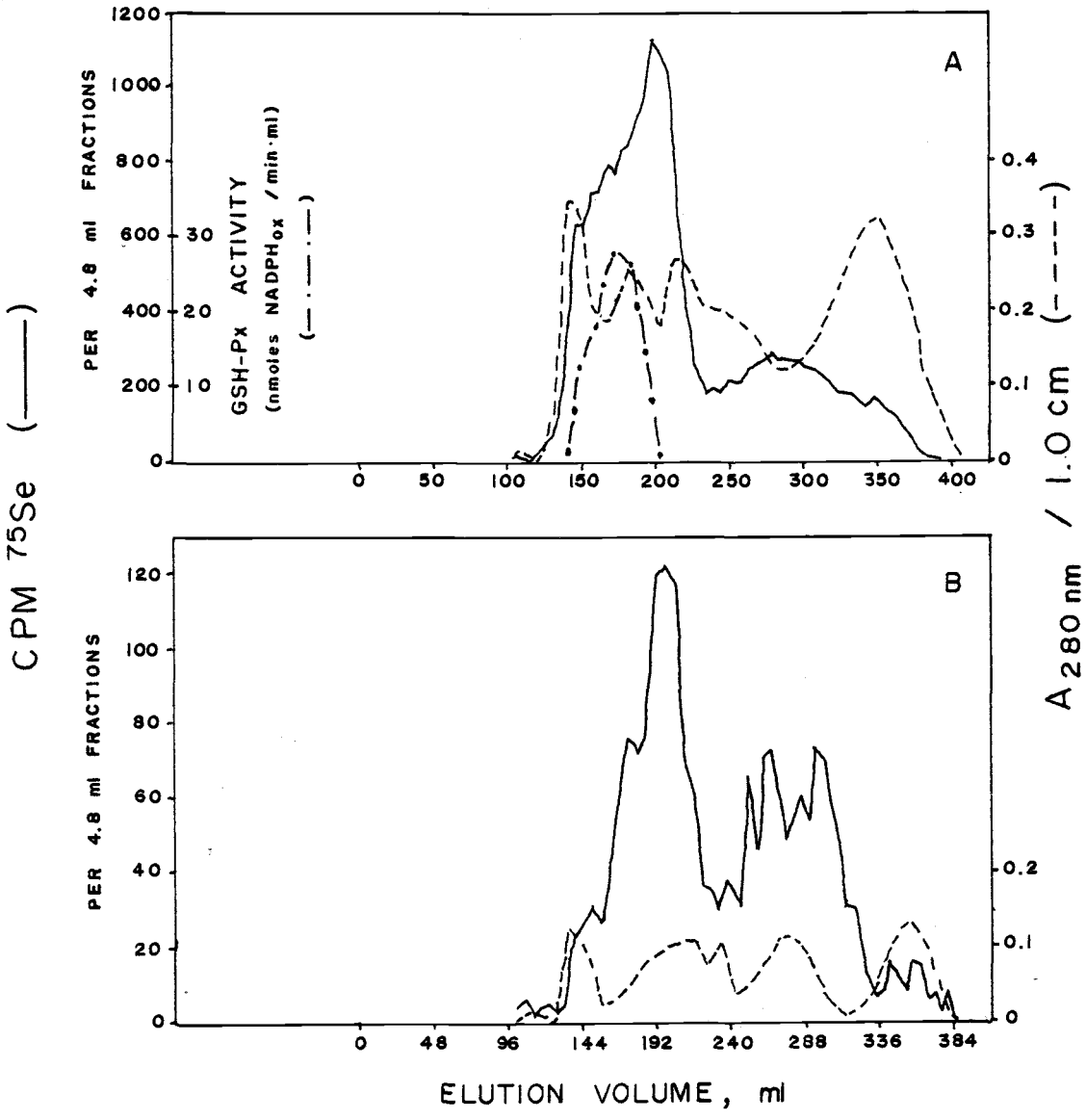


Figure 8. Sephadex G-150 gel filtration of seminal vesicle cytosol (A), and Cowper's glands cytosol (B) from a selenium-adequate ram (2 x 115 cms columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.01% NaN_3, pH 6.3).

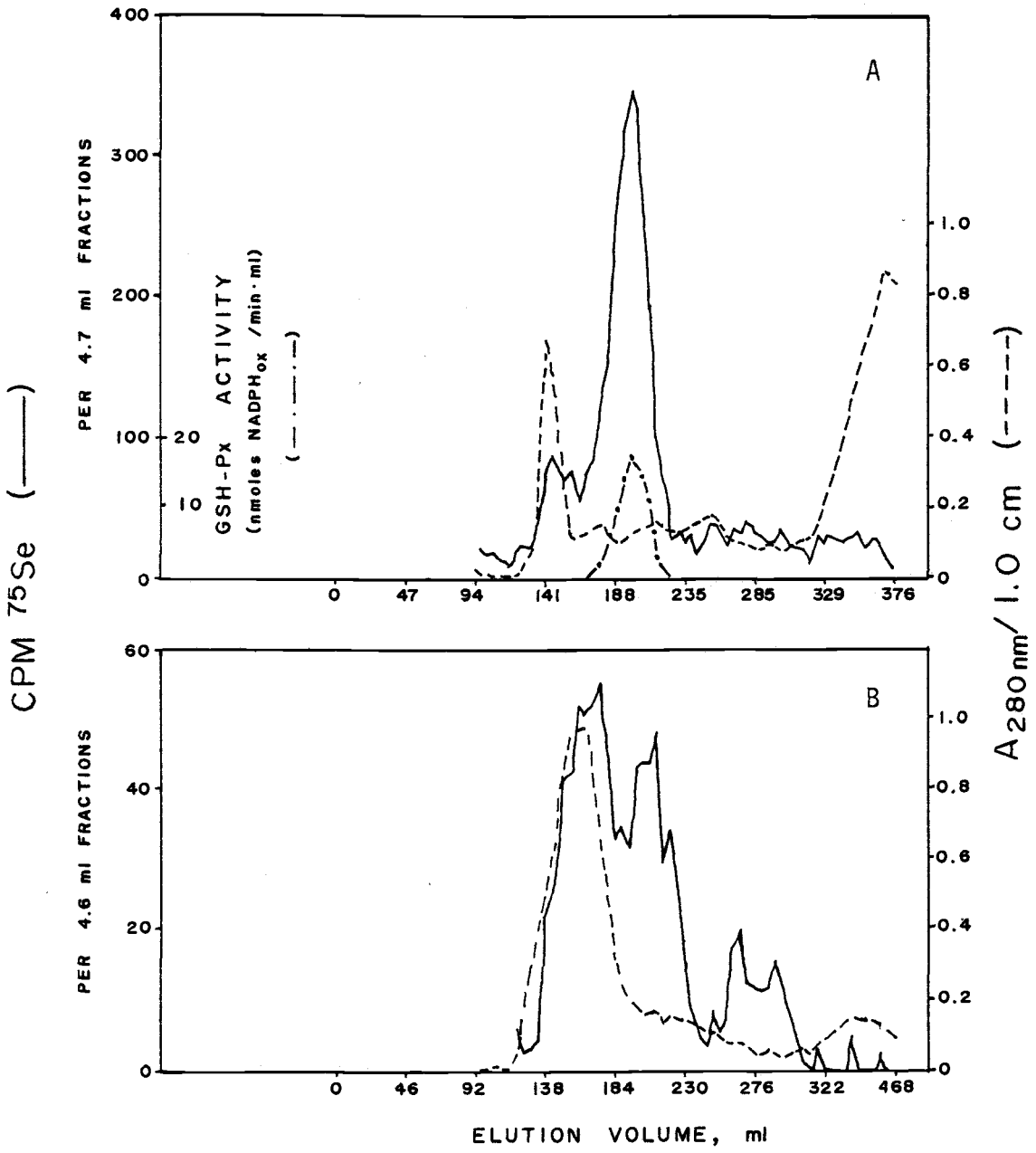


Figure 9. Sephadex G-150 gel filtration of seminal vesicle cytosol (A), and Cowper's glands cytosol (B) from bull (2 x 115 cms columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.01% NaN_3, pH 6.3).

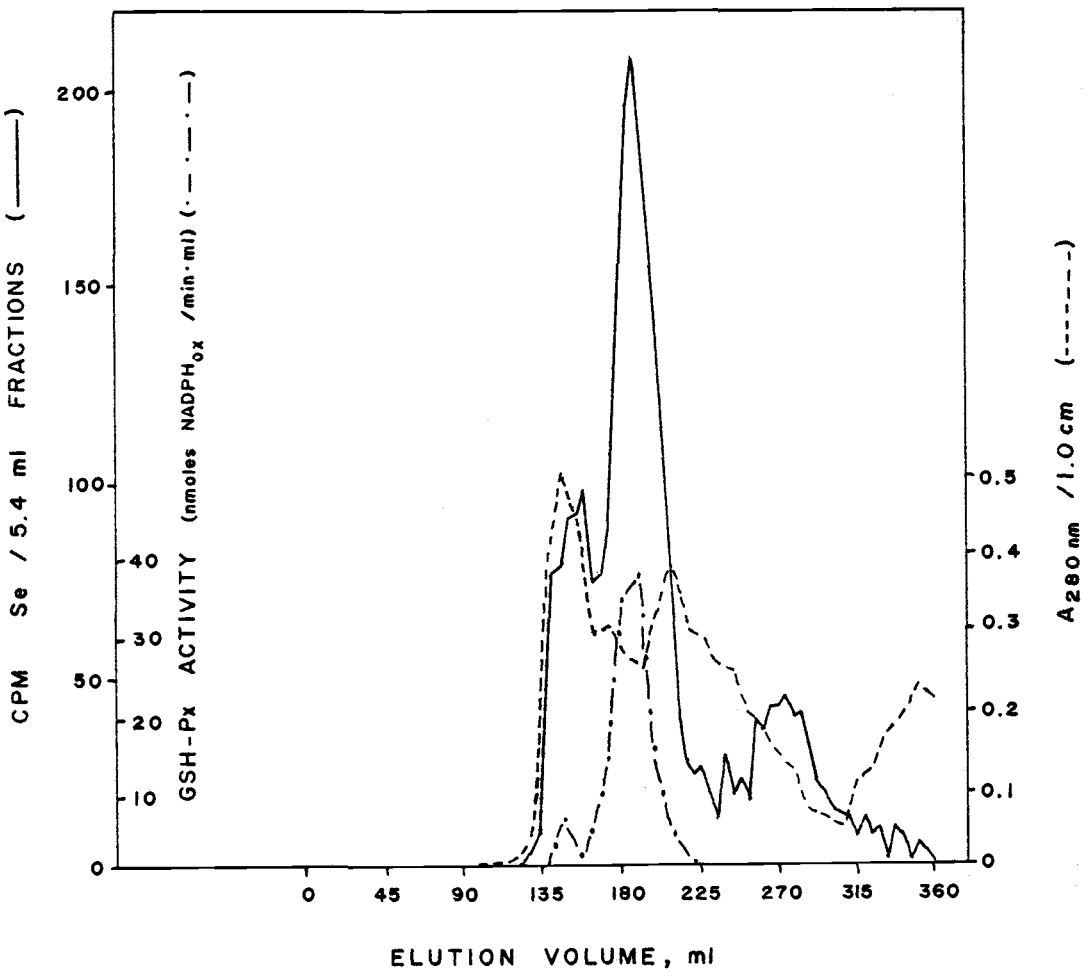


Figure 10. Sephadex G-150 gel filtration of bull prostate gland cytosol (2 x 115 cms columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.01% NaN_3, pH 6.3).

