AN ABSTRACT OF THE THESIS OF

Salaheldin Eltigani Abdelgadir for the degree of Master of Science in Animal Science presented on March 28, 1983.

Title: EFFECTS OF SELENIUM AND GLUTATHIONE PEROXIDASE ON THE BIOSYNTHESIS OF PROSTAGLANDIN E_2 and $F_{2\alpha}$ BY THE RAM VESICULAR GLAND

Abstract approved: Redacted for Privacy

Dr. J.E. Oldfield

This study was conducted to investigate the involvement of selenium and glutathione peroxidase in prostaglandin biosynthesis by the ram vesicular gland.

Vesicular glands were collected from healthy intact rams covering a wide range of selenium nutrition and were analyzed for selenium concentrations, glutathione peroxidase activities and levels of prostaglandin E_2 and prostaglandin $F_{2\alpha}$.

This study showed a statistically highly significant positive relationship between selenium concentrations and glutathione peroxidase activities, a statistically significant negative relationship between glutathione peroxidase activities and prostaglandin $F_{2\alpha}$ levels and a statistically positive relationship between prostaglandins E_2 and $F_{2\alpha}$, but it did not show any significant relationships between gluthathione peroxidase activities and prostaglandin E_2 levels, between selenium concentrations

and prostaglandin E_2 levels and between selenium concentrations and prostaglandin $F_{2\alpha}$ levels.

The results of this study do not favour the direct involvement of selenium and glutathione peroxidase in prostaglandin biosynthesis by the ram vesicular gland. Glutathione peroxidase appears not to be an indispensible enzyme in this process or an obligatory requirement for its continuation. However glutathione peroxidase may have an indirect effect on prostaglandin biosynthesis by protecting the prostaglandin endoperoxide synthetase enzyme against harmful effects of lipid peroxides.

The negative effect of glutathione peroxidase on prostaglandin $F_{2\alpha}$, showed in this study agrees with and confirms a previous report. The significance and reasons for this negative relationship remain to be determined.

Effects of Selenium and Glutathione Peroxidase on the Biosynthesis of Prostaglandin E_2 and $F_{2\alpha}$ by the Ram Vesicular Gland

by

Salaheldin Eltigani Abdelgadir

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of
Master of Science

Completed March 28, 1983

Commencement June 1983

APPROVED:

Redacted for Privacy

Professor of Animal Science in charge of major

Redacted for Privacy

Head of Department of Animal Science

Redacted for Privacy

Dean of Graduate School

Date thesis is presented March 28, 1983

Typed by Lisa Harris for Salaheldin Eltigani Abdelgadir

To my wife Afaf ,

for her immense patience,

magnificent devotion. ,

encouragement and love .

ACKNOWLEDGEMENTS

Many people have contributed to the successful excution of this project, foremost among these is Dr. James E. Oldfield, my major professor. His expert advice, guidance and encouragement were what I needed most to complete this project. For this I shall be indebted to him forever.

Many thanks go to Dr. Arthur S. Wu, Dr. Wilbert Gamble, Dr. Philip D. Whanger and Dr. Wilson E. Schmisseur for serving as members of my graduate committee and for their valuable constructive criticisms and advice.

I gratefully acknowledge the technical assistance of Dr. Lloyd Swanson and Mary Zelinski who helped me with the development and execution of the radioimmunoassay.

Judy Butler, Mike Beilstein and Dr. Frank W. Adams of the Department of Agricultural Chemistry were of immense help to me in the analysis of tissue glutathione peroxidase activity and selenium concentration. Their assistance is gratefully acknowledged.

Finally I wish to extend my gratitute to Mr. Elmer Humphrey of Bellfountain, Oregon for providing me with the ram vesicular glands used in this study.

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INTRODUCTION

The concentration of selenium by certain organs and its high affinity for specific metabolic systems has received considerable attention recently. The male reproductive tract appears to be one of the major systems for selenium metabolism in mammals. Brown and Burk (1973) reported the retention of more than 40% of injected ^{75}Se in the testis-epididymis complex, three weeks after injection. Despite this high concentration of selenium by the male reproductive tract, its exact function(s) in male reproduction remain to be determined.

Perhaps the best documented function of selenium in the mammalian body is in the glutathione peroxidase enzyme (Rotruck et al., 1973), which functions in cell membrane lipid peroxidation (Cohen and Hochstein, 1963). Although glutathione peroxidase activity has been detected in the testis, accessory sex glands and seminal plasma, this does not appear to account for all selenium in the male reproductive tract. For example the sperm cell has been found to be very rich in selenium content and virtually negative in glutathione peroxidase activity (Pond 1981).

The male reproductive tract is also one of the richest tissues in prostaglandins (Bindra and Bindra, 1977). It has been suggested that glutathione peroxidase plays some role in prostaglandin biosynthesis (Nugteren and Hazelhof, 1973). If this is true it would account at least partially for the significance of selenium in mammalian reproduction.

LITERATURE REVIEW

Glutathione Peroxidase: Discovery and Physical Characteristics

Glutathione peroxidase (GSH:H_2O_2 oxidoreductase, E.C. 1.11.1.9.) referred to hereafter as GSH-Px was first described by Mills (1957) who isolated a heat labile erythrocyte factor which acts with glutathione to protect hemoglobin from oxidative breakdown and called it glutathione peroxidase since it catalyzes the oxidation of reduced glutathione by hydrogen peroxide.

Rotruck et al. (1973) studied glucose-dependent susceptability to peroxide-induced hemolysis in selenium-deficient rats and discovered selenium as a key component of GSH-Px.

Recent purifications of GSH-Px indicated a molecular weight of 75,000-100,000 daltons based on different tissues from different species. Flohe´ et al. (1973) purified the enzyme from bovine erythrocytes and reported a molecular weight of 84,000 daltons. The enzyme from ovine erythrocytes has a molecular weight of 88,000 daltons (Oh et al., 1974), from human erythrocytes 95,000 daltons (Awasthi et al., 1975) and from rat liver 76,000 daltons (Nakamura et al., 1974).

According to Flohe´ et al. (1971) each molecule of GSH-Px consists of four subunits. Recent studies (Flohe´ et al., 1973; Oh et al., 1974; Awasthi et al., 1975 and

Flohe´ et al., 1976) found four atoms of selenium in the molecule - probably one atom in each subunit.

Mechanism of action of GSH-Px

GSH-Px functions in close association with glutathione (GSH) which is a tripeptide, (γ-glutamylcysteinylglycine) that serves as a hydrogen donor in the following reaction:

$$H_2O_2 + 2GSH \xrightarrow{GSH\text{-}Px} 2H_2O + GSSG$$

This protective system has a lasting effect only when the product of the reaction GSSG (glutathione disulfide or oxidized glutathione) is continuously reduced to GSH (Mills and Randall, 1958). This reduction of glutathione disulfide could be achieved by glucose-6-phosphate, fructose-6-phosphate, ribose-5-phosphate as well as some other substrates (Butler and Buttenweiser, 1957). The complete glucose-linked glutathione peroxidase pathway for detoxification of hydrogen peroxide was first established by Cohen and Hochstein (1963). The first step in the pathway sequence which is catalyzed by GSH-Px is the reduction of hydrogen peroxide to water through the oxidation of glutathione to glutathione disulfide. In the second step glutathione reductase reduces glutathione disulfide to glutathione and oxidizes $NADPH_2$ into NADP. Glucose-6-phosphate dehydrogenase reduces glucose-6-phosphate to

6-phosphogluconate and maintains a continuous supply of $NADPH_2$. Glucose-6-phosphate in turn is produced from glucose through the action of hexokinase as illustrated in figure 1.

It is the presence of glucose in the reaction medium which led to the discovery of selenium as a component of GSH-Px (Rotruck 1981).

Biochemical functions and site of action of GSH-Px

When it was first discovered, GSH-Px was believed to protect proteins, particularly hemoglobin against oxidative breakdown by hydrogen peroxide (Mills 1957). The red blood cells are known to produce hydrogen peroxide by various mechanisms such as the reaction between ascorbic acid and oxyhemoglobin (Mills and Randall, 1958) and the decomposition of oxygen anion by superoxide dismutase (McCord and Fridorich, 1968). The pronounced reactivity of hydrogen peroxide and superoxide provides the basis of such important biochemical reactions as hydroxylation (Coon et al., 1972), biosynthesis of thyroxin (Serif and Kirkwood, 1958), α-oxidation of fatty acids (Mead and Levis 1963) and oxidation of alcohols (Oshino et al., 1973). Yet the reactivity of the oxygen species mentioned is high enough to attack unspecificially a variety of biological structures susceptible to oxidative damage (Loschen et al., 1974 and Cohen and Heikkila, 1974). The cellular

device for regulation of steady state levels of these peroxides and oxygen species consists of the heme-containing peroxidases among which catalase is of particular importance and the selenoenzyme GSH-Px, as well as some other mechanism, (Flohe´ et al., 1976). Catalase was considered the major enzyme responsible for reducing hydrogen peroxide until Cohen and Hochstein's (1963) demonstration of GSH-Px as the first line of defense against oxidative damage by hydrogen peroxide or lipid peroxide produced in various cells of the body. They provided evidence that GSH-Px linked to hexose-shunt activity, rather than catalase, protects hemoglobin from oxidation to methemoglobin by hydrogen peroxide. They showed that catalase-deficient erythrocytes were protected against the toxic effects of hydrogen peroxide by sustained GSH-Px activity. O'Brien and Frazer (1966) reported that catalase had no effect on the lipid peroxide produced in the liver. Little and O'Brien (1968) demonstrated that glutathione oxidation accompanying lipid peroxidation in subcellular fractions involves GSH-Px. They suggested that unlike catalase, the GSH-Px system is the major mechanism in many cells for the detoxification of lipid peroxides.

