AN ABSTRACT OF THE THESIS OF

MOHAMMAD ATTA ALAYAN for the MASTER OF SCIENCE

in BIOCHEMISTRY AND BIOPHYSICS presented on June 10,1983

Title: SELENIUM METABOLISM IN THE CHICK AND EFFECTS OF VITAMIN E AND SELENIUM ON HEAT STRESS

Abstract approved: Redacted for Privacy

Dr. Philip D. Whanger

An *in vitro* study was conducted to investigate the metabolism and distribution of Se^{75}-selenite and Se^{75}-selenomethionine (SEM) in chick blood. Se^{75}-selenite is taken up by RBC (13% within 20 minutes) and expelled into the plasma to become bound to proteins. In contrast, Se^{75}-SEM showed a more gradual and continuous buildup in RBC with time, according to a hyperbolic type function. Chromatography of RBC lysates on Sephadex G200 established that Se^{75} from selenite is more specifically incorporated into GSH-Px (MW 150,000), whereas Se^{75} from SEM is mostly incorporated into Hb (MW 66,000). The elution pattern of plasma Se^{75}-selenoproteins from Se^{75}-selenite or Se^{75}-SEM on Sephadex G200 showed that radioactivity is associated with two peaks corresponding to MW >440,000 and 89,000.

Binding of Se^{75}, from selenite or SEM, to plasma proteins was dependent on the presence of RBC. Addition of GSH and GSSG reductase to plasma gave the same effects as RBC on binding of Se^{75} from selenite, but not from SEM, to plasma proteins.

To study the nature of this bond, the resistance of plasma protein bound Se^{75} (from selenite or SEM) to extraction by TCA or exhaustive dialysis was studied and indicated that the element was tightly bound to plasma proteins. Treatment with β-mercaptoethanol followed by TCA, however, caused release of most of the Se^{75} plasma selenoproteins (from both Se sources), suggesting that Se^{75} is present in proteins as a selenotrisulfide bond (Pr-S-Se^{75}-S-Pr). This was confirmed by chromatography of the released radioactivity on an amino acid analyzer. Based on these results, an <u>in vitro</u> model of selenium metabolism in chick blood is postulated.

After chicks were intubated with Se^{75}-selenite or Se^{75}-selenomethionine (SEM), the distribution and metabolism of radioactivity, as judged by incorporation into cytosolic proteins of various tissues, were studied. Gel filtration studies indicated that Se^{75} from selenite is more specifically incorporated into erythrocyte GSH-Px (MW 150,000) than Se^{75}-SEM. It was observed that chick hemoglobin (Hb, MW 66,000) bound Se^{75} from both sources, and possessed peroxidase activity. A mechanism was postulated to explain Hb peroxidase activity. Results of chromatographing plasma on Sephadex G200 indicated that proteins corresponding to MW of 338,000 are the major carriers of Se^{75} from selenite or SEM 6 hours after dosing, however proteins possessing a MW of 89,000 are the important Se^{75} carriers 96 hours after intubation.

Compared to tissues, the liver and kidney contained the highest concentrations of Se^{75}. Gel filtration chromatography of tissue cytosols on Sephadex G200 indicated that the majority (34-80%) of Se^{75}, from selenite or SEM, is associated with the enzyme GSH-Px 24 hours after dosing. Pancreatic cytosol of chicks dosed with Se^{75}-SEM had the majority of radioactivity associated with a peak corresponding to MW 44,500. Evidence for non-glutathione peroxidase selenoproteins were detected in various tissues. These included high molecular weight (>440,000) selenoproteins in cytosols of all tissues examined except in bone marrow, a peak (MW 44,500) specific to selenite but not SEM in liver cytosols, a low molecular weight (MW 25,100) protein in kidney and spleen cytosols from both Se^{75} treatments, and two distinct testicular selenoproteins (MW 35,000 and 263,000) from both Se^{75} treatments. A hypothetical pathway for the incorporation of Se^{75} into selenocysteine-containing proteins (i.e., GSH-Px) is presented to account for these observations.