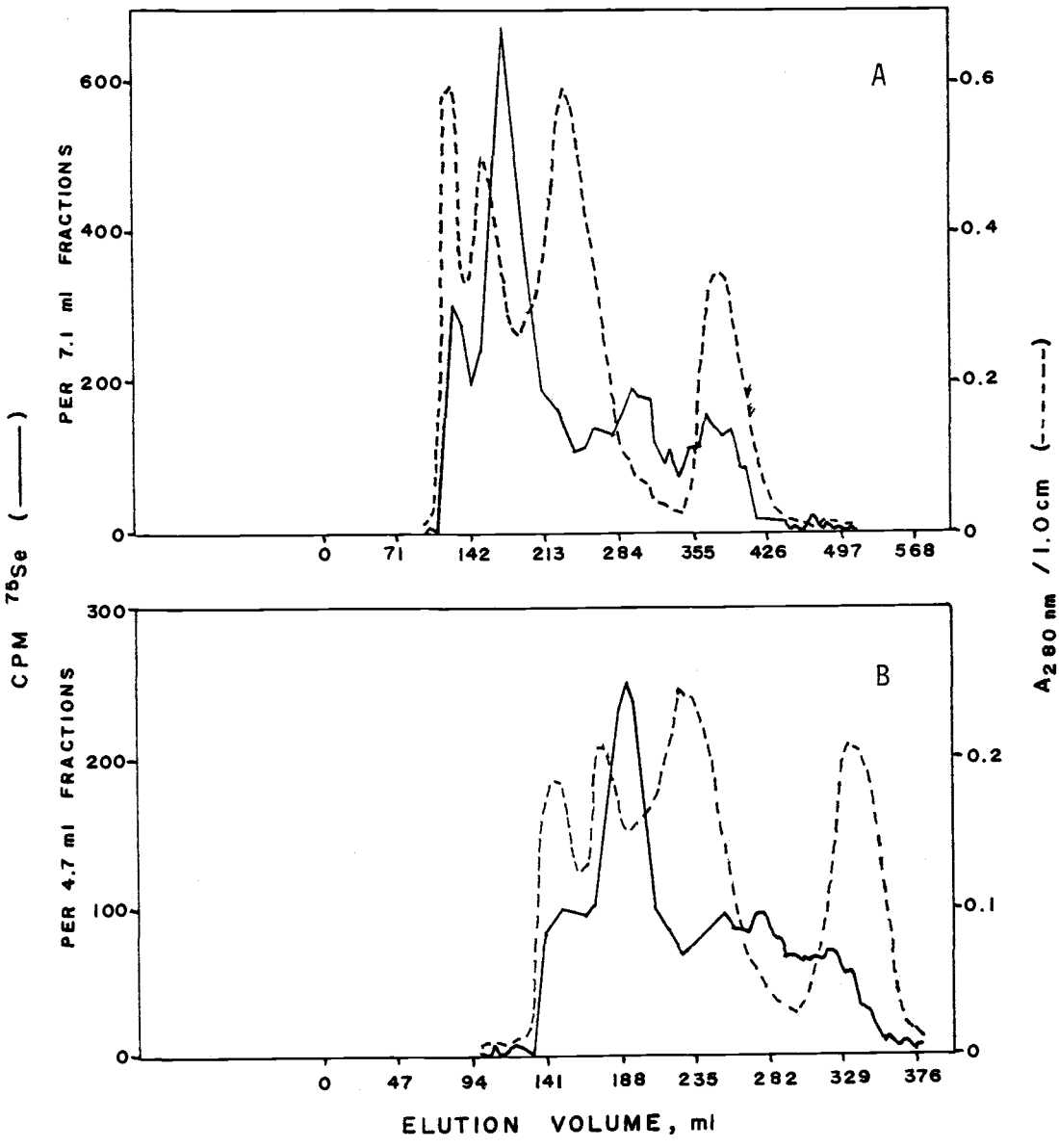


Figure 11. Sephadex G-150 gel filtration of liver cytosol from a selenium-adequate ram (A), and a bull (B) (2 x 115 cms columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.1% NaN_2, pH 6.3). (ram liver date from Beilstein, 1980).

elution profiles.

Discussion

A similarity existed between Ve/Vo ratios of the ^{75}Se-labeled gel filtration peaks from the ram testis cytosol and four of the five bull testis cytosol peaks. The additional peak in bull testis cytosol had a Ve/Vo ratio of 1.90. Cummins and Martin (1967) assessed the strength of selenium binding to protein by alkaline dialysis. Ionically bound selenium can be displaced from its binding site by increasing the pH of its environment. McConnell et al. (1979) found that 85% of the radioactivity was lost from ^{75}Se-labeled rat testis cytosol upon overnight dialysis against 0.5M NaOH, whereas, under denaturing or reducing conditions, at a neutral pH, relatively little label was lost. Dialysis experiments with bull and ram testis cytosol (Figures 5 and 7) suggest a greater binding stability of selenium with the peak having GSH-Px activity (Ve/Vo 1.52 for ram and Ve/Vo 1.48 for bull), though caution is required in interpretations based upon the limited amount of data.

Semen from a number of animals is known to possess GSH-Px activity (Li, 1975; Brown et al., 1977). Data of this study suggest that the prostate gland may contribute a large amount of the GSH-Px in seminal plasma. Cowper's glands apparently do not contribute significantly to seminal GSH-Px. In both the ram and bull these glands did not accumulate ^{75}Se to the degree that the seminal vesicles or prostate gland did. Even though seminal vesicles may contribute to seminal GSH-Px, it should be noted that GSH-Px has

been suggested to be required for prostaglandin synthesis (Lands et al., 1971; Nugteren and Hazelhof, 1973), which occurs to the greatest extent in seminal vesicles. Thus, seminal vesicle GSH-Px may not be available for export into the seminal plasma.

III. ^{75}SE INCORPORATION IN SEMEN OF RAM AND BULL

Introduction

Selenium-75 has been shown to be incorporated into developing spermatozoa of rats, mice, and bulls (Burk et al., 1972; Gunn et al., 1967; Smith et al., 1979). Data for bulls were thought to indicate selenium incorporation by spermatids (Smith et al., 1979). Gunn and Gould (1970) interpreted data from rat experiments as evidence of selenium incorporation in spermatogenesis earlier than the spermatid stage.

Selenium-dependent glutathione peroxidase (GSH-Px; E.C. 1.11.1.9) activity has been found in semen of ram, dog, goat and human, but it has not been detected in boar or rabbit semen (Li, 1975). GSH-Px activity has been detected in bull seminal plasma but not the spermatozoa (Brown et al., 1977; Smith et al., 1979).

Methods and Materials

Semen was collected periodically during a 63-day period by electroejaculation from the same selenium-deficient and selenium-adequate rams as described in Part II Methods. Sperm were separated from seminal plasma by low speed centrifugation and washed with 0.9% NaCl. Sperm counts were made for all ejaculates. Bull semen was obtained from a bull injected with ^{75}Se-selenite (Smith et al., 1979) as described in Part II Methods. It was shipped to our laboratory packed in dry ice. Radioactivity of samples was measured

by a Beckman 8000 Gamma Counter with an efficiency of 42%. Glutathione peroxidase activities were determined by the coupled enzyme procedure of Paglia and Valentine (1969) as modified by Whanger et al. (1977). Either columns of Sephadex G-150 (2 x 115 cms) or Sephacryl S-200 (2 x 100 cms) were used in the gel filtration studies of seminal plasma. Columns were standardized with Blue Dextran, bovine serum albumin, γ-globulin, ovalbumin, soybean trypsin inhibitor, and riboflavin. Samples were eluted with 0.1M sodium phosphate buffer containing 0.03% EDTA and 0.01% NaN_3. Column effluents were continuously monitored at 254 nm by an ISCO UA-2 absorbance monitor. Seminal plasma and selected column fractions were assayed for protein content by the method of Lowry et al. (1951) using bovine serum albumin as standards.

Results

Two concentration peaks of isotope were found in ejaculated sperm over a 63-day observation period in both the selenium-deficient ram (-Se ram) and the selenium-adequate ram (+Se ram) (Figure 1). Concentration peaks occurred in the -Se ram at approximately 32 and 52 days, whereas those for the +Se ram were at approximately 39 and 52 days. By day 21 in the -Se ram, the sperm contained 94% of ^{75}Se counts in whole semen and this level was maintained throughout the remainder of the observation period. In the +Se ram the percentage of counts in the sperm increased more slowly, reaching a plateau after about 35 days.

Chromatographic elution profiles for ram and bull seminal

Figure 1. cpm ^{75}Se in ejaculated sperm and percent of total ejaculated counts in sperm for a selenium-deficient and a selenium-adequate ram, injected intramuscularly with 500 µCi Se-75.

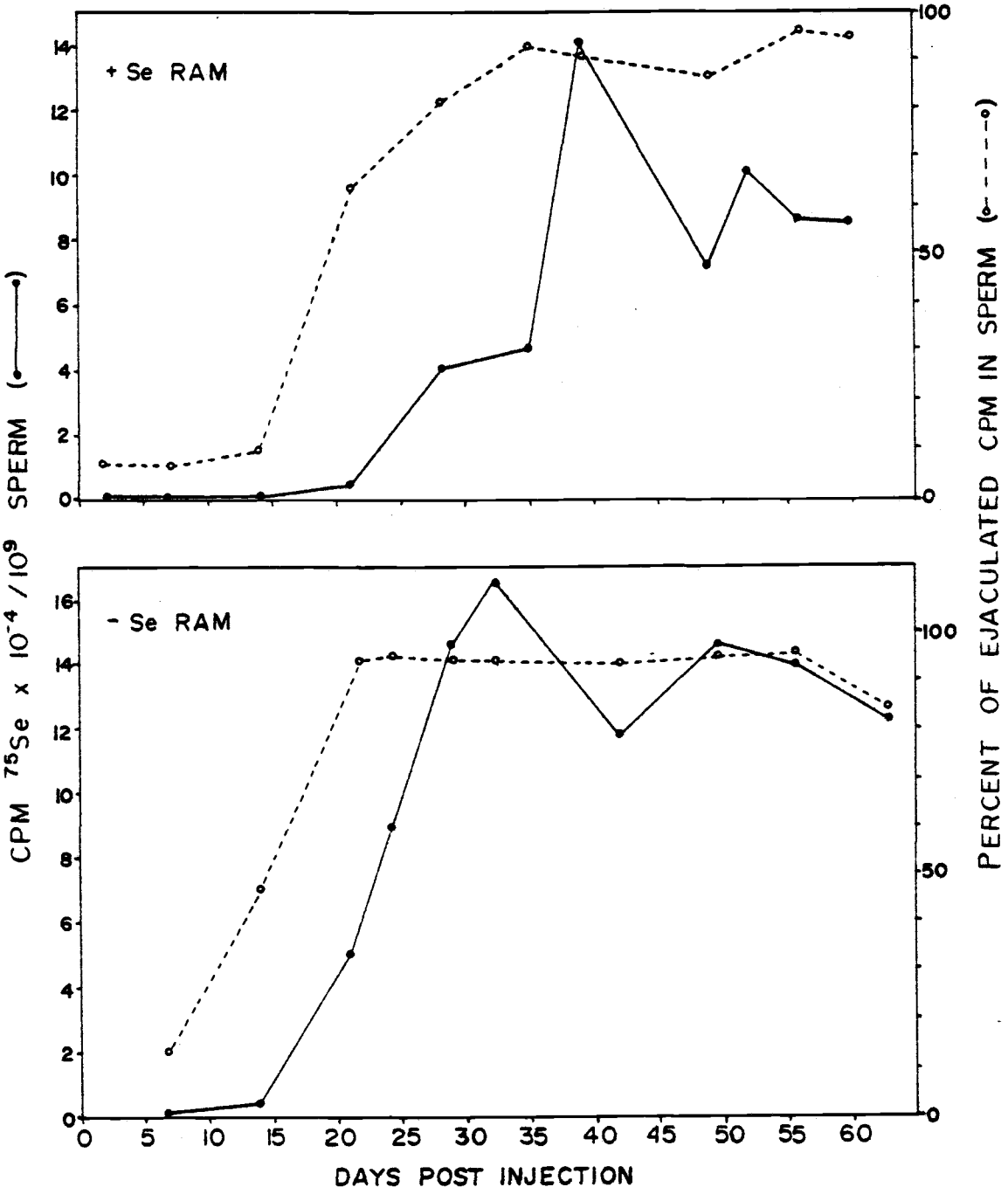
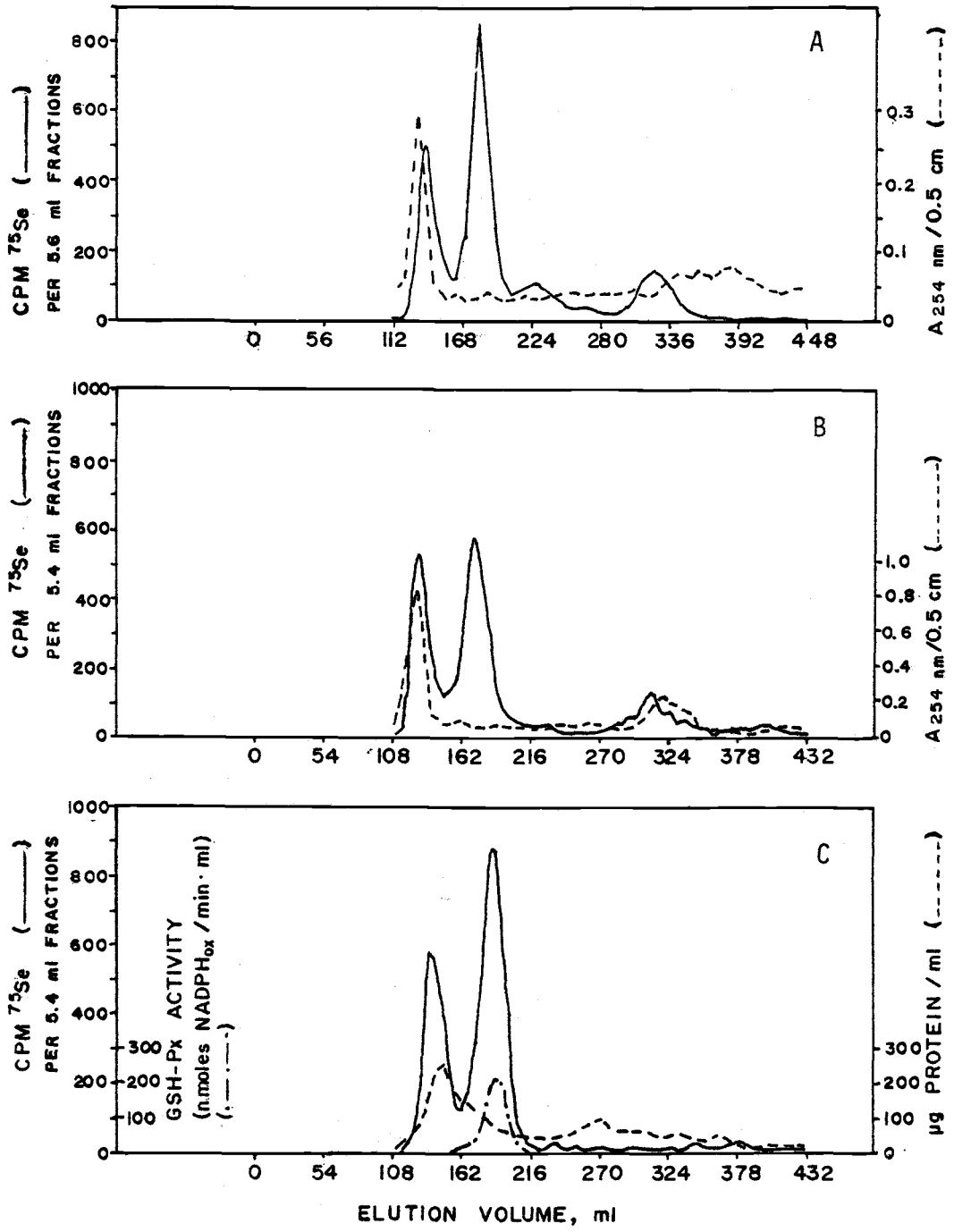