Christophersen (1968) isolated and identified the products formed by the reduction of lipid peroxide by GSH-Px and suggested that the enzyme shows no specificity

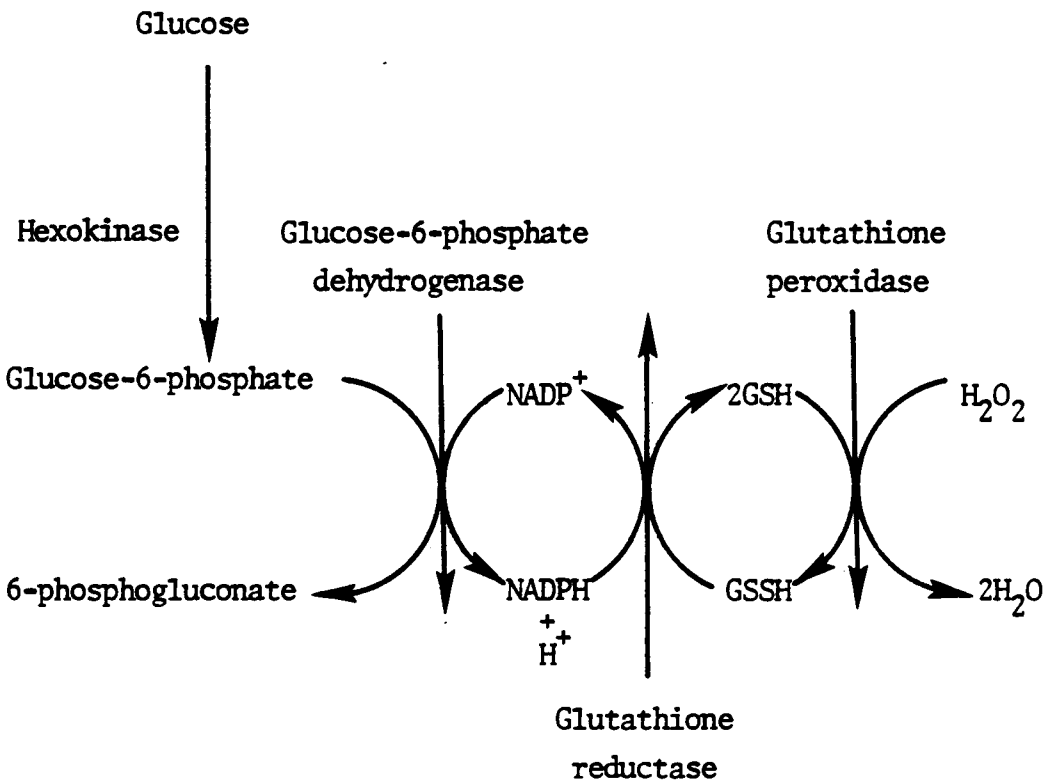

Figure 1. The Detoxification of Hydrogen Peroxide via the Glucose Linked Glutathione Peroxidase Pathway (from Cohen and Hochstein 1963). The abbreviations GSH and GSSG refer to Reduced Glutathione and Oxidized Glutathione, respectively.

for the position of the hydro-peroxide groups or of the double bonds and that it therefore can reduce the hydroperoxides of all polyunsaturated fatty acids which occur in subcellular membranes thus protecting them from oxidative damage.

It has been well established that hydrogen peroxide is produced by a variety of enzymes not only within the peroxisomes but also in the microsomal, mitochondrial, and soluble fractions of the cell (Boveris et al., 1972). The cellular localization of GSH-Px was investigated by Green and O'Brien (1970). They indicated that 60% of the GSH-Px was recovered in the soluble fraction of the cell, 28% from the mitochondrial fraction, and the rest from the microsomal and lysosomal fractions. They concluded that GSH-Px apparently is not associated with the peroxisomes but is truly mitochondrial.

The role of GSH-Px in prostaglandin biosynthesis was extensively debated by Nugteren and Hazelhof (1973), and Lands and co-workers (Lands et al., 1971; Smith and Lands, 1972a; Smith and Lands, 1972b, and Vanderhoek and Lands, 1973). This role will be discussed later.

Distribution of Selenium in the Male Reproductive Tract

The concentration of selenium by certain organs and its high affinity for certain systems has received considerable attention recently. The male reproductive tract appears to be one of the major systems for selenium

metabolism in mammals. Brown and Burk (1973) studied the retention of ^{75}Se by 16 different tissues in the rat and reported that the testis-epididymis complex retained 41.8% of the total body ^{75}Se three weeks after injection. Rosenfeld (1964) demonstrated that, with the exception of the kidneys, testes accumulated the highest concentration of ^{75}Se administered to rats. This finding was supported by Anghileri and Marques (1965) who reported that mice testis accumulated selenium progressively over a 7 day period while other organs reached a peak and declined within the first day, and by Smith et al. (1979) who found that for a total of 14 tissues in the bull, the epididymis and testis retained the greatest amount of selenium with the exception of the kidney, 23 days postinjection. Pond (1981) showed high affinity of selenium for both bull and ram reproductive organs and Alabi (1982) found high levels of selenium in the boar, bull and ram reproductive tracts.

The accessory sex glands also retained high levels of selenium. Anghileri and Marques (1965) reported high selenium concentrations in seminal vesicles of the rat, and Hansson and Jacobsson (1966) using whole body autoradiography of mice showed that the seminal vesicles contained the highest concentration of ^{75}Se in all tissues 4 days after injection. Smith et al. (1979) found that the prostate and vesicular glands of the bull

contained levels of selenium exceeded only by the testis, epididymis and the kidneys. Bartle et al. (1980) indicated that the accessory sex glands appear to be more responsive to changes in selenium status than blood.

The ability of each sex gland to accumulate selenium differs among species. Among the accessory glands of the ram the vesicular gland retained the highest level of selenium followed by Cowper's gland, and among the bull's accessory glands the prostate retained the highest level of selenium followed by the seminal vesicles and Cowper's gland (Pond, 1981). The distribution of selenium in reproductive tissues of the boar, bull and ram was studied by Alabi (1982). For all the species studied, the testis and epididymis retained the highest level of selenium followed by the seminal vesicles and the prostate. The ampulla and Cowper's gland had the least selenium among the accessory sex glands.

The testis and the accessory sex glands produce spermatozoa, seminal plasma and some hormones. High levels of selenium were detected in the spermatozoa of all species studied. Incorporation of ^{75}Se by the sperm cells has been demonstrated in the rat (Brown and Burk, 1973; Calvin 1978; Wu et al., 1979), mouse (Gunn, et al., 1976), bull (Smith and Senger, 1978; Neimi et al., 1979; and Smith et al., 1979), and ram (Pond 1981). Autoradiography of the sperm cell showed ^{75}Se to be heavily

localized in the midpiece region (Brown and Burk, 1973; Wu et al., 1979; and Pond 1981).

The Function of Selenium in the Male Reproductive Tract

Despite this high affinity, the exact function(s) of selenium in male mammalian reproduction is not yet fully understood.

Selenium may function in male reproduction in three different ways:

1. In the sperm cell: - Gunn et al. (1967) and Wu et al. (1969) were the first to recognize the specific importance of selenium in the process of spermatogenesis in mice and rats respectively. Different functions have been suggested for selenium in the sperm cell. A structural function has been postulated first by Wu et al. (1973) who demonstrated the breakage of the sperm flagella in selenium-deficient rats and suggested that selenium may be essential in the formation of the sperm flagellum or the element could be important to the quality of the membrane that envelops the axial filaments. This has been supported by Calvin (1978) and Smith et al. (1979). The selenium component might be essential for the assembly of the mitochondrial membrane of the sperm (Wu et al., 1979; Pallini and Bacci 1979; and Calvin and Cooper, 1979). A physiological function has been suggested by Gunn et al. (1967) Brown and Burk (1973) and McConnell et al. (1979). The incorporation of

selenium in the sperm cell is reported to be in a polypeptide of 15,000-21,000 daltons depending on the species. Although this selenopolypeptide does not exhibit GSH-Px activity, its molecular weight is similar to that of one subunit of this enzyme (Calvin, 1978). The nature and function of this selenopolypeptide in the sperm cell remain to be determined. Some selenium - containing proteins or enzymes other than GSH-Px have been described in both mammalian and microbial tissues. Whanger et al. (1973) isolated and purified a selenium-containing protein from heart and semitendinosus muscles of lambs. This selenoprotein has a molecular weight of 10,000 daltons, contains a heme group and its properties closely resemble those of cytochrome C. A selenium-containing protein having a formate dehydrogenase activity has been isolated from E. coli (Shum and Murphy, 1972) and from Clostridium thermoaceticum (Andreesen and Ljungdahl, 1973). A low molecular weight 12,000 daltons, acidic selenoprotein having a glycine reductase activity has been isolated and purified from Clostridium sticklandii (Turner and Stadtman, 1973). These reports might indicate the participation of selenium containing proteins in electron transport, oxidation reduction reactions and energy production.

2. In the seminal plasma: - The sperm cell is capable of producing hydrogen peroxide as a result of

deamination of certain amino acids (Tosic and Walton, 1950). GSH-Px is secreted in the seminal plasma by the accessory sex glands and can protect the sperm against hydrogen peroxide. Alabi (1982) showed high GSH-Px activity in the accessory sex glands of the bull, ram and boar.

3. In Prostaglandin biosynthesis: - It has been postulated that GSH-Px catalyzes one of the steps in the biosynthesis of prostaglandin by the vesicular gland, (Nugteren and Hazelhof, 1973).

Prostaglandins: Discovery and General Properties

The prostaglandins (PG) form a class of natural products with diverse and potent biological activities. The natural PG's are unsaturated hydroxylated fatty acids, all derivatives of prostanic acid (Crabbe, 1977). They are synthesized from certain essential fatty acids by a microsomal enzyme system known as prostaglandin synthetase which is widely distributed in mammalian tissues. They do not appear to be stored freely in tissues, but instead are synthesized and released on demand (Bindra and Bindra, 1977).

The first indication of the presence of a specific biologically active compound in human accessory sex glands came from Columbia University in New York in 1930, where Kurzrok and Lieb showed that the human seminal fluid could induce strong muscle contractions or

relaxations when applied to the human uterus depending on whether or not the woman had given birth. A few years later Goldblatt (1933) in England and von Euler (1934) in Sweden demonstrated independently the presence of a vasodepressor agent and a musle-stimulating factor in sheep vesicular glands and human seminal plasma. von Euler (1935) suggested that the biological activity was due to lipid-soluble material with acidic properties and called it "prostaglandin" in the belief that the origin of the newly discovered substance was the prostate gland. The isolation and crystallization of PGE_1 and $PGF_{1\alpha}$ from sheep vesicular seminal extracts was achieved in 1957 by Bergstrom and Sjovall.

Although human seminal plasma is the richest known vertebrate source of prostaglandins, they have been detected in low concentrations in virtually all tissues investigated, which suggests that they play a fundamental role in cellular metabolism and cell function. Their biological effects are elicited at exceedingly low concentrations on the order of nanograms per milliliter, ranking them amongst the most potent substances found in nature (Bindra and Bindra, 1977).

The Role of GSH-Px on Prostaglandin Biosynthesis

Samuelsson (1967) reported that production of PGE_1 from arachidonic acid by the sheep vesicular gland

system was stimulated by addition of reduced glutathione (GSH). This effect of glutathione on PGE production was supported by van Drop (1967) and Lapidus et al. (1968).

Lands et al. (1971) investigated the effect of both glutathione and GSH-Px on the biosynthesis of PGE and PGF. They reported that glutathione and GSH-Px selectively accelerate PGE production at the expense of PGF.

Smith and Lands (1972a) reported that oxygenation of fatty acids by lipoxygenase of freshly prepared homogenates of sheep vesicular gland was inhibited by GSH-Px in the presence of glutathione. They suggested that a hydroperoxide product necessary for the functioning of lipoxygenase was removed by GSH-Px and indicated that GSH-Px had no direct effect on the maximal velocity but only on the rate of attaining that velocity (Smith and Lands, 1976b).