During a study on the effects of high levels of Se and/or Vitamin E on performance under heat stress, a significant depression in gain and feed consumption was found, regardless of treatment. However, feed efficiency significantly improved in heat stressed chicks fed diets high in selenium and Vitamin E when compared to feed efficiency in animals on other dietary regimes. Significantly lower hematocrit levels were found in heat stressed chicks, as compared to control chicks, which were raised under conventional conditions. Se levels in chick blood, liver, kidney, and pancreas, and plasma Vitamin E levels

were directly related to dietary Se or Vitamin E content. Dietary Vitamin E supplementation counteracted the effect of heat stress in depressing blood selenium levels, however, the opposite trend was observed in the other organs studied. The pancreas in heat stressed chicks was larger than chicks raised at conventional temperatures.

Selenium Metabolism in the Chick and Effects of Vitamin E and Selenium on Heat Stress

by

Mohammad Atta Alayan

A THESIS

submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Master of Science

Commencement June, 1984

APPROVED:

Redacted for Privacy

Professor of Biochemistry in charge of major

Redacted for Privacy

Chairman of the department of Biochemistry and Biophysics

Redacted for Privacy

Dean of Graduate School

Date thesis is presented June 10,1983

Typed by C. Joy Adams for Mohammad Atta Alayan

DEDICATED WITH LOVE ...

To my father, my mother, and the rest of my family, who have given me all the love, support and help I needed. I am truly fortunate to have such wonderful people in my life to help and encourage me to achieve my ambitions.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation and thanks to Dr. Philip D. Whanger for his intensive help, guidance, and patience during the research and preparation of the thesis. His personal input and understanding were important for the completion of this thesis and will be always remembered.

I am grateful to Dr. George A. Arscott for his cooperation, help, and guidance in conducting the animal studies. Gratitude is also expressed to all the members of the Poultry Science Department for their friendly cooperation.

I am indebted to Mr. M.A. Beilstein and Mrs. Judy Butler for their help, suggestions, and friendship.

Appreciation is also extended to Mr. Sitir Hasanian and Ms. Maysoon Salama for their help and support in performing the chick dissections used in Chapter IV.

I am grateful to Kuwait Institute for Scientific Research for financing my study at Oregon State University.

Finally, I am thankful to Mrs. C. Joy Adams for her patience and cooperation in typing this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SELENIUM METABOLISM IN THE CHICK AND EFFECTS OF VITAMIN E AND SELENIUM ON HEAT STRESS

CHAPTER II
HISTORICAL BACKGROUND

It is possible that a man by the name of Arnold de Villanova was the first to observe and describe the element which came to be known as selenium. In "Rosarius Philosophorum", written around the beginning of the fourteenth century,[1] he described the vaporization of sulfur and the appearance on the walls of the container of a deposit which he called "sulfur rubeum". It was not until 1817, however, that a reliable account of the isolation and identification of the new element, named selenium, was published by J. J. Berzelius.[2] The new element was given the name, selenium, for the moon, because of its similarity to tellurium which, 35 years earlier, had been named for the earth. Although Berzelius was thrilled, as anyone would be, at the discovery of a new element, he was apparently too busy with other things to do anything with it, and it remained on chemists' shelves for many years--a chemical curiosity that gave little hint of its potential power as one of the most reactive substances on earth, as evidenced from its schizopherenic chemical personality, behaving as a metallic non-metal and non-metallic metal. This behavior is what makes this element interesting, but difficult to work with in the laboratory. Because of its unique and unusual properties, many, many diverse uses have been found for this element.

The importance of selenium as a nutritionally essential element went unnoticed for many years, due mainly to the almost infinitesimal

quantities in which it functioned, and to the lack of a sensitive method for its detection. It is unfortunate, also, that when it was "noticed", it was at toxicity levels. This toxicity manifested itself in, what was then, the Dakota Territory in the loss of horses and this loss was attributed by Franke and others[3] to selenium.

The history of selenium as a nutritionally essential element began in 1951 when Klaus Schwarz[4] reported the existence of a "hitherto unrecognized factor" against necrotic liver degeneration and named it "Factor 3".