+ Se RAM
- Se RAM
CPM ^{75}Se x 10^{-4} / 10^{9} SPERM (●——●)
PERCENT OF EJACULATED CPM IN SPERM (o- - - -o)
DAYS POST INJECTION
0
5
10
15
20
25
30
35
40
45
50
55
60
2
4
6
8
10
12
14
16
50
100

Figure 1

plasma are given in Figure 2. Sephacryl S-200 gel filtration of seminal plasma from the -Se ram resulted in four ^{75}Se peaks with Ve/Vo ratios of 1.06, 1.40, 1.73, and 2.42. The first two peaks were the predominant ones. In only one of the +Se ram ejaculates was the seminal plasma volume and specific activity high enough to permit column chromatography. The Sephadex G-150 elution pattern for this sample was similar to the Sephacryl S-200 patterns of the -Se ram, showing two major peaks. Bull seminal plasma ^{75}Se radioactivity eluted in two peaks from Sephadex G-150. These peaks corresponded to the major peaks from ram seminal plasma (Ve/Vo ratios of 1.06 and 1.40). The peaks at 1.40 Ve/Vo eluted in the same position as did erythrocyte GSH-Px standards. However, GSH-Px activity was only detected in eluant of bull seminal plasma. In seminal plasma of the -Se ram, the percent contribution of each peak to total seminal plasma radioactivity varied with time after injection (Figure 3). When normalized to semen volume, ^{75}Se cpm for each peak reached a maximum near the 49th day post injection (Figure 4).

The radioactivity per mg protein in seminal plasma of the -Se ram reached a peak after 49 days (Figure 5). This trend was not as apparent in +Se ram seminal plasma, but the peak appears to be around 38 days. GSH-Px activity per mg protein varied greatly in both rams during the observation period (Figure 6). The total GSH-Px activity (nmoles NADPH ox/min) per ml seminal plasma had a value in the +Se ram of 385, while the activity in bull seminal plasma was 2908. However,

Figure 2. Gel filtration of seminal plasma from a selenium-deficient ram, a selenium-adequate ram, and bull injected intramuscularly with 500 μCi Se-75 (Sephacryl S-200 column - 2 x 100 cms; Sephadex G-150 column - 2 x 115 cms; columns eluted with 0.1M phosphate buffer containing 0.03% EDTA and 0.01% NaN_3, pH 6.3).



A selenium-deficient ram seminal fluid, day 42; Sephacryl S-200

B selenium-adequate ram seminal fluid, day 39; Sephadex G-150

C bull seminal fluid; Sephadex G-150

Figure 2

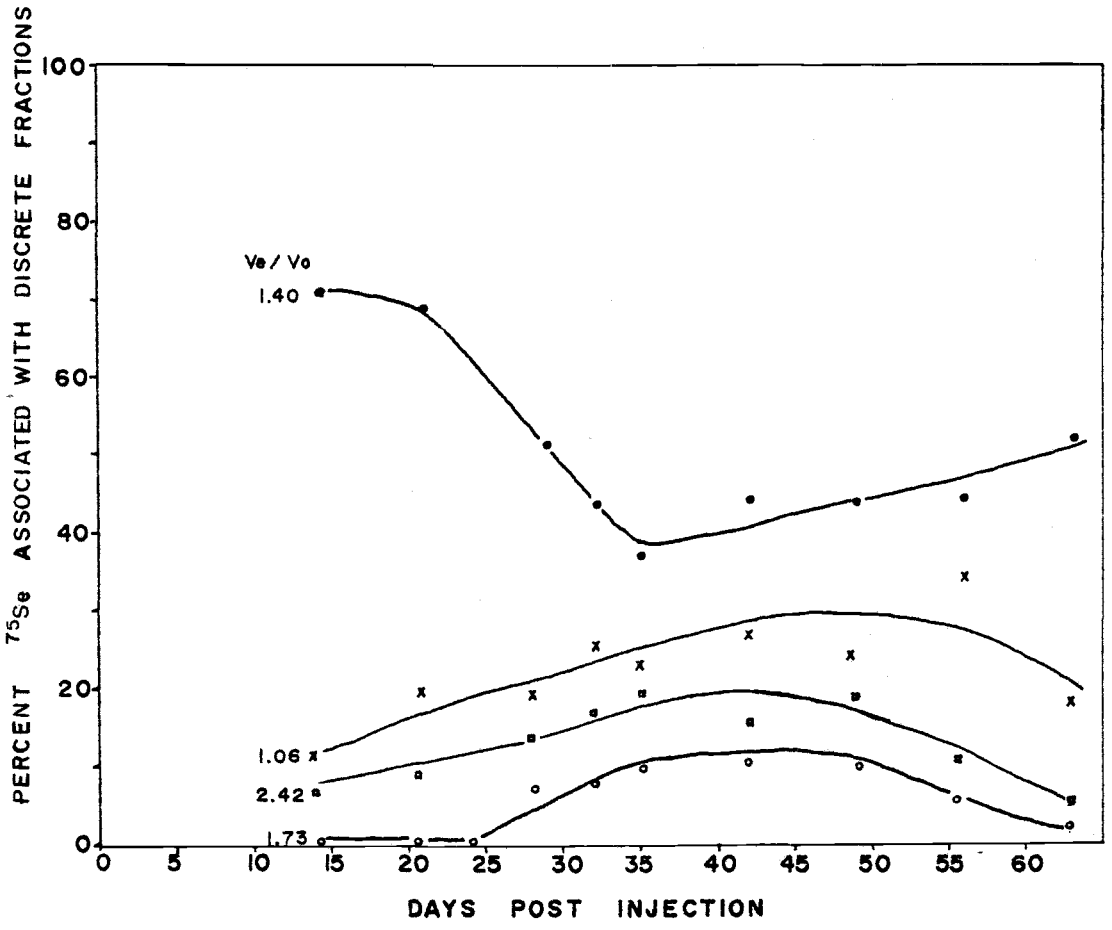


Figure 3. Distribution of ^{75}Se in Sephacryl S-200 gel filtration fractions of seminal plasma from a selenium-deficient ram injected intramuscularly sith 500 µCi Se-75 (percent determined by weight).

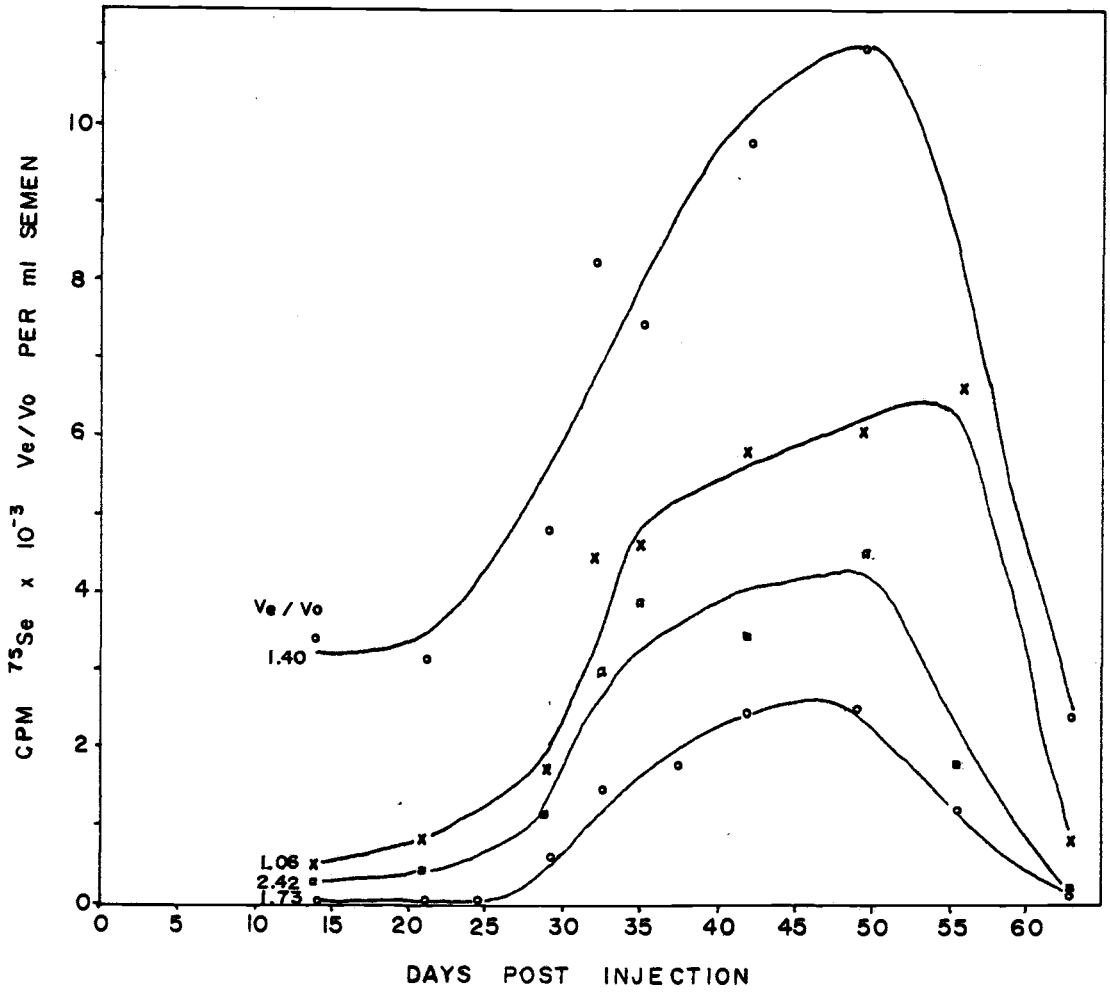


Figure 4. cpm ^{75}Se in Sephacryl S-200 gel filtration fractions of seminal plasma from selenium-deficient ram injected intramuscularly with 500 µCi Se-75. Counts normalized per ml semen.