Nugteren and Hazelhof (1973) isolated and identified 15-hydroperoxy-prostaglandin R_2 as a necessary intermediate in prostaglandin biosynthesis. The authors postulated that the 15-hydroperoxy group of the prostaglandin is reduced by GSH-Px to form prostaglandin R_2 which is in turn converted into PGE_2 and $PGF_{2\alpha}$ by other enzymes. Thus GSH-Px may catalyze an indispensible step in the biosynthesis of prostaglandins. Hamberg

et al. (1974) isolated a compound identical to 15-hydroperoxy-prostaglandin R_2 which they called prostaglandin G_2. This PGG_2 rapidly decomposes with concomitant formation of malondialdehyde, the unpleasant compound already known as a breakdown product of peroxidized lipids. In this context, the proposed physiological role of GSH-Px would be to channel the labile peroxide intermediates into the correct pathway, thereby preventing the formation of undesirable by-products (Flohe´ et al., 1976). If GSH-Px catalyzes such a reaction in prostaglandin biosynthesis it would suggest that the role of the enzyme in regulating metabolism and biomembrane function is greater than had been previously thought. Moreover our understanding of the biological role of the individual prostaglandins would be enhanced.

Further research is necessary to explain the mystery of selenium and GSH-Px in the male reproductive tract. Does selenium function solely through GSH-Px or does it have other function(s)? Is GSH-Px stimulatory to prostaglandin biosynthesis as suggested by Samuelsson (1967), or is it only stimulatory to PGE at the expense of PGF (Lands et al., 1971)? Is it an indispensible part of prostaglandin biosynthesis (Nugteren and Hazelhof, 1973) or inhibitory to prostaglandin biosynthesis through the inhibition of the lipoxygenase enzyme (Smith and

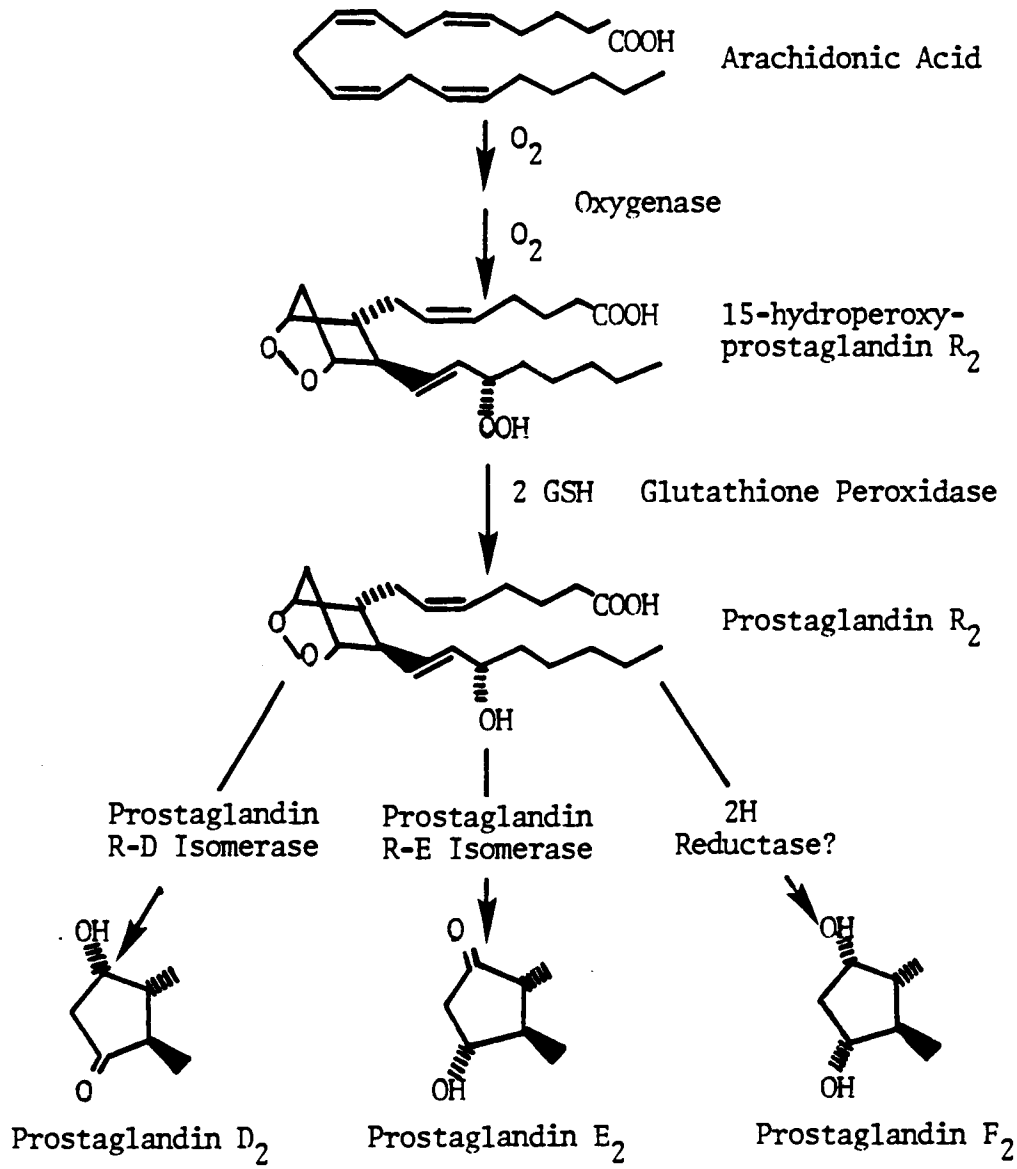

Figure 2. The Involvement of Glutathione Peroxidase in Prostaglandin Biosynthesis by the Ram Vesicular gland System as suggested by Nugteren and Hazelholf 1973.

Lands, 1972)? Or is it only regulatory to prostaglandin biosynthesis through the direction of reactions away from the formation of prostaglandin endoperoxides (Flohe´ et al., 1967)?

This research is directed toward providing answers to some of these questions.

MATERIALS AND METHODS

This research was designed to investigate the effects of selenium and glutathione peroxidase (GSH-Px) on prostaglandin biosynthesis. The male was selected for this study to provide a simple model and to avoid the effects of different stages of the estrous cycle and pregnancy in the female on prostaglandin levels. The ram seminal vesicle is one of the richest tissues in prostaglandins (Bindra and Bindra, 1977 and Crabbe, 1977). Hence ram vesicular glands were chosen for this study.

Reproductive organs from a random group of 94 healthy, intact rams were collected at slaughter at a ranch about 20 miles south of Corvallis. The vesicular glands were dissected and connective tissue was removed within 10 minutes after slaughter. The left vesicular gland was divided into two portions, each one was placed into a clean glass vial, flushed with liquid nitrogen, and placed in dry ice until they reached the laboratory where they were stored at -60°C for the determination of prostaglandin E_2 and prostaglandin $F_{2\alpha}$. The right vesicular gland was also divided into two portions. Those were used for determinations of selenium levels and GSH-Px activities. For all these parameters each assay was done in duplicate for all samples.

Technique of Glutathione Peroxidase (GSH-Px) Assay

A known weight (0.5-1.0 wet weight) of the ram vesicular gland was homogenized in 5 volumes of 0.05 M sodium phosphate buffer (pH 6.3) using a Potter-Elvejhem homogenizer with the pestel driven by an electric drill motor. The homogenates were centrifuged at 19000 x g for 30 minutes at 4°C using a Sorval rotor type SS-34. Aliquots of the supernatants were assayed for both GSH-Px activity and protein content.

GSH-Px activities were determined according to Paglia and Valantine (1967), as modified by Whanger et al. (1977). For each assay 0.8 ml reaction mixture which contained 0.125 M phosphate buffer, (pH 7.0) with 4.5 mM EDTA; 4.7 mM sodium azide, 2.8 nmoles NADPH, 49.9 nmoles reduced glutathione and 0.67 units glutathione reductase was used. The sample (0.1 ml) was added to the cuvette, followed by the freshly prepared reaction mixture (0.8 ml). The reaction was initiated by addition of 0.1 ml of freshly prepared 0.25 mM hydrogen peroxide (H_2O_2) solution. GSH-Px activities were then measured spectrophotometrically at 340 nm using a Hitashi 100-80A spectrophotometer. The blank cuvette contained double distilled water instead of the sample. For each sample and blank, the rate of NADPH oxidation was measured 10 times over a period of 10 minutes and a curve of nmoles of NADPH oxidized per minute was drawn. The values of

the blanks were subtracted from those of the samples before enzyme units were calculated. GSH-Px activity was expressed as nanomoles NADPH oxidized per minute per mg of protein. The protein content of the tissue homogenate was determined according to Lowry et al. (1951) using bovine serum albumin as the standard.

Selenium Level Determination

Selenium was determined fluorometrically according to the method described by Brown and Watkinson (1977). To a known weight (0.5-1.0 g wet weight) of vesicular gland tissue, 10 ml of concentrated nitric acid, and 3 ml of perchloric acid were added, and the samples were predigested overnight at room temperature. Then hot plate digestion to white fumes was carried out followed by 15 minutes white fume digestion, addition of 1 ml concentrated hydrochloric acid and another 15 minutes white fume digestion on the hot plate, after which the flasks were removed from the heat and cooled. A standard curve was prepared from 0.303 ppm sodium selenite solution which was treated the same way. After cooling, 15 ml of 0.009 M EDTA was added to each flask followed by two drops of indicator (cresol red and brom cresol green). Titration to yellow (pH 2-3) was then performed with 5N ammonium hydroxide, and selenium content was measured by an Alpkem Autoanalyzer II (AA II) designed

for selenium analysis as described by Brown and Watkinson (1977). Peak heights were measured in mm, a standard curve was plotted and selenium content of samples were calculated from the standard curve in µg per gram of wet tissue weight.

Protocol for Prostaglandin Extraction

Tissue prostaglandin was extracted according to the method described by Jaffe and Behrman (1974). A known weight of vesicular gland tissue (100-120 mg wet weight) was homogenized in a mixture of 1.0 ml phosphate buffered saline (0.01 M phosphate, 0.15 M sodium chloride, pH 7.4) and 3.0 ml of an ethyl acetate: isopropanol:0.2 N HCl (3:3:1; v/v/v) extraction solution. Fifty microliters containing 4000 cpm of either prostaglandin E_2 or prostaglandin $F_{2\alpha}$ as radioactive tracer were added, followed by 2.0 ml of ethyl acetate, and 3.0 ml of distilled water. After mixing, the aqueous and organic phases were separated by centrifugation. The organic phase was removed and dried at 55°C in air. This is called the crude lipid extract.

Column Chromatography for Separation of Prostaglandins

Separation of prostaglandins was achieved by silicic acid column chromatography as described by Caldwell et al. (1971) and modified by Jaffe and Behrman (1974). Columns were prepared in 10 ml disposable glass pipettes with a

glass bead inserted at their tips, by addition of 0.5 g silicic acid (100-200 mesh) suspended in 2 ml benzene: ethyl acetate (60:40 vol/vol; solvent-1). The columns were allowed to drain and then eluted with 5 ml benzene: ethyl acetate:methanol (60:40:20; vol/vol/vol; solvent-2) and 1.2 ml of solvent 1, after which they were allowed to completely drain. The crude lipid extract was dissolved in 0.2 ml of benzene:ethyl acetate:methanol (60:40:10; vol/vol/vol; solvent-3), vortexed and 0.8 ml of solvent-1 added. The crude lipid extract was then applied to the prepared column with a Pasteur pipette. The column was allowed to drain and then eluted with 6 ml of solvent 1. This fraction (F-1) contained neutral lipids, fatty acids, sterols, steriods, prostaglandin A and prostaglandin B. The column was then eluted with 12 ml of benzene:ehtyl acetate:methanol (60:40:2; vol/vol/vol; solvent-4) and this fraction (F-2) contained prostaglandin E. The final elution was with 3 ml of solvent-2 and this fraction (F-3) contained prostaglandin F. Recovery efficiency of prostaglandin from samples was calculated as the radioactive tracer recovered in the final fraction as percentage of the amount originally added to the tissue homogenate, 57.2% ± 9.9 was the mean recovery efficiency for both prostaglandins.

Radioimmunoassay of Prostaglandin E_2 and $F_{2\alpha}$

Radioimmunoassay of both prostaglandin E_2 and

prostaglandin $F_{2\alpha}$ was performed according to a modification of the method described by Jaffe and Behrman (1974).

Rabbit anti-prostaglandin E-BSA, and rabbit anti-prostaglandin $F_{2\alpha}$-BSA sera were purchased from Miles Research Products (Indiana). Tritiated prostaglandin E_2[5, 6, 8, 11, 12, 14, 15-^{3}H (N)] - and prostaglandin $F_{2\alpha}$ [5, 6, 8, 9, 11, 12, 14, 15-^{3}H(N)]- were purchased from New England Nuclear Company while non-radioactive prostaglandin E_2 and $F_{2\alpha}$ were bought from Sigma Chemical Company.

For each set of assays of prostaglandin E_2 and $F_{2\alpha}$, three tubes labelled zero control, blank and total tube, each containing 0.1 ml of 0.1 M sodium phosphate buffer (pH 7.5) which contains 0.15 M sodium chloride, 0.015 M sodium azide and 0.1% gelatine were prepared. To assay tubes 0.1 ml of standards or samples were added followed by 0.5 ml of the appropriate antibody. To the zero control tube 0.5 ml of the antibody was added while the blank and total tubes received 0.5 ml of the buffer. All tubes were incubated at 4°C for 30 minutes. 0.1 ml of tritiated prostaglandin E_2 of prostaglandin $F_{2\alpha}$ of 100,000-150,000 dpm/ml was added to all tubes followed by incubation at 4°C for 60 minutes. 1.0 ml dextran coated charcoal solution (0.63% w/v Neutral Norit and 0.063% w/v Dextran T-70 in buffer) was added to each tube excluding the total tube to which 1.0 ml buffer was

added. All tubes were then mixed, incubated at 4°C for 10 minutes and centrifuged at 3,000 rpm for 15 minutes. 0.7 ml of the supernatant was removed, added to 6 ml of scintillation fluid and counted in Packard TRI carb 2425 liquid scintillation spectrometer. The bound fraction was calculated as follows:

$$\% \text{ bound fraction} = \frac{\text{cpm in sample} - \text{cpm in blank}}{\text{cpm in zero control} - \text{cpm in blank}} \times 100$$

Levels of prostaglandin E_2 and $F_{2\alpha}$ were then calculated from the standard curves which were plotted as % bound vs picogram prostaglandin/tube of standard using a Hewlet-Packard 41CV calculator.

Radioimmunoassay performance was measured according to Hafs et al. (1977). A non-specific binding of 2.28% ± 0.57 and 3.0% ± 0.51 was obtained for prostaglandin E_2 and prostaglandin $F_{2\alpha}$ respectively. The accuracy of the assay was measured by the addition of aliquots of tissue extracts containing known amounts of PGE_2 and $PGF_{2\alpha}$ to standard tubes containing 0, 7.5, 15, 31, 63 and 125 pg of the appropriate prostaglandin and the calculation of the percentage agreement between the estimates obtained by radioimmunoassay and the quantities actually present. Accuracies of 94.6 ± 9.2 and 95.3 ± 7.5 were obtained for Prostaglandin E_2 and Prostaglandin $F_{2\alpha}$ respectively. The precision was

measured as the coefficient of variation of the error associated with multiple estimations of the hormone within and among assays. An intraassay coefficient of variation of 11.40% and 2.40% and interassay coefficient of variation of 7.85% and 28.0% were obtained for PGE_2 and $PGF_{2\alpha}$ respectively. The sensitivity was measured as the smallest quantity of hormone which can be measured reliably expressed as the smallest standard significantly different from the zero control. For both PGE_2 and $PGF_{2\alpha}$ the sensitivity was 7.5 pg. Figures 3 and 4 illustrate typical standard curves for PGE_2 and $PGF_{2\alpha}$ respectively.

Statistical Analysis

Standard statistical analysis in this study such as linear regression, analysis of the variance and coefficient of variation were performed according to Steel and Torrie (1980).

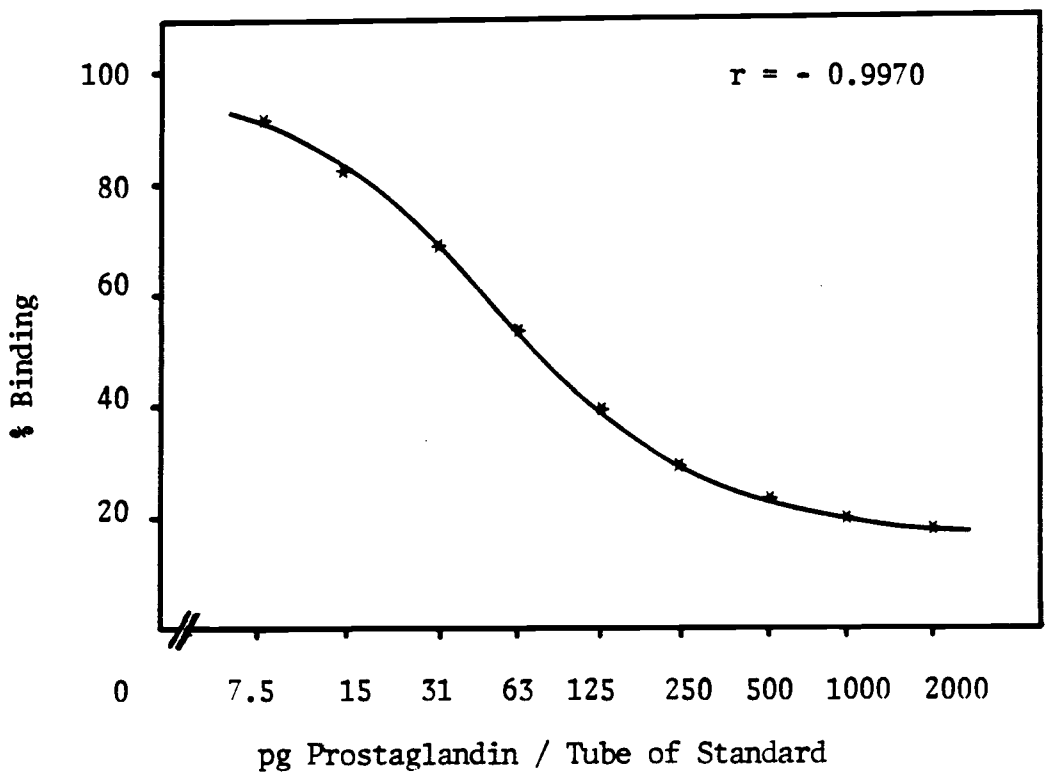

Figure 3. A Typical Standard Curve for Prostaglandin E_2 Radioimmunoassay.

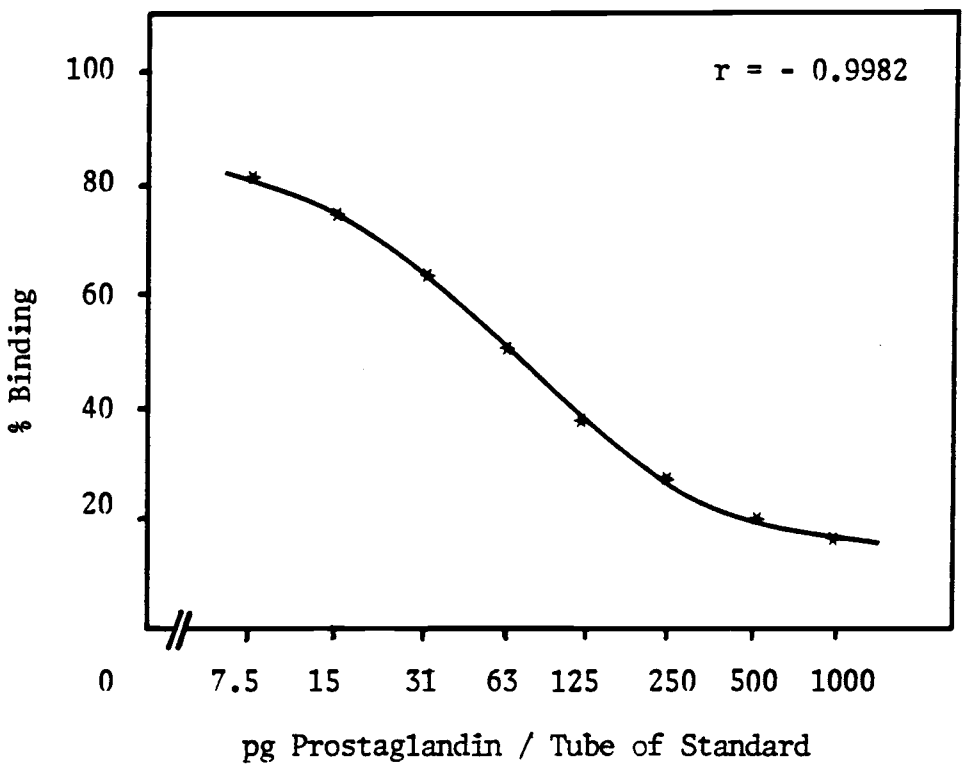

Figure 4. A Typical Standard Curve For Prostaglandin $F_{2\alpha}$ Radioimmunoassay.