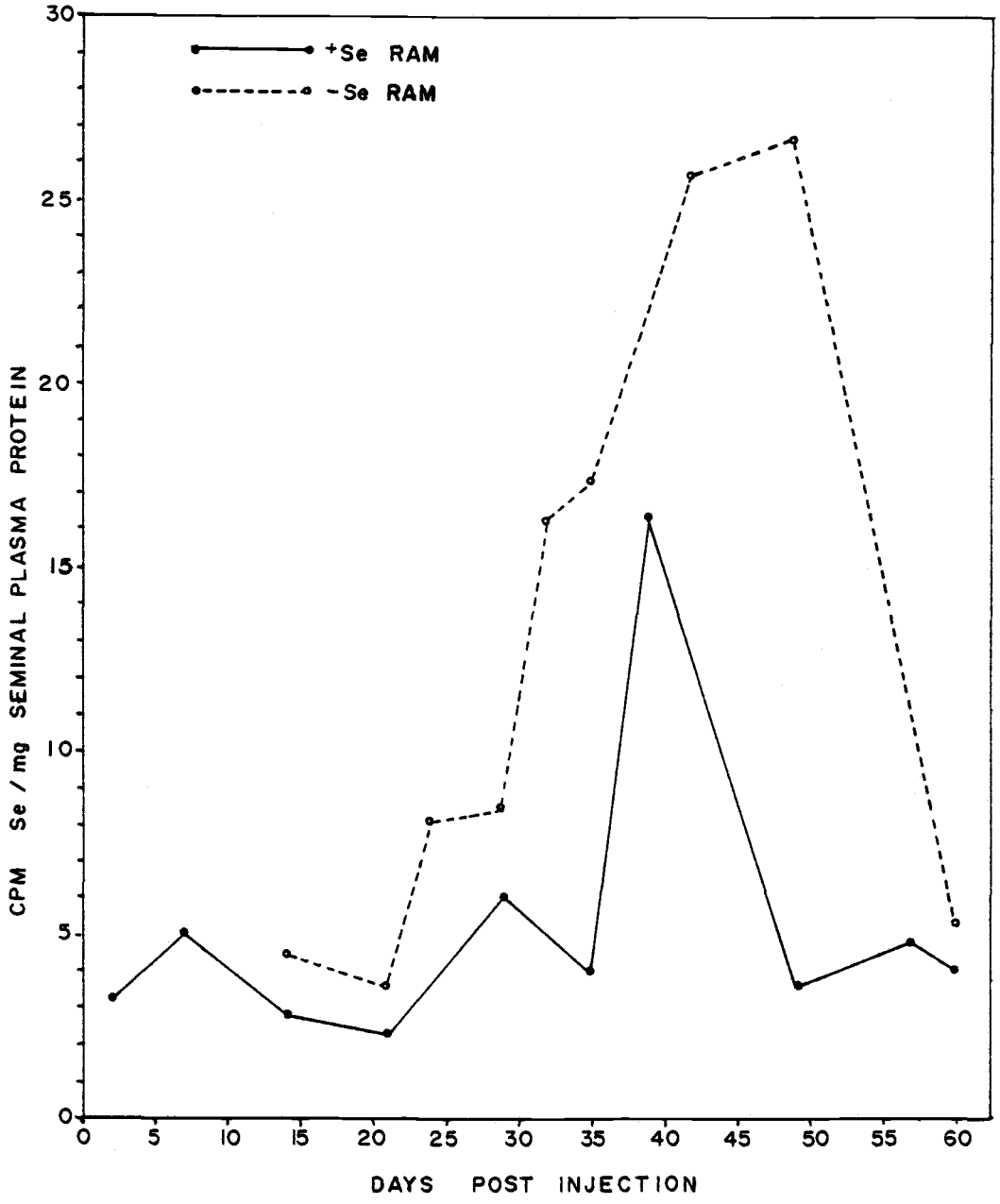
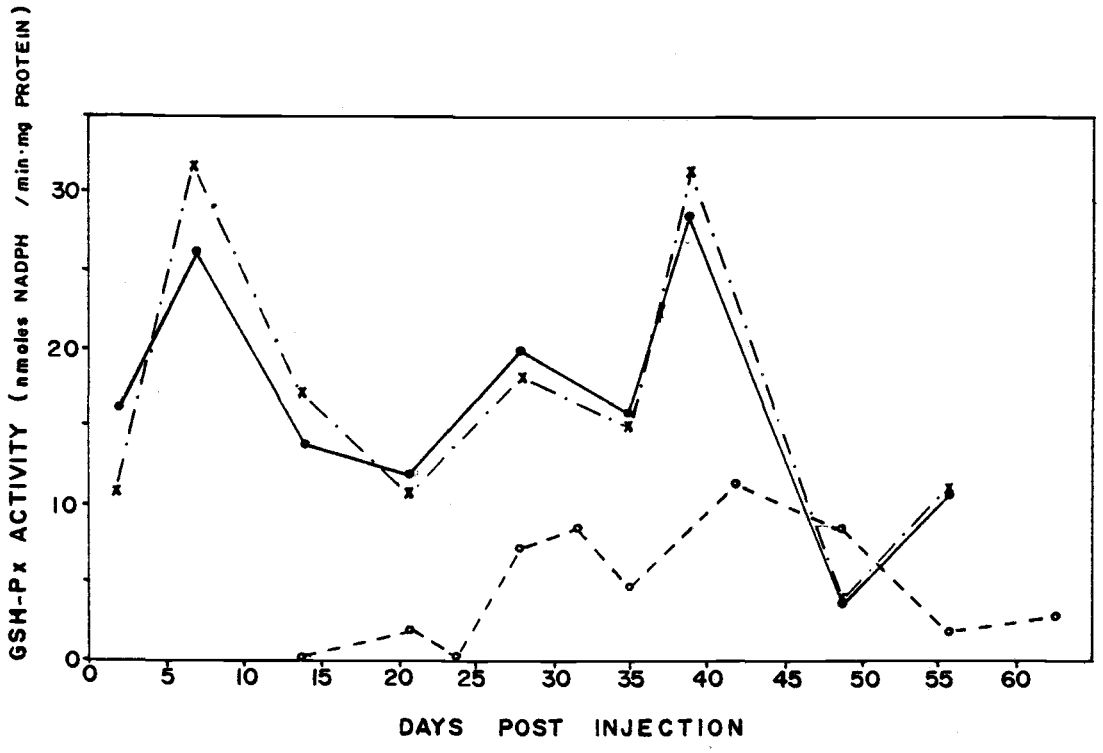


Figure 5. cpm ^{75}Se per mg protein in seminal plasma for a selenium-deficient ram and a selenium-adequate ram injected intramuscularly with 500 μCi Se-75.



- - - - - - selenium-deficient ram; H_2O_2 as substrate

—•—•—•—•—• selenium-adequate ram; +butyl O_2 as substrate

———————— selenium-adequate ram; H_2O_2 as substrate

Figure 6. Glutathione peroxidase activity (nmoles $NADPH_{ox}$/ min·mg protein in seminal plasma of a selenium-deficient ram and a selenium-adequate ram.

when expressed on a per mg protein basis, the values of these samples were comparable at 28.97 for the ram, and 27.73 for the bull.

The maximal ^{75}Se concentration (44,000 cpm/ml) in whole blood of the -Se ram was reached 2-5 hours after injection (Figure 7). This was 11 fold less than the maximal concentration obtained by semen.

Discussion

Patterns of selenium incorporation in spermatogenesis will reflect either exposure of spermatogenic cells to selenium or their uptake efficiencies for the available selenium. To date it is not known whether microenvironmental selenium concentrations vary within the seminiferous tubules or if selenium is equally available to cells throughout the tubule. Also uncertain is whether all spermatogenic cells have equal efficiencies of selenium incorporation.

^{75}Se was used in this study to monitor selenium incorporation into developing sperm cells of a selenium-deficient and a selenium-adequate ram. It is reasonable to assume that following exposure to an initially high ^{75}Se pulse spermatogenic cells of the ram continued to be exposed to ^{75}Se, though at a lower level, for a period of weeks thereafter, as indicated by selenium levels in the blood (see Figure 7).

The high percentage (~93%) of total semen counts seen in the sperm collected from the -Se ram between days 21 and 56 suggests

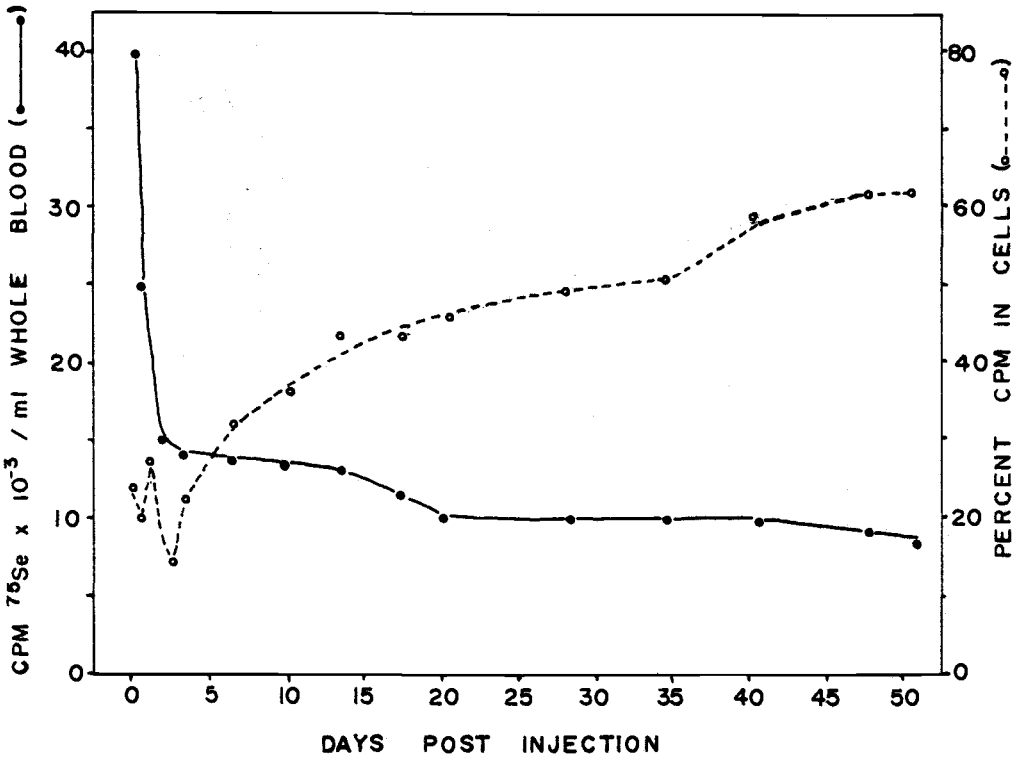


Figure 7. Distribution of ^{75}Se in whole blood of a selenium-deficient ram injected intramuscularly with 500 µCi Se-75.

an incorporation of selenium in spermatocyte to spermatid stages of spermatogenesis. The peaks of radioactivity reached in spermatozoa at 30 and 49 days post injection indicates greater incorporation prior to the spermatid stage.