RESULTS

Effect of Selenium on GSH-Px

The relationship between selenium concentrations and GSH-Px activities was tested in Figure 5 which shows the regression of GSH-Px against selenium. The solid line represents the regression line while the two broken lines represent the 95% confidence-interval band. A moderately high correlation coefficient of 0.68 was calculated between selenium concentrations and GSH-Px activities, and was found to be statistically significant ($P<0.01$).

Effect of Selenium on PGE_2 and $PGF_{2\alpha}$

The relationships between selenium concentrations and PGE_2 levels and between selenium concentrations and $PGF_{2\alpha}$ levels were tested in figures 6 and 7 respectively. Figure 6 shows the regression of PGE_2 against selenium. A correlation coefficient of 0.06 was calculated between selenium and PGE_2 and the relationship between these two parameters was found to be statistically insignificant ($P<0.05$). Figure 7 shows the regression of $PGF_{2\alpha}$ against selenium. The correlation coefficient between these two parameters was found to be 0.02 and the relationship between them was also statistically not significant ($P<0.05$).

Effect of GSH-Px on PGE_2 and $PGF_{2\alpha}$

The relationships between GSH-Px activities and PGE_2 levels and between GSH-Px activities and $PGF_{2\alpha}$ levels were tested in figures 8 and 9 respectively. Figure 8 shows the regression of PGE_2 against GSH-Px. A correlation coefficient of 0.06 was calculated between these two parameters and the relationship between them was not significant ($P<0.05$). Figure 9 shows the regression of $PGF_{2\alpha}$ against GSH-Px. In this case a negative correlation coefficient of -0.25 was calculated, and the relationship between $PGF_{2\alpha}$ and GSH-Px was found to be statistically significant ($P<0.05$).

Effect of PGE_2 on $PGF_{2\alpha}$

Figure 10 shows the regression of $PGF_{2\alpha}$ against PGE_2. A positive correlation coefficient of 0.43 and a statistically significant relationship ($P<0.01$) was calculated between these two prostaglandins.

Table 1 lists selenium concentrations, GSH-Px activities, PGE_2 and $PGF_{2\alpha}$ levels of the 94 ram vesicular glands collected for this study. Table 2 lists the numbers of observations, ranges, means, standard deviations and variances of these parameters.

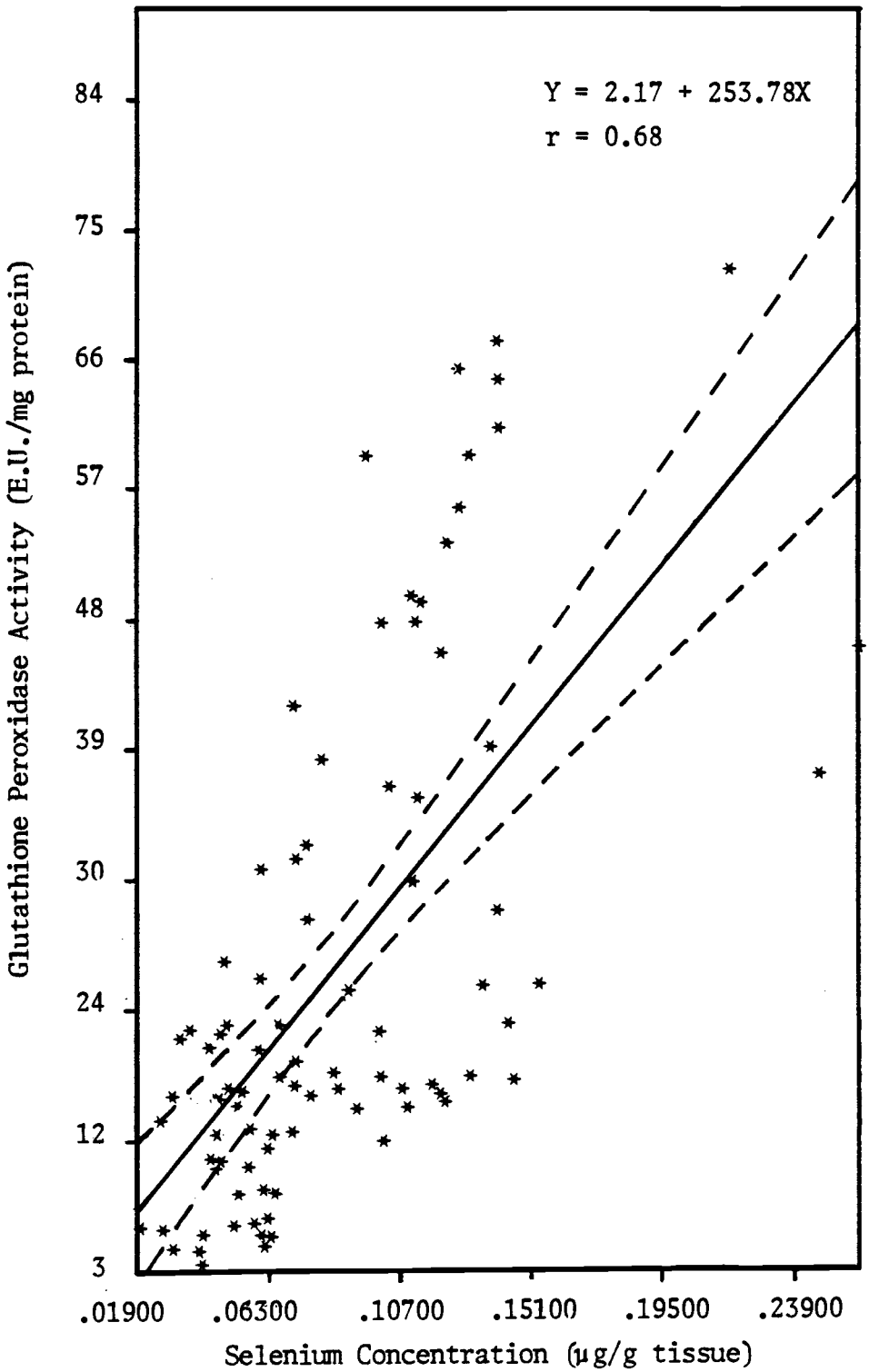

Figure 5. Regression of GSH-Px Against Selenium.

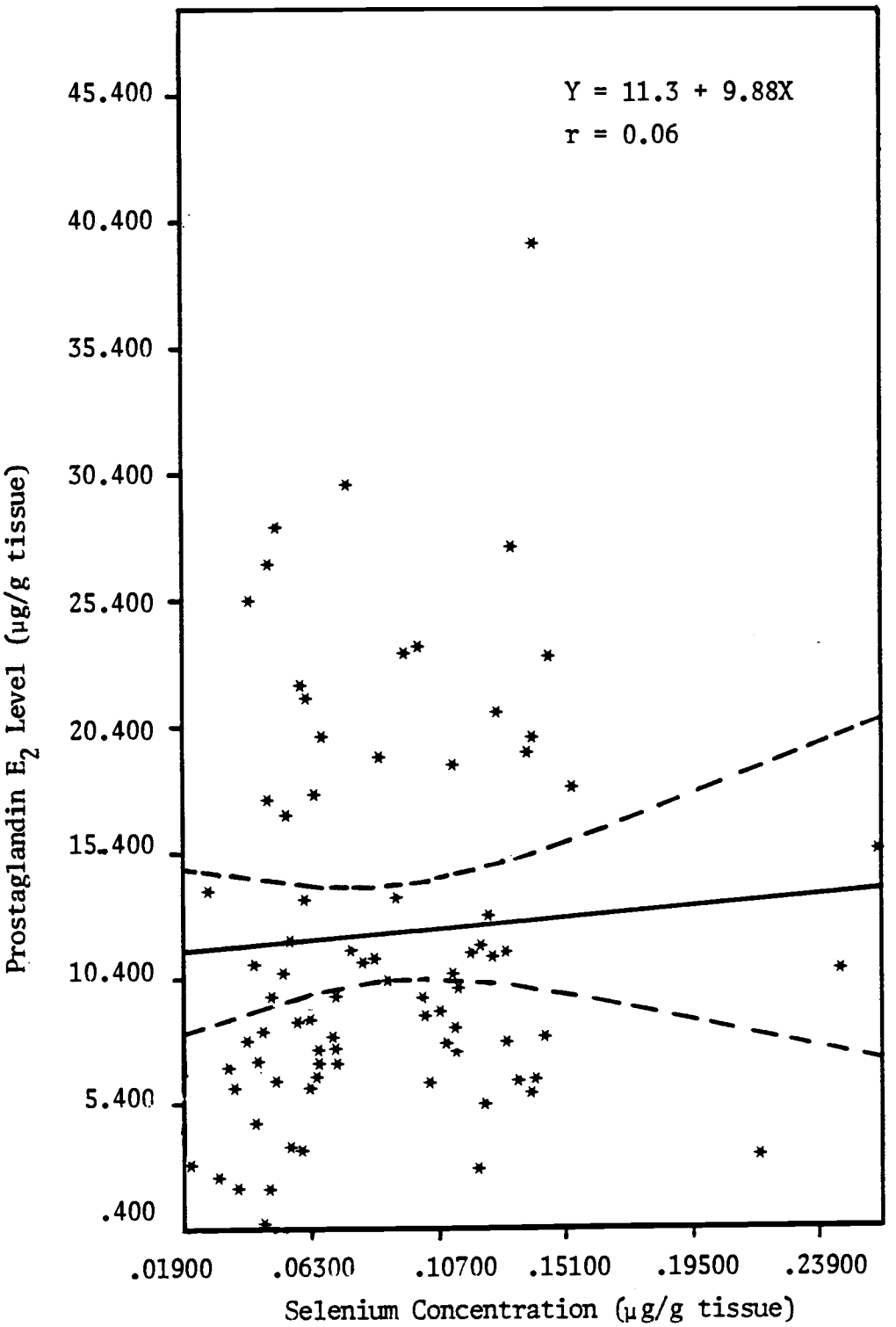

Figure 6. Regression of Prostaglandin E_2 Against Selenium.

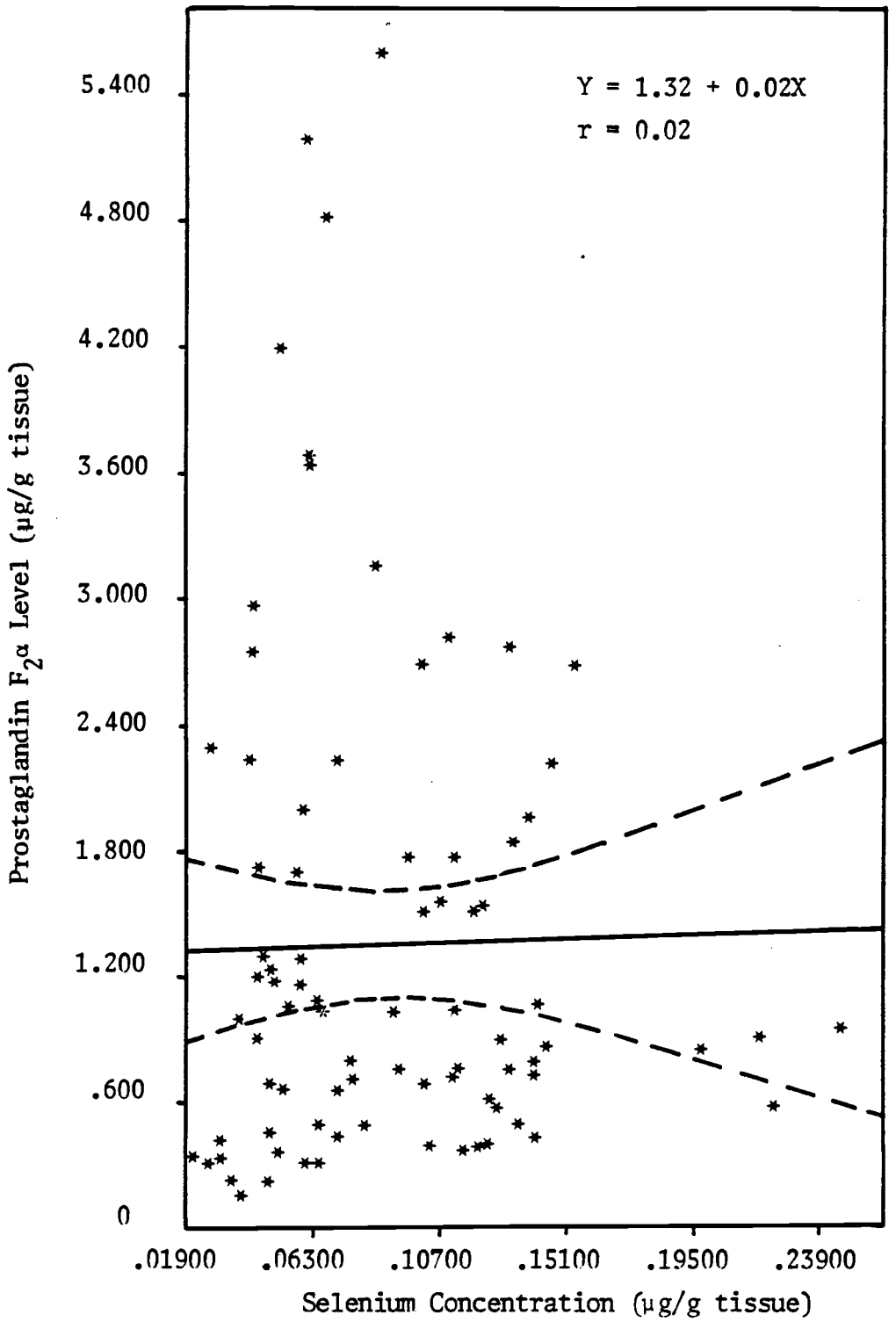

Figure 7. Regression of Prostaglandin $F_{2\alpha}$ Against Selenium.

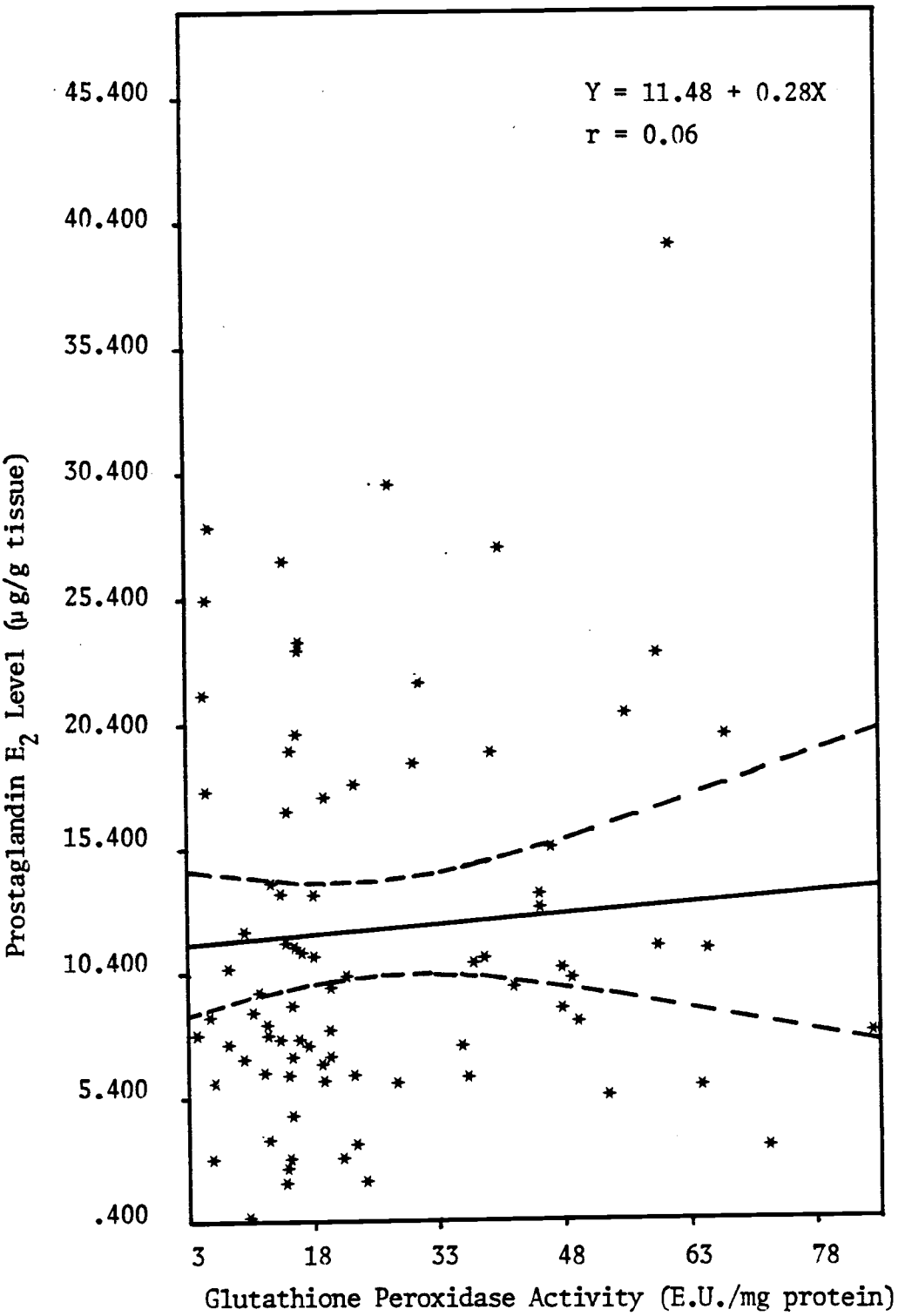

Figure 8. Regression of Prostaglandin E_2 Against GSH-Px.

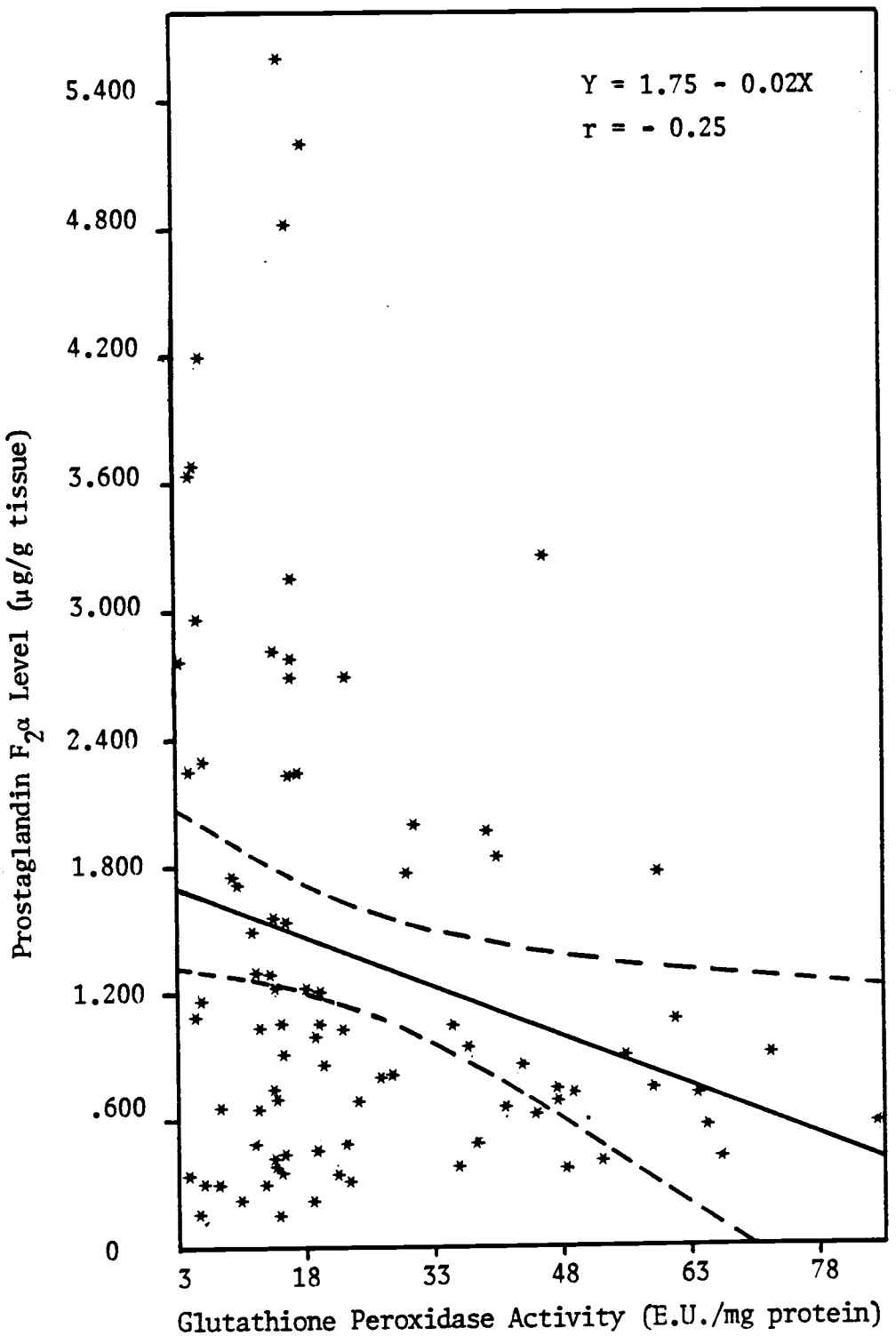

Figure 9. Regression of Prostaglandin $F_{2\alpha}$ Against GSH-Px.