The delay in appearance of ^{75}Se peaks in semen collected from the +Se ram, and the lower percentages in total semen counts found in sperm between 21 and 35 days (Figure 1) is probably due to the difference in selenium status between the two animals. A higher level of cold selenium in the seminiferous tubules at the time of ^{75}Se administration would reduce ^{75}Se uptake, and this would presumably be most dramatic in cells in latter stages of spermatogenesis as they would already have been saturated with selenium.

Thus, the hypothesis proposed is that selenium is equally distributed in the seminiferous tubule and is incorporated in all spermatogenic stages up to and including the spermatid with a high efficiency in the ram, but with one stage having a much higher level of incorporation. While this explanation most simply accounts for the data, other possibilities cannot be ruled out.

In the bull (Smith et al., 1979) seminal plasma counts reached a maximum 10 days after ^{75}Se injection and declined thereafter, whereas sperm counts peaked at 24 days post injection and appeared to stabilize for three weeks thereafter. The maximum seminal plasma counts were approximately twice the sperm counts. The seminal plasma counts in rams tended to parallel counts in the

sperm, and at peak ^{75}Se levels the sperm counts were nearly tenfold those of the seminal plasma.

The different patterns of ^{75}Se distribution in semen of rams (this study) and bulls (Smith et al., 1979) may be a reflection of species differences. Bull seminal plasma shows a much higher GSH-Px activity than does the ram (2908 nmoles NADPH ox/min·ml vs. 385), however, based on protein content the activities are very similar. It is not known why the patterns of incorporation in seminal plasma differ between ram and bull. It is possible that the similarity between ^{75}Se radioactivity in sperm and seminal plasma of rams results from leakage of selenoproteins from spermatozoa as a consequence of damage to the sperm cells by dilution and centrifugation (Mann, 1964). Since ^{75}Se counts in bull sperm remain stable throughout the length of the spermatogenic cycle, this could be interpreted as an incorporation of selenium not only by spermatids, but also by cells in earlier spermatogenic stages.

Gel filtration of seminal plasma from bull and ram showed similar patterns with respect to the large molecular weight peaks, but in seminal plasma of bull the lower molecular weight components were either absent or much lower. The significance of changes in the concentration of label in ram seminal plasma with time is unknown. Further work is required to elucidate the roles of these non-glutathione peroxidase selenium components.

IV. ^{75}SE AUTORADIOGRAPHY OF RAM SPERM

Introduction

Wu et al. (1969) showed decreased epididymal sperm concentrations and abnormal sperm in second generation selenium deficient rats. The majority of sperm showed impaired motility and breakage near the midpiece. Electron micrography revealed damage in the membrane system near the midpiece (Wu et al., 1979). This condition, responsive to selenium but not vitamin E or other antioxidants, suggested a specific involvement of selenium in sperm maturation and structural integrity (Wu et al., 1973). Brown and Burk (1973) demonstrated by autoradiography the presence of selenium in the midpiece of rat spermatozoa.

Calvin (1978) reported that intratesticular administration of ^{75}Se led to its incorporation primarily in tail keratin of rat sperm. He isolated a selenopolypeptide from this fraction which was suggested to play a critical function in sperm tail assembly. Smith et al. (1979) found glutathione peroxidase activity only in seminal plasma of the bull and postulated a structural role for selenium in bull spermatozoa. Pallini and Bacci (1979) reported selective localization of selenium in bull sperm mitochondria where it was bound to a structural polypeptide and was believed to function in the stabilization of the outer mitochondrial membrane.

Objectives of this study were to define patterns of selenium

distribution in ram spermatozoa as a function of time following ^{75}Se administration and to identify subcellular regions of selenium localization.

Methods and Materials

^{75}Se-labeled semen was collected from the same selenium-deficient and selenium-adequate rams as described previously (Methods section, Part II and Part III). Sperm were separated from seminal plasma by centrifugation at 1000 x g for 15 min, washed once with 0.9% NaCl, then fixed at 5° C for at least 24 hours in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.0, followed by fixation for 24 hours in 1% osmium tetroxide in 0.1M phosphate buffer, pH 7.3. They were then dehydrated with ascending concentrations of acetone and embedded in Epon (Wu et al., 1979; Hayat, 1970). Sections were mounted on either Formvar-coated 200 mesh copper grids, or uncoated 300 mesh copper grids. Sections were stained with lead citrate and carbon coated (Hayat, 1970). Under safelight, Kodak NTE emulsion was warmed to 50° C and diluted tenfold with doubled distilled water. Aliquots of diluted emulsion were distributed into small watchglasses on a hot plate maintained at 50° C. Grids were dipped in diluted emulsion then rapidly placed horizontally on filter paper to dry (about 5 min). Grids were then placed into separate disposable glass pipets, wrapped in multiple layers of aluminum foil and stored in an evacuated desiccator at 5° C for the exposure period (usually 2 to 6 weeks). After exposure, grids were carried in the disposable

pipets through the development procedure recommended by Salpeter and Bachmann (1964). All solutions used in development were filtered through 0.45μ Millipore filters. Control grids included sperm from a nonradioactive ram taken through the entire exposure-development procedure, and radioactive sperm taken as far as the staining process. Grids were examined under a Phillips Model 300 transmission electron microscope.

Results

Radioactive exposure of the NTE emulsion was primarily over membranes (Figures 1 and 2). In addition, in nuclei and to a lesser extent the central regions of the midpiece, areas of heavy ^{75}Se exposure were observed (Figures 3 and 4). These areas of nuclear localization appeared random. Neither control showed any of these labeling patterns (Figures 5, 6, and 7). Electron autoradiographs of spermatozoa collected throughout the experimental period revealed no detectable differences in ^{75}Se localization. Thus, whether selenium incorporation occurs at one or more spermatogenic stages, the location of selenium in mature spermatozoa appears unaffected. However, this does not negate the possibility that initial patterns of ^{75}Se incorporation are different at various stages of spermatogenesis.

Discussion

The electron autoradiographic pattern of ^{75}Se distribution in ram spermatozoa resembles that reported for vole sperm

Figure 1. ^{75}Se electron autoradiograph of longitudinal section through ram sperm head. The plasma membrane is detached. Arrows indicate labeling in membranes. magnification = 20,000.

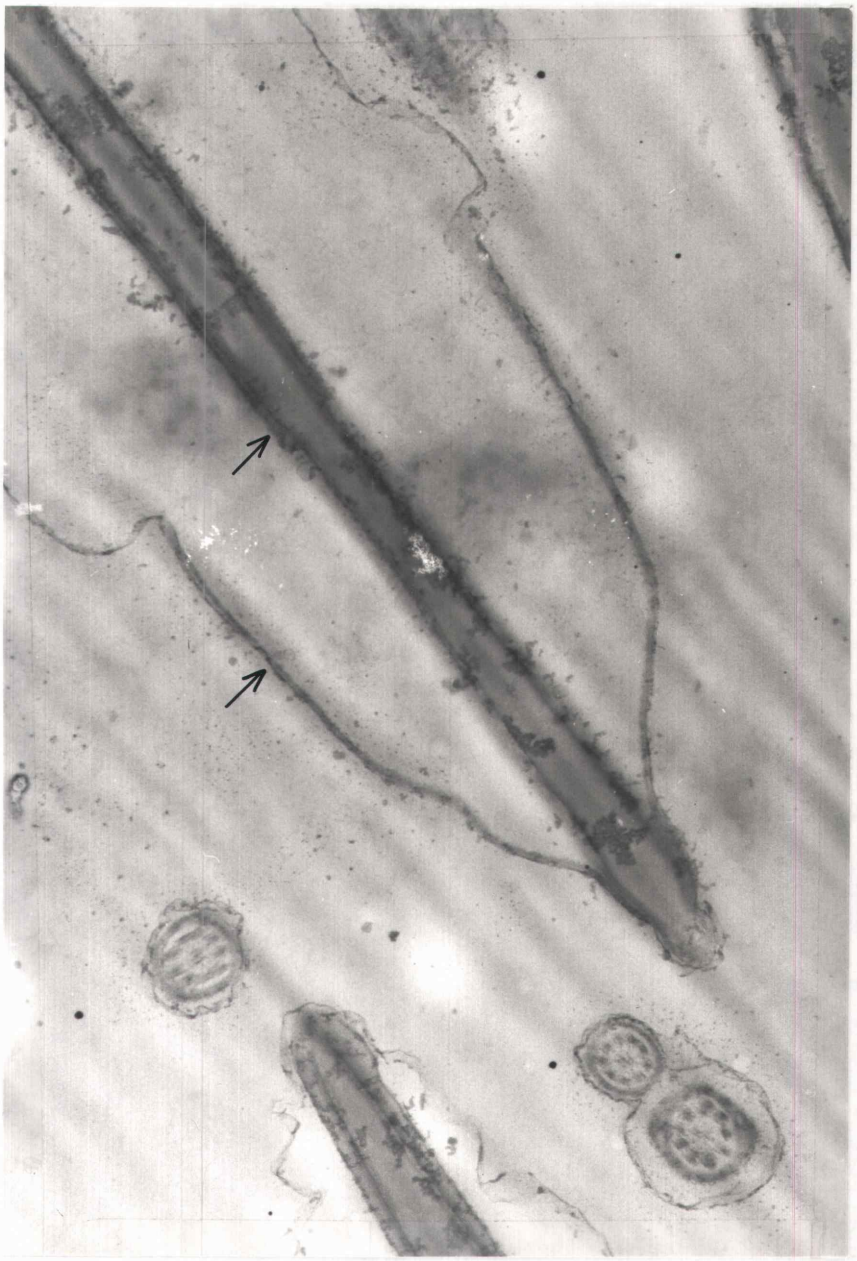


Figure 1

Figure 2. ^{75}Se electron autoradiograph of longitudinal section through ram sperm midpiece. Arrow indicates labeling in mitochondrial membranes. magnification = 25,000.

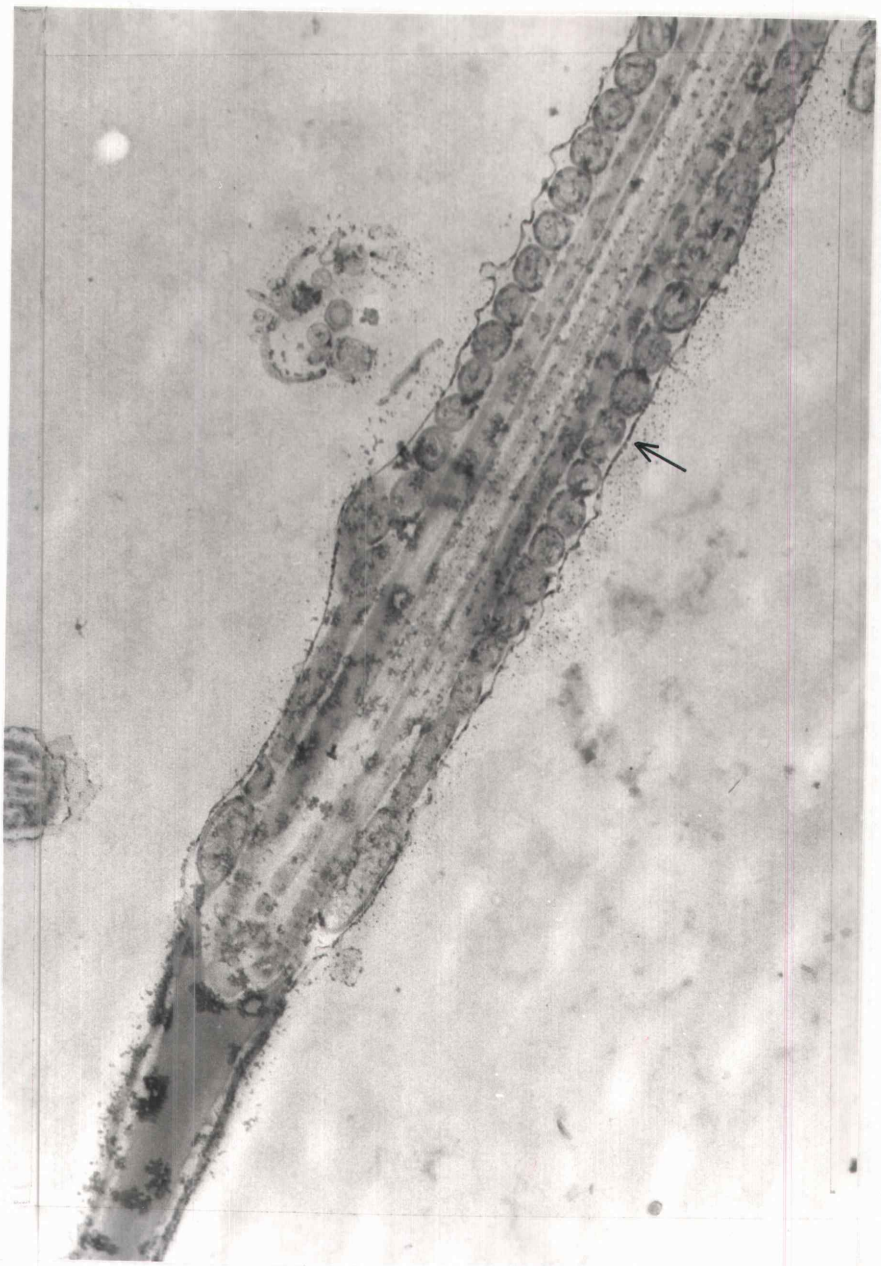


Figure 2

Figure 3. ^{75}Se electron autoradiograph of longitudinal sections through ram sperm heads. Arrow indicates areas of labeling within a sperm head. magnification = 35,000.

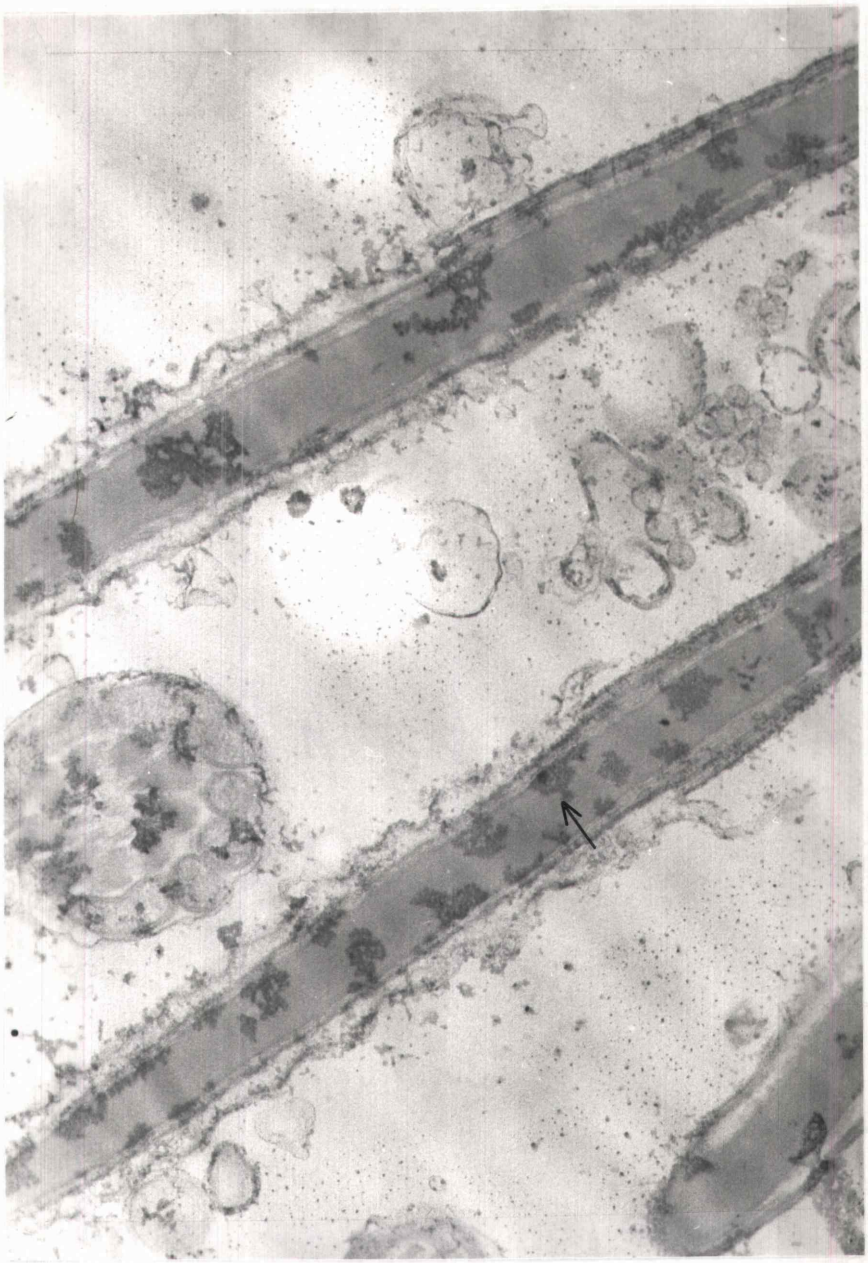


Figure 3

Figure 4. ^{75}Se electron autoradiograph of longitudinal section through ram sperm midpiece. Arrows indicate labeling in axonemal complex (AC) and in mitochondria (M). magnification = 70,000.

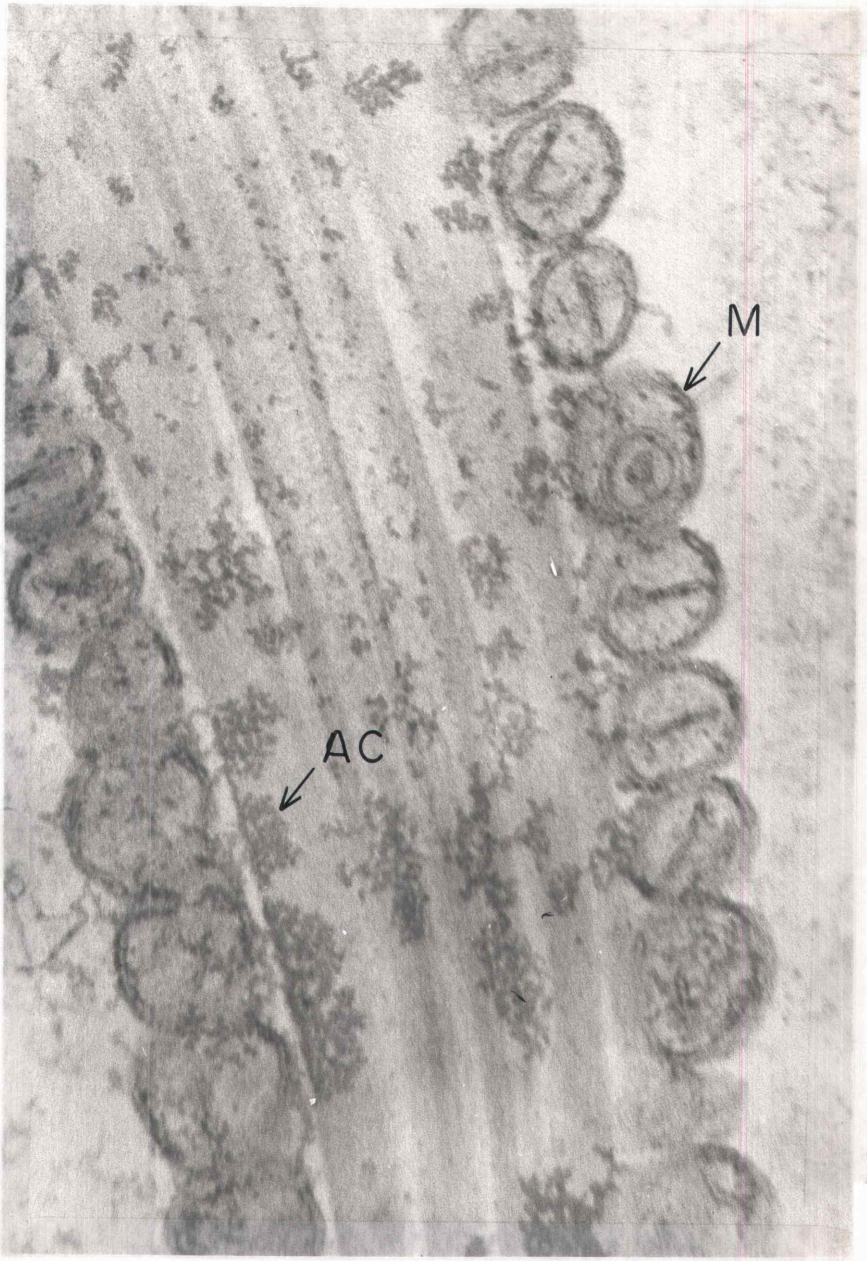


Figure 4

Figure 5. ^{75}Se electron autoradiographic control: ^{75}Se-labeled ram sperm carried through staining procedure, but not coated with emulsion. magnification = 24,000.

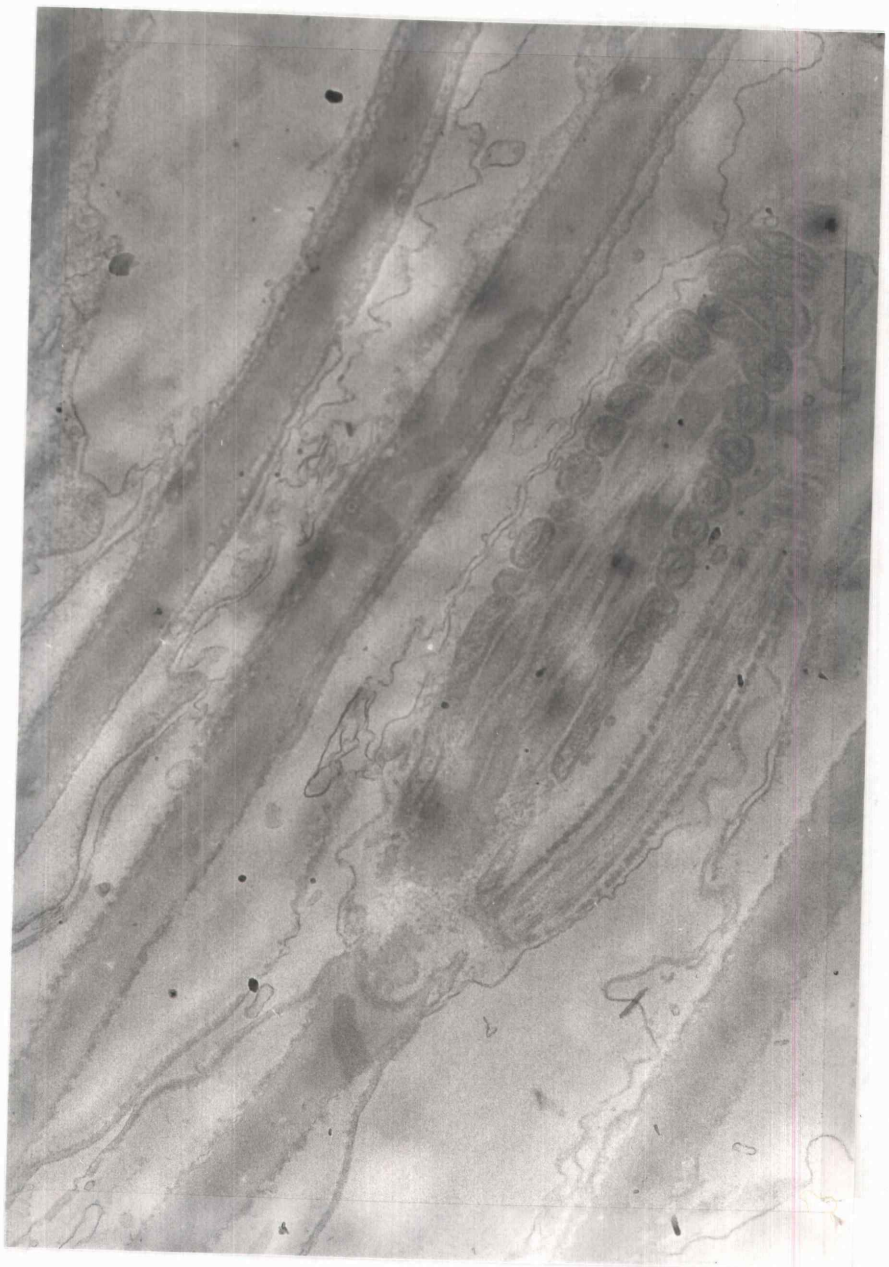


Figure 5

Figure 6. ^{75}Se electron autoradiographic control: ^{75}Se-labeled ram sperm carried through staining procedure, but not coated with emulsion. magnification = 75,000.