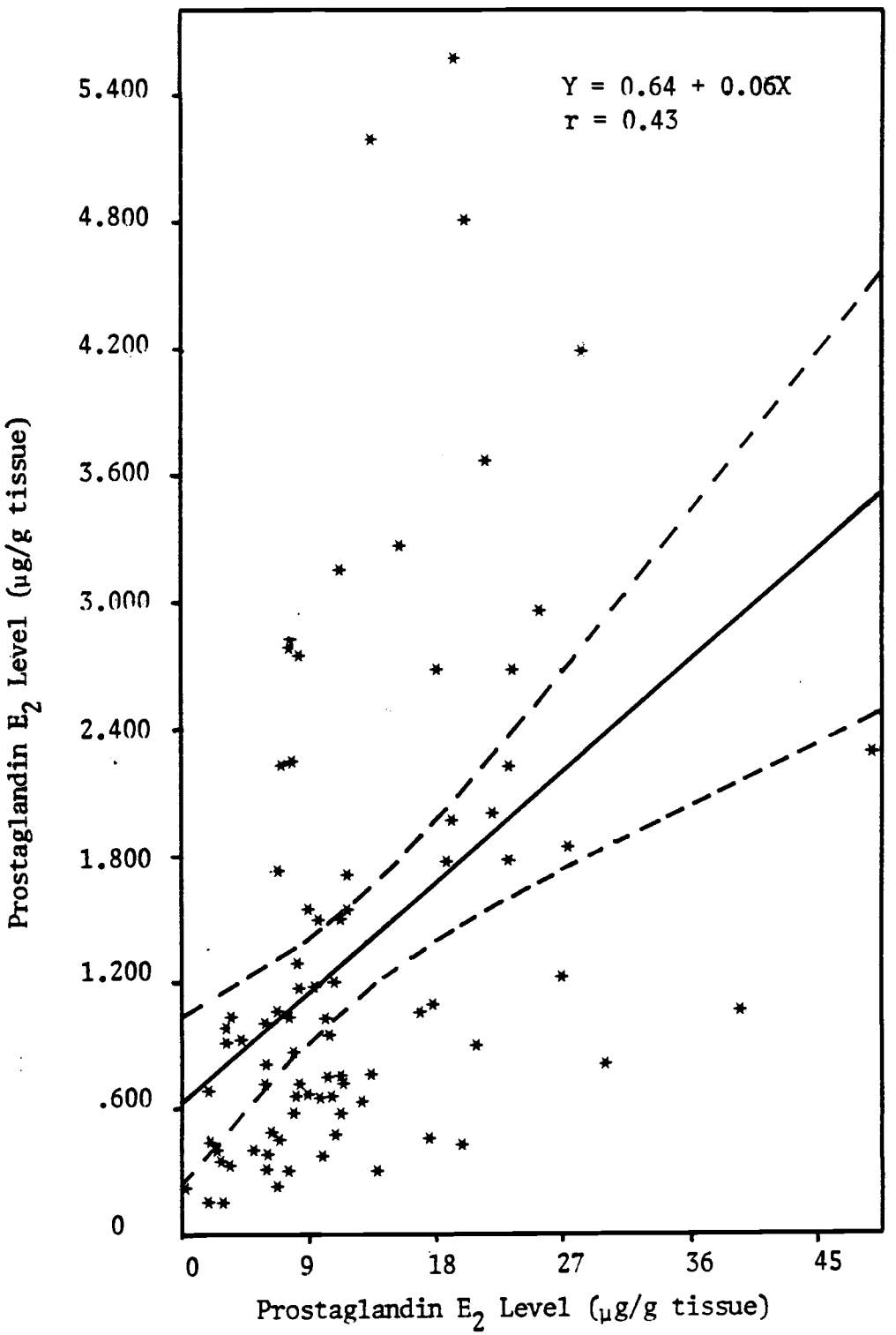

Figure 10. Regression of Prostaglandin $F_{2\alpha}$ Against Prostaglandin E_2.

DISCUSSION

This study has demonstrated a relationship between selenium concentrations and GSH-Px activities in the ram vesicular gland, which was statistically significant ($P<0.01$), although the correlation coefficient of 0.68 was only moderately high, a clustering of values at or near the 0.06µg/g level may account in part for the lack of higher correlation. Elsewhere DeVore and Greene (1982) reported a significant relationship and a correlation coefficient of 0.65 between GSH-Px activities and selenium concentrations in bovine semitendinosus muscles with a cluster of values at the 0.5µg/g region. They calculated cluster selenium concentrations of bovine muscles on a dry weight basis whereas selenium concentrations of the ram vesicular glands in this study were expressed on a wet weight basis. This might account for the difference in selenium concentrations at which the clusters of values occur in these two studies. The cluster region may coincide with selenium border-line deficiency (DeVore and Greene, 1982; and Hoffman et al., 1973). Since vesicular glands in this study were drawn randomly from a population of rams from the Western Oregon area where most grains and forages are low in selenium (Scott et al., 1976), more values would be expected to fall at low rather than high selenium levels. Nevertheless the highly

significant positive relationship between GSH-Px and selenium was expected, since each molecule of GSH-Px contains four atoms of selenium (Flohe´ et al., 1973 and Oh et al., 1974). This study substantiates the fact that selenium functions in the male reproductive system in the seminal plasma through GSH-Px but it does not exclude other functions. Under aerobic conditions ram spermatozoa produce organic peroxides which cause a decline in sperm respiration and motility (Jones and Mann 1973). These spermatozoa which lack GSH-Px activity entirely (Pond, 1981) or exhibit it in relatively small amounts (Alabi 1982) may need an additional source of GSH-Px to protect themselves against harmful effects of these lipid peroxides. This protection could be provided by the GSH-Px in seminal plasma which is produced by the accessory sex glands, thus maintaining sperm function and survival.

Although this study has demonstrated a statistically significant negative relationship between GSH-Px activities and $PGF_{2\alpha}$ levels, it did not demonstrate any significant relationship between selenium and PGE_2, selenium and $PGF_{2\alpha}$ and GSH-Px and PGE_2.

This negative correlation between GSH-Px activities and $PGF_{2\alpha}$ levels coincides with and supports a previous report that GSH-Px selectively decreases PGF production (Lands et al., 1971). However this study disagrees

with the same report on the acceleration of PGE production by GSH-Px.

Furthermore this study does not support the suggestion that GSH-Px functions in prostaglandin biosynthesis. Nugteren and Hazelhof (1973) isolated and identified 15-hydroperoxy-prostaglandin R_2 as a necessary intermediate in prostaglandin biosynthesis in the ram vesicular gland and postulated that the 15-hydroperoxy group is reduced by GSH-Px to form prostaglandin R_2 which is then converted into PGE_2 and PGF_2 by other enzymes. An investigation of the ram vesicular gland system for prostaglandin biosynthesis by this study failed to detect any significant positive relationship between GSH-Px activities and prostaglandin levels. GSH-Px may possibly catalyze the reduction of 15-hydroperoxy prostaglandin R_2 - or simply prostaglandin G_2 as called by other authors (Hamberg et al., 1974 and Flohe´ et al., 1976) - into prostaglandin R_2 but it is not an obligatory requirement for this process (Diplock 1981).

In 1977 a research team that includes Nugteren, one of the authors who suggested a role for GSH-Px in prostaglandin biosynthesis, isolated prostaglandin endoperoxide synthetase enzyme from sheep vesicular glands and purified it to homogeneity (Van Der Ouderaa 1977). They found that the same enzyme performs both

cyclooxygenase and peroxidase activities. This finding was in full agreement and support of a previous work of Miyamoto et al., 1976 who isolated and purified prostaglandin endoperoxide synthetase enzyme from bovine vesicular glands and found that it displayed both cyclooxygenase and peroxidase activities. Both reports could demonstrate that a single homogenous enzyme is responsible for the oxygenase as well as the peroxidase reactions in the metabolic pathway of prostaglandin synthesis in both ovine and bovine vesicular glands. It remains remarkable that the specific peroxidase and oxygenase activities of this enzyme were of the same magnitude. Accordingly the same enzyme which converts arachidonic acid into prostaglandin G_2 by its cyclooxygenase activity could convert prostaglandin G_2 into prostaglandin R_2 by its peroxidase activity (Van Der Ouderaa et al., 1977). This might explain why prostaglandin G_2 is a highly unstable compound, since it is converted into another compound by the same enzyme which catalyzes its production and since this conversion occurs at the same rate at which prostaglandin G_2 is produced. Figure 11 A and B illustrates the metabolic pathways for prostaglandin biosynthesis by the ram vesicular gland as illustrated by Nugteren and Hazelhof (1973) and Van Der Ouderra et al. (1977) respectively. The figure shows that the