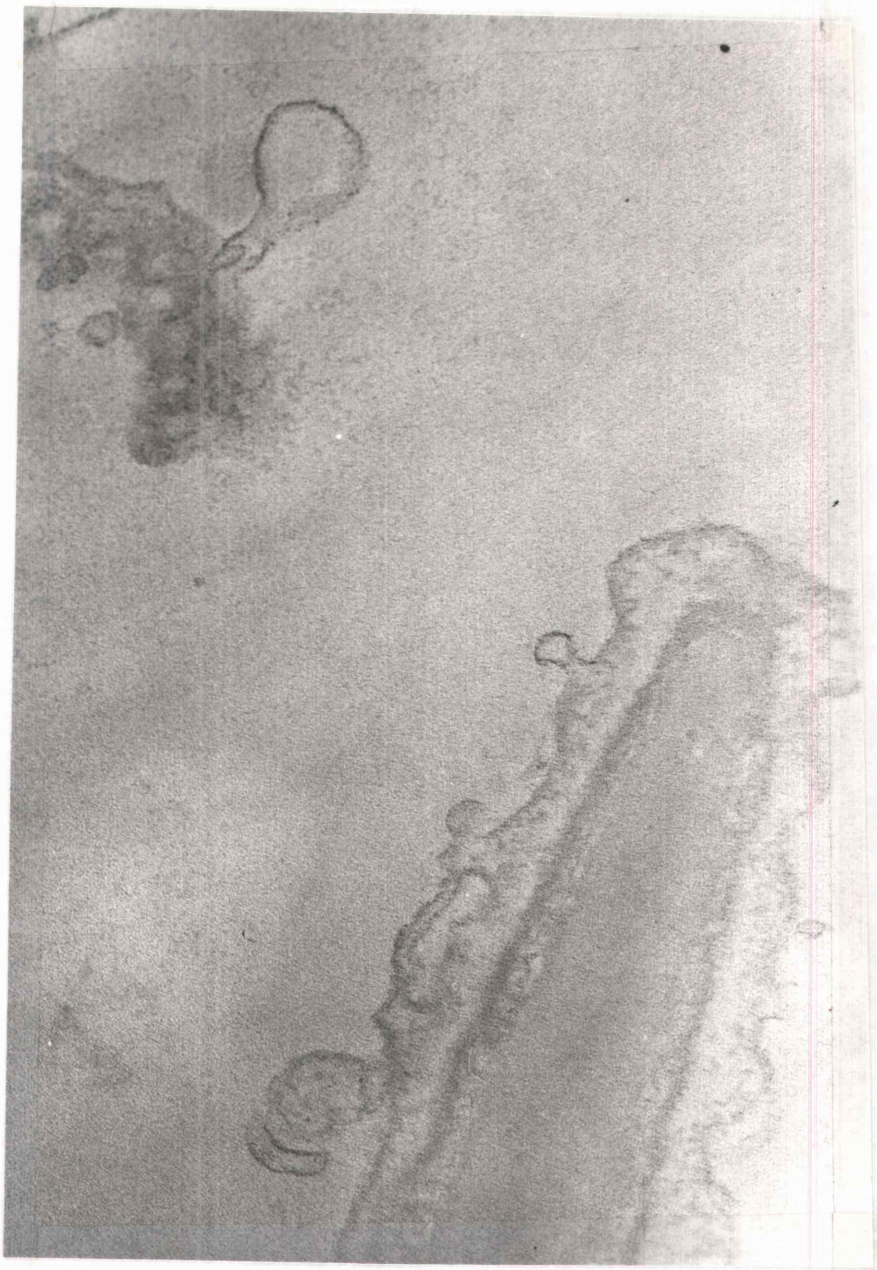


Figure 6

Figure 7. Electron autoradiographic control; nonradioactive ram sperm taken through exposure-development process. magnification = 35,000.

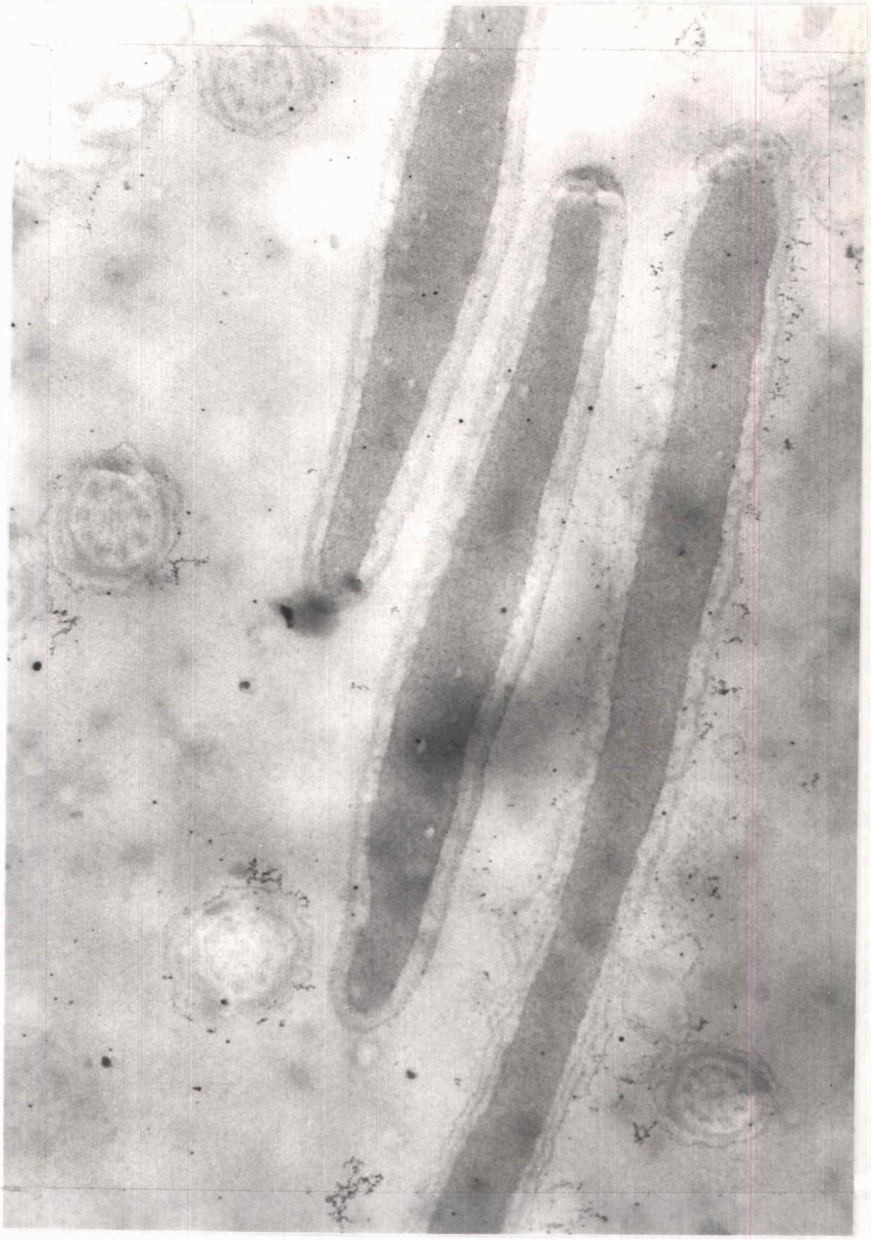


Figure 7

(Whitfield, 1978). In contrast, autoradiographic work of Brown and Burk (1973) showed high ^{75}Se concentrations in the midpiece of rat sperm and comparatively little ^{75}Se associated with the sperm head. Calvin (1978) found only 2-3% of radioactive label in isolated heads of rat sperm. Furthermore, Pallini and Bacci (1979) coupled electron microscopic observations with cytochrome oxidase measurements in an isopycnic centrifugation study and concluded that the presence of selenium in the nuclear fraction of bull spermatozoa resulted from mitochondrial contamination. Calvin and Cooper (1979) found the majority of ^{75}Se label associated with the outer mitochondrial membrane in rat sperm. Results of this study, while showing ^{75}Se association with mitochondrial membranes, did not indicate a preferential incorporation in these organelles. The label was distributed among all membranes.

Several differences exist between the experiments cited. One difference is the route of administration and another is possibly the selenium status of animals involved. The route of ^{75}Se administration in this study was via intramuscular injection, whereas Cavin (1978) and Calvin and Cooper (1979) used an intratesticular injection. That the mode of administration could produce different results is questionable since, in this study, the stage of developing spermatozoa at the time of ^{75}Se exposure apparently failed to alter final intracellular distribution. Also, Pallini and Bacci (1979) did not use radioactive selenium to study selenoproteins in bull sperm, thereby eliminating route of admini-

stration as a possible contributing factor to intracellular selenium localization.

The selenium status of animals has been shown to affect assimilation and retention patterns of ^{75}Se in rat tissues (Burk et al., 1972). Selenium-adequate animals given a radioactive pulse of selenium would be expected to show less labeling due to dilution with cold selenium and a preponderance of the label associated with proteins having high turnover rates. However, in the present study, similar patterns of ^{75}Se labeling were observed in the selenium-deficient ram and the selenium-adequate ram. Finally, a species difference in selenium utilization may explain discrepancies between studies of ^{75}Se incorporation into spermatozoa. However, to date sufficient data are not available from a variety of species to draw conclusions.

In the ram, it appears that selenium is incorporated in membranes throughout the spermatozoa. This study also emphasizes the need for investigation of the role of selenium in nonmitochondrial sperm structures, specifically the ram sperm nucleus.

V. DISTRIBUTION OF ^{75}SE IN RAM SPERMATOZOA

Introduction

Calvin (1978) and Calvin and Cooper (1979) have shown that, of the total radioactivity in rat spermatozoa 24-28 days following an intratesticular injection of ^{75}Se, over 79% was found in the tail and less than 3% in the head. The ^{75}Se appeared to concentrate in mitochondrial vesicles and was solubilized by incubation in a solution of sodium dodecyl sulfate (SDS) and dithiothreitol (DTT). Gel electrophoresis revealed only one radioactive component, having a molecular weight of approximately 17,000 daltons. In bull sperm, Pallini and Bacci (1979) found selenium to be selectively localized in the mitochondria as a component of a SDS-insoluble protein complex, believed to stabilize the outer membrane. The polypeptide to which selenium was bound had a molecular weight of about 20,000 daltons. The selenium distribution within spermatozoa of other species has not been examined.

Methods and Materials

^{75}Se-labeled sperm, collected from a selenium-adequate ram by electroejaculation, were sonicated in 0.1M phosphate buffer, pH 6.3, until cleavage of heads from tails was approximately 99% complete, as estimated by phase microscopy. Sonicated samples were applied to discontinuous sucrose density gradients (1.80, 2.05, and 2.20 M sucrose layers in 0.02M phosphate buffer, pH 6.0, con-

taining 10^{-3}M EDTA) and centrifuged at 4° C for 60 min at 91,000 x g. The two upper layers were collected in one ml fractions and the head pellet was recovered according to the methods of Calvin (1976). Heads and tails were counted using a hemocytometer. Head and tail suspensions were recentrifuged. Pellets were diluted to two ml with 0.0125M TRIS-HCl buffer containing 2% sodium dodecyl sulfate (SDS), 0.025M βmercaptoethanol (βME), 0.045% NaCl, pH 7.1, and incubated for three hours at 36° C. Following incubation, samples were centrifuged at 12,000 x g for 20 min to remove insoluble material and the supernatants applied to Sephacryl S-200 columns (1.5 x 90 cms) equilibrated with 0.05M phosphate buffer, containing 1% SDS, 1% βME, 0.1M NaCl, pH 7.0

Results

The amount of ^{75}Se label associated with ram sperm heads, collected 21 and 18 days post injection, was 62% and 57%, respectively (Table II). Incubation in SDS/βME released about 32% of counts from the head pellets and 83% from the tail pellets (Table III). Gel filtration of the soluble fractions from SDS/βME incubation resulted in one predominant peak (Figure 1), which eluted at a Ve/Vo of 1.18. However, this may not accurately reflect monomolecular protein size due to SDS micelle formation, thereby increasing the effective elution size.

Discussion

Pallini and Bacci (1979) presented evidence that selenium was

Table II. ^{75}Se distribution in heads and tails of ram sperm

Sperm collection date	cpm/10^9 heads	cpm/10^9 tails	% cpm in heads	% cpm in tails	% recovery
21 days post injection	3,224	1,961	62	38	111
28 days post injection	24,228	17,943	57	43	104

Table III. Solubility of head and tail ^{75}Se-labeled proteins with SDS/βME incubation*

sperm collection date	HEADS		TAILS	
	cpm in supernatant (% total cpm)	cpm in pellet (% total cpm)	cpm in supernatant (% total cpm)	cpm in pellet (% total cpm)
21 days post injection	2,722 (27%)	7,161 (72%)	3,841 (80%)	918 (19%)
28 days post injection	2,260 (40%)	3,294 (59%)	6,160 (84%)	1,096 (15%)

* incubated 3 hours at 36° C in 0.0125M TRIS-HCl, 2% sodium dodecyl sulfate (SDS), 0.025M β mercaptoethanol (βME), 0.045% NaCl, pH 7.1.

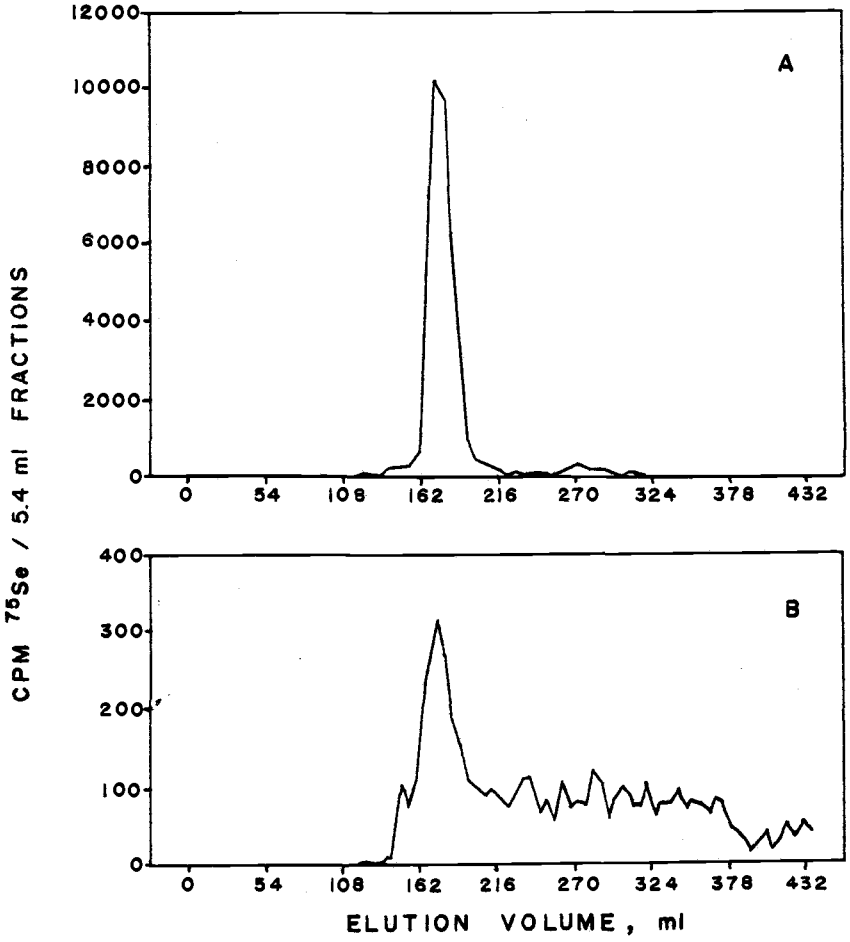


Figure 1. Sephacryl S-200 gel filtration of SDS/βME-soluble fraction of ram sperm tails (A) and ram sperm heads (B) (see text for procedure); (1.5 x 90 cms columns, eluted with 0.05M phosphate buffer containing 1% SDS, 1% βME, 0.1M NaCl, pH 7.0).

not present in the nuclear fraction of bull spermatozoa. Also, Calvin (1978) and Calvin and Cooper (1979) found less that 3% of ^{75}Se-label in the head fraction following cleavage of rat sperm heads and tails. High percentages of ^{75}Se measured in ram sperm heads may be indicative of species differences. It should be noted that electron autoradiographic analysis of ram sperm heads revealed the presence of ^{75}Se. Additional work is required to confirm and further resolve the intraspermatozoon distribution of selenium in the ram. ^{75}Se in the soluble fraction of both sperm heads and tails eluted in similar positions, but further characterization of SDS/βME-insoluble selenium containing molecules is required.

VI. SUMMARY AND CONCLUSIONS

Selenium accumulates in ram and bull testes and is incorporated into developing spermatozoa. Smith et al. (1979) presented evidence that selenium is incorporated by spermatids of the bull. In contrast, findings of this study suggest that in the ram selenium is incorporated prior to and during the early spermatid stage. Gunn and Gould (1970) argued that selenium incorporation in rat sperm probably occurs during the secondary spermatocyte or early spermatid stage. Selenium does not appear to be incorporated by mature spermatozoa of the ram (present study), bull (Smith et al., 1979), or rat (Gunn and Gould, 1970).

Electron autoradioagraphy of ram sperm indicated an affinity of membranes for selenium, but selenium was also incorporated into the nucleus. These findings differ from those of bull and rat studies in which selenium was specifically incorporated into the fibrous material of the outer mitochondrial membrane. Neither the bull nor rat showed significant amounts of selenium in the head portion of sperm (Pallini and Bacci, 1979; Calvin, 1978; Calvin and Cooper, 1979). Selenopolypeptides isolated from bull and rat sperm mitochondria were believed to serve in a structural capacity and suggested to be required for assembly of the mitochondrial sheath and maintenance of membrane integrity.

The different distribution of selenium in ram sperm suggests that the role of selenium in spermatozoa varies between different

species of animals. To date, the essentiality of selenium for sperm function has only been demonstrated in the rat (Wu et al., 1973). High testicular and sperm selenium concentrations in other animals suggests that they have a requirement for selenium in sperm. However, deficiencies in other animals, while affecting reproduction, have not been noted to specifically affect sperm function or male reproductive processes. Possibly this is because animals have not been depleted for a period sufficient to manifest such problems (see Buchanan-Smith et al., 1969). Significant species differences have been reported in the seminal activities of the selenoenzyme, glutathione peroxidase (Li, 1975). Further investigation is needed to determine if such differences exist in the intracellular distribution and function of spermatozoon selenium. GSH-Px activity was not detected in spermatozoa of ram (present study), bull (Brown et al., 1977; Smith et al., 1979), or rat (Calvin and Cooper, 1979).

Selenium supplementation of rams may be warranted since selenium is likely involved in the function and/or maintenance of structural integrity of the sperm. For maximum benefit, data of this study suggest that supplementation of rams should precede the breeding season by at least 42 days, as judged by the peak of incorporation of selenium into sperm. The work of Smith et al. (1979) indicates a similar time period to insure high selenium concentrations in ejaculated sperm. However, it is not yet known what selenium levels are required for optimal sperm function in

either the ram or bull, or what relationship, if any, exists between sperm function and sperm selenium concentrations.

In addition to its presence in bull and ram sperm, selenium is also found in seminal plasma. Gel filtration of ^{75}Se-labeled ram seminal plasma resulted in four radioactive peaks. Two peaks predominated, one of which eluted in the region of GSH-Px. In bull seminal plasma, two ^{75}Se peaks (corresponding to the predominant peaks in ram seminal fluid) were obtained by gel filtration; one showing GSH-Px activity. The GSH-Px activity per ml of bull seminal plasma was approximately 10-fold higher than the highest per ml activity seen in ram seminal plasma. However, there is considerably more protein in bull seminal plasma than in the ram. Thus, when expressed on a per mg protein basis, the GSH-Px activities of ram and bull were comparable. Presumably, peroxidase activity in seminal plasma is important in reducing oxidative damage to the sperm, and its consequent immobilization (see Mann, 1964; Tabor and Rosenthal, 1956). The significance of higher total GSH-Px activity in bull seminal plasma is not known.

The GSH-Px found in bull seminal plasma may be contributed by the prostate gland. This accessory gland showed high GSH-Px activity in the cytosol. Seminal vesicles of bull and ram also showed high GSH-Px activity. However, seminal vesicles are known to possess high prostaglandin synthesis activity, which has been reported to involve GSH-Px (Nugteren and Hazelhof, 1973). Therefore, GSH-Px in these glands may function in prostaglandin synthesis

rather than, or perhaps in addition to being secreted into seminal plasma. Cowper's gland cytosol from the bull and ram showed a low level of GSH-Px activity.

GSH-Px involvement in prostaglandin synthesis may also have significance to reproductive success. Normally, prostaglandin E (PGE) levels exceed those for prostaglandin F (PGF) in seminal plasma. GSH-PX has been found to accelerate production of PGE but decreases that of PGF (Lands et al., 1971; Nugteren and Hazelhof, 1973). In humans, Karim (1972) presented evidence that PGE in semen may assist sperm transport, while PGF is antagonistic to this action. He also suggested that a correlation existed between semen prostaglandin levels and fertility. If this is the case, then it should be expected that PGE levels will reflect the selenium status of the animal. Vincent (1974) postulated a role for prostaglandins in some selenium deficiency symptoms.

Gel filtration of ram testes cytosol gave four ^{75}Se peaks. In the Se-adequate ram, the GSH-Px peak predominated, but in the Se-deficient ram the GSH-Px peak was second to the higher molecular weight peak (Ve/Vo 1.09). The lower molecular weight peaks also changed, relative to the Ve/Vo 1.09 peak, with selenium status of the animal. Whether this reflects different patterns of selenium utilization in Se-deficient and Se-adequate rams cannot be concluded from these data. It has been demonstrated that ^{75}Se uptake and retention, as well as whole body and intracellular distribution varies with selenium status of the animal (Lopez

et al., 1969; Wright and Mraz, 1964; Brown and Burk, 1973). McConnell et al. (1979) found that, with time after injection, there was a shift in molecular weight components in rat testis cytosol to which ^{75}Se was bound. Five ^{75}Se peaks were obtained from gel filtration of bull testis cytosol. As in the Se-adequate ram, the predominant peak was the GSH-Px peak. The role of selenium in testicular cells is not yet understood.

Cummins and Martin (1967) showed that alkaline dialysis removed ionically bound selenite from protein. Data of dialysis experiments on ram and bull cytosols indicate that selenium-protein binding in the ram is most stable in gel filtration peaks eluting at Ve/Vo 1.52 and 2.30. The peak at 1.52 possessed GSH-Px activity. The peak eluting at Ve/Vo 2.85 was least stable, showing 72% to 88% loss of radioactivity upon 24 hour storage or dialysis. The peak eluting at Ve/Vo 1.09 had an intermediate binding stability, losing about 59% of the ^{75}Se with dialysis at pH 11.0.

The binding stability of selenium for bull testis cytosol proteins differed considerably from that in the ram. Upon storage, radioactivity lost from the gel filtration peak at Ve/Vo 1.11 was apparently gained by the peak eluting at Ve/Vo 2.78. Dialysis at pH 6.3 or pH 11.0 resulted in a loss of radioactivity from all protein peaks, with the exception of the protein eluting at Ve/Vo 1.11, which gained ^{75}Se when dialysed at pH 11.0.

Further experimentation is needed to delineate the functions of selenium in the male reproductive system. The present study

demonstrates the incorporation of selenium into ram and bull testes, accessory glands, and sperm. It is postulated that selenium is directly involved in ram spermatozoa, not only in the midpiece, but also in the nuclear region. Selenium may also indirectly affect sperm function through its role in GSH-Px of the seminal plasma and accessory glands.

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