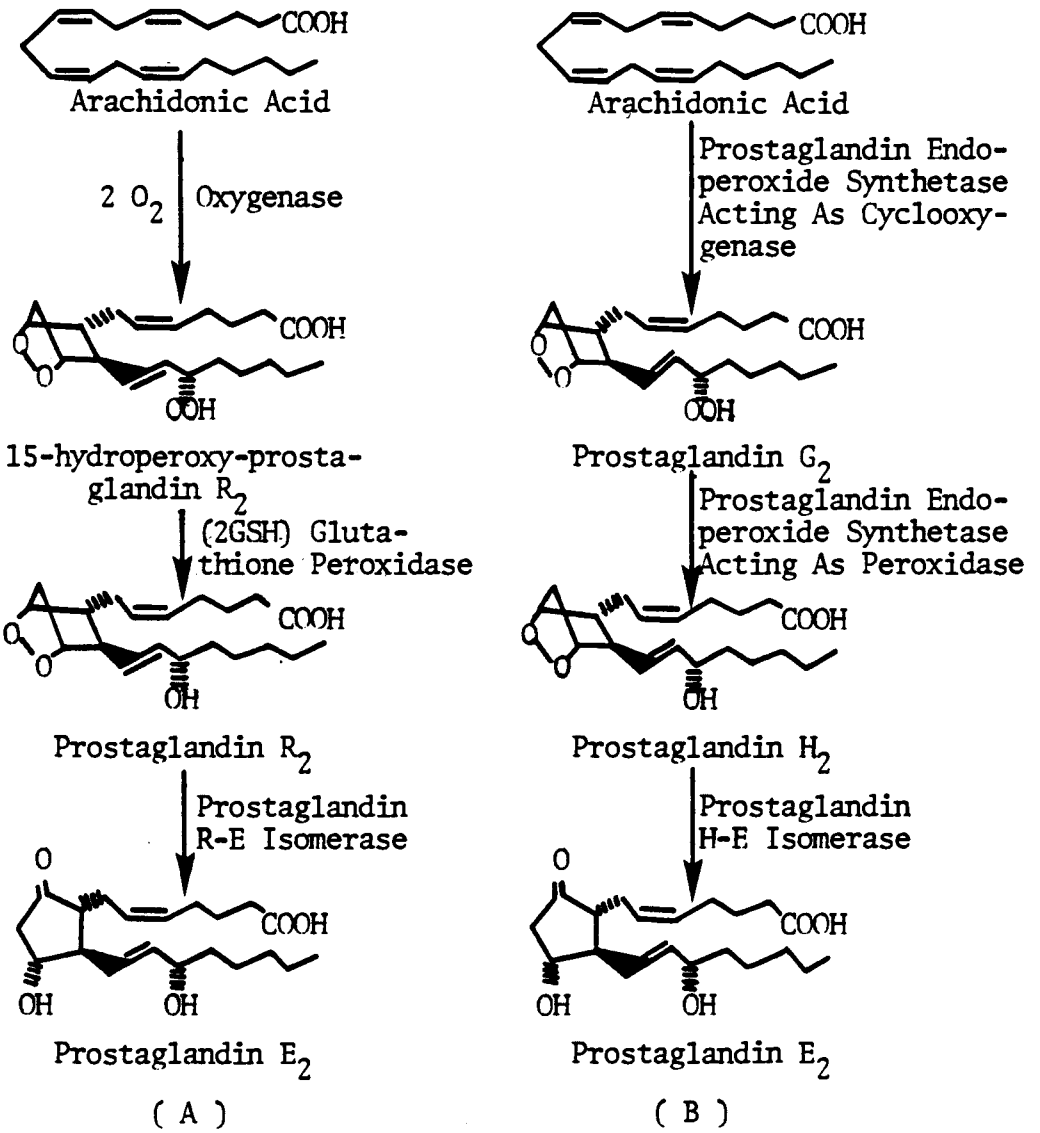

Figure 11. The Metabolic Pathway for Prostaglandin Biosynthesis by the Ram Vesicular Gland as depicted by Nugteren and Hazelhof (1973) (A) vs. Van Der Ouderaa, et al. (1977) (B) 15-Hydroperoxy-prostaglandin R_2 and Prostaglandin R_2 in (A) are identical to Prostaglandin G_2 and Prostaglandin H_2 in (B) respectively except that the structures in (A) are in the cis form while those in (B) are in the trans form.

suggestion of Nugteren and Hazelhof that glutathione peroxidase function in prostaglandin biosynthesis is contradicted by the findings of Van Der Ouderaa et al. that the prostaglandin endoperoxide synthetase exhibits at the same magnitude both cyclooxygenase and peroxidase activities. In this system it seems that GSH-Px has no role in prostaglandin biosynthesis per se, although it may have an indirect effect on prostaglandin biosynthesis. Van Der Ouderaa (1977) reported that the addition of H_2O_2 or ROOH strongly enhances the inactivation of prostaglandin endoperoxide synthetase. This coincides with the inhibitory effects of fatty acid hydroperoxides on prostacyclin formation which was identified earlier by Gryglewski et al., 1976. GSH-Px, therefore may protect the prostaglandin endoperoxide synthetase against the harmful effects of H_2O_2. This may explain the very low and statistically insignificant positive relationship between GSH-Px and PGE_2 but it does not explain the significant negative relationship between GSH-Px and $PGE_{2\alpha}$. Further research is needed to elaborate on and confirm this phenomenon.

Christ-Hazelhof et al. (1976) reported that serum albumins accelerate the decomposition of prostaglandin endoperoxides and that glutathione-S-transferase in the presence of glutathione converts the endoperoxides into a mixture of prostaglandin $F_{2\alpha}$, E_2 and D_2. This might

explain the reports (Samuelsson 1967, Van Dorp 1967 and Lapidus et al., 1968) that glutathione increases PGE production by the sheep vesicular gland synthetase system. Furthermore this glutathione-S-transferase has a GSH-Px activity with respect to lipid hydroperoxides and is identical to the so called selenium-independent GSH-Px (Prohaska and Ganther, 1977).

This study has also demonstrated a statistically significant positive relationship between PGE_2 and $PGF_{2\alpha}$ levels in the ram vesicular glands. Since both prostaglandins are synthesized by the same metabolic pathway and since both of them are made of prostaglandin R_2, this kind of relationship is expected.

SUMMARY AND CONCLUSIONS

Selenium and glutathione peroxidase do not appear to play a direct role in prostaglandin biosynthesis by the ram vesicular gland system. The results of this study do not agree with the suggestion of Nugteren and Hazelhof (1973) that glutathione peroxidase catalyzes the reduction of 15-hydroperoxy-prostaglandin R_2 into prostaglandin R_2. Hence glutathione peroxidase does not appear to be an indispensible enzyme in prostaglandin biosynthesis.

The role that selenium plays in male reproduction remains to be clearly defined. Further research is also needed to elaborate on the negative effect of glutathione peroxidase on prostaglandin $F_{2\alpha}$ biosynthesis and the significance of this effect.

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APPENDIX

Table 1. Selenium Concentrations, Glutathione Peroxidase Activities and Prostaglandin E_2 and Prostaglandin $F_{2\alpha}$ Levels in the Ram Vesicular Glands.

Number of Sample	Selenium Concentration µg/g tissue	Glutathione peroxidase (Enz. unit/ mg protein)	Prostaglandin E_2 (µg/g tissue)	Prostaglandin $F_{2\alpha}$ (µg/g tissue)
1	0.124	45.54	12.88	0.62
2	0.050	19.84	9.72	1.19
3	0.037	19.35	6.01	1.00
4	0.081	34.44	11.03	0.48
5	0.038	14.85	2.03	0.15
6	0.112	30.06	18.82	1.78
7	0.044	15.45	4.56	0.91
8	0.101	19.67	-	-
9	0.031	15.07	2.46	0.43
10	0.140	64.58	5.81	0.73
11	0.072	42.02	9.72	0.65
12	0.076	32.38	-	-
13	0.072	31.55	-	-
14	0.096	59.30	23.29	1.78
15	0.060	30.87	22.07	2.00
16	0.055	15.24	16.85	1.06
17	0.049	14.71	26.83	1.23
18	0.054	14.15	-	-
19	0.032	4.46	-	0.34
20	0.028	5.92	48.90	2.30
21	0.046	12.38	8.18	1.30

Number of Sample	Selenium Concentration µg/g tissue	Glutathione peroxidase (Enz. unit/ mg protein)	Prostaglandin E_2 (µg/g tissue)	Prostaglandin $F_{2\alpha}$ (µg/g tissue)
22	0.045	10.70	-	-
23	0.061	18.17	13.49	5.20
24	0.048	19.48	17.47	0.46
25	0.019	5.79	2.97	0.15
26	0.115	49.11	10.09	0.37
27	0.035	19.04	6.78	0.22
28	0.047	10.43	0.46	0.22
29	0.048	24.39	1.98	0.69
30	0.028	13.37	13.90	0.30
31	0.140	67.16	19.95	0.42
32	0.119	15.38	11.40	1.51
33	0.087	15.45	19.23	5.60
34	0.102	11.92	9.58	1.50
35	0.073	11.35	8.79	-
36	0.060	23.23	3.54	0.32
37	0.121	15.16	2.72	0.38
38	0.104	36.56	6.19	0.38
39	0.063	6.46	6.03	0.30
40	0.093	14.26	13.55	0.75
41	0.135	22.83	6.28	0.49
42	0.091	22.49	10.27	1.03
43	0.065	12.19	6.44	0.49
44	0.077	14.96	11.50	0.71
45	0.044	18.38	10.96	1.20

Number of Sample	Selenium Concentration µg/g tissue	Glutathione peroxidase (Enz. unit/ mg protein)	Prostaglandin E_2 (µg/g tissue)	Prostaglandin $F_{2\alpha}$ (µg/g tissue)
46	0.108	15.44	9.06	1.55
47	0.144	20.16	8.05	0.86
48	0.072	15.73	7.01	0.44
49	0.071	12.54	7.97	0.65
50	0.131	16.51	7.75	2.78
51	0.021	21.81	2.95	0.35
52	0.085	16.62	11.23	3.16
53	0.113	47.94	10.51	0.74
54	0.051	15.23	6.23	0.35
55	0.138	39.31	19.29	1.97
56	0.066	19.93	6.92	1.04
57	0.110	14.34	7.63	2.82
58	0.102	47.94	8.85	0.68
59	0.057	12.80	3.53	1.04
60	0.053	8.16	10.54	0.66
61	0.218	72.46	3.25	0.91
62	0.223	85.26	7.95	0.58
63	0.067	16.22	19.98	4.82
64	0.062	5.02	21.55	3.68
65	0.072	17.43	7.44	2.24
66	0.128	55.79	20.90	0.89
67	0.101	16.35	23.60	2.69
68	0.146	16.22	23.20	2.23

Number of Sample	Selenium Concentration µg/g tissue	Glutathione peroxidase (Enz. unit/ mg protein)	Prostaglandin E_2 (µg/g tissue)	Prostaglandin $F_{2\alpha}$ (µg/g tissue)
69	0.065	5.27	17.70	1.09
70	0.052	5.98	28.30	4.20
71	0.133	40.51	27.50	1.85
72	0.057	10.10	11.92	1.71
73	0.076	27.40	30.00	0.80
74	0.122	14.63	11.66	1.55
75	0.127	65.37	11.28	0.57
76	0.141	61.24	39.60	1.08
77	0.247	37.47	10.70	0.94
78	0.123	53.38	5.35	0.40
79	0.045	9.83	7.00	1.73
80	0.140	27.99	6.02	0.80
81	0.042	3.39	8.10	2.76
82	0.261	46.24	15.52	3.26
83	0.059	5.96	8.60	1.16
84	0.065	8.07	7.50	0.30
85	0.131	59.43	11.40	0.74
86	0.042	5.44	25.40	2.97
87	0.113	35.77	7.40	1.04
88	0.062	8.44	-	-
89	0.113	49.66	8.38	0.72
90	0.154	23.00	17.96	2.69
91	0.062	4.65	-	3.64

Number of Sample	Selenium Concentration µg/g tissue	Glutathione peroxidase (Enz. unit/ mg protein)	Prostaglandin E_2 (µg/g tissue)	Prostaglandin $F_{2\alpha}$ (µg/g tissue)
92	0.198	43.94	-	0.85
93	0.041	4.29	7.86	2.25
94	0.059	14.20	-	1.29

Table 2. Number of Observations, Range, Mean, Standard Deviation and Variance of Selenium, Glutathione Peroxidase, Prostaglandin E_2 and Prostaglandin $F_{2\alpha}$ of the Ram Vesicle Glands used in this Study.

	Number of Observations	Range	Mean	Standard Deviation	Variance
Selenium	94	0.019-0.261	0.088	±0.049	0.24
Glutathione peroxidase	94	3.39-85.26	24.52	±18.33	335.89
PGE_2	84	0.46-48.9	12.18	±8.56	73.31
$PGF_{2\alpha}$	87	0.15-5.60	1.36	±1.18	1.39