In the same year, M. L. Scott[5] reported that a severe turkey leg abnormality, produced experimentally by adding cod liver oil to a practical diet low in Vitamin E, could be partially prevented by supplementing the diet with Vitamin E, but completely prevented by the addition of 10% dried brewers yeast to the diet.

Progress toward elucidation of the nature of the unidentified factor in yeast gained considerable impetus when both Klaus Schwarz and Milt Scott were invited to become members of the Research Advisory Board of the Brewers Yeast Council.

The discovery of Se as the factor responsible for prevention of exudative diathesis in chicks and liver necrosis in rats was a serendipitous event. In an attempt to characterize the chemical nature of Factor 3, Schwarz produced a fraction which had a very strong odor when concentrated. A person who had lived in South Dakota entered Schwarz's laboratory and inquired about the source of the strong garlic odor in the lab. Schwarz replied that it was his Factor

3 concentrate. The person responded that the odor smelled like the breath of cows in South Dakota which had been consuming seleniferous herbage, and suggested that Schwarz run a selenium assay on the concentrate.

Indeed, not only was the concentrate of Factor 3 high in selenium, but Schwarz and Foltz (1957)[6] immediately showed that inorganic selenium salts were potent in preventing liver necrosis, subsequently Scott et al.[7] in the same year showed that selenium would prevent exudative diathesis.

Soon after this discovery, selenium metabolism became an attractive area to both nutritionists and biochemists. Muth et al. (1959)[8], at Oregon State University, and Proctor et al. (1958)[9], at Cornell University, showed that selenium would prevent a long term problem, muscular dystrophy in sheep, also known as "stiff lamb disease". Afterward, some New Zealand work, by Andrews et al. (1968),[10] demonstrated that selenium deficiency had been responsible for muscular dystrophies in lambs, white muscle disease in calves, and reproductive problems and "ill thrift" in adult sheep and cattle.

Despite the recognition that exudative diathesis was a major problem in broilers in the United States, and despite the application supported by the wealth of evidence submitted by the American Feed Manufacturers Association (AFMA) to the Food and Drug Administration (FDA) to approve the addition of selenium to poultry and livestock feeds, the FDA officials were in a quandary. This was partly attributed to the observation reported by Nelson et al.[11] in 1943,

describing liver tumors following cirrhosis caused by selenium in rats. Even though this work was subject to many serious questions, and in spite of the fact that Tinsley et al.[12] in 1967, at Oregon State University, using over 500 tumor-susceptible rats and performing over 6,000 nicropsies, found no evidence that selenium was a carcinogen, the FDA did not approve the addition of this nutrient to feeds until 1974.

After the voluminous widespread studies on the metabolic effects of selenium, scientists became more interested in investigating its action. The discovery that selenium constitutes an integral part of the enzyme glutathione peroxidase helped solve the "selenium dilemma". As soon as Rotruck and coworkers[13] in 1973 made their report in "Science", Noguchi et al.[14,15] initiated a series of experiments which demonstrated that selenium was acting in glutathione peroxidase in the prevention of exudative diathesis.

Now, the interrelationship among Se, Vitamin E, antioxidants, and sulfur amino acids in ameliorating the same nutritional disorders can be rationalized. Vitamin E and antioxidants would help prevent the formation of harmful fatty acid hydro-peroxides, in accordance with their well-known effects in scavenging free radicals. Sulfur amino acids (as precursors of glutathione) and selenium as an integral part of glutathione peroxidase, bring about the decomposition of peroxides once formed.

It was natural that the profound biological effects caused by selenium in animals raised questions about the potential for similar

effects in humans. Some interesting relationships were found. In the early 1970s, Dr. Frost[16] conducted regional surveys, on the possibility of an inverse relationship between dietary selenium levels and incidence of certain forms of cancer in humans. A number of definitive studies have followed, and the relationship appears to be more causal than coincidental.[17,18] It seems quite interesting that a substance once reported as a carcinogen could serve in cancer therapy.

It is interesting that a selenium responsive muscle degenerative syndrome, Keshan's Disease, has been reported in humans in China.[19] This disease was essentially eradicated by widespread selenium supplementation.

Earlier in New Zealand, Donald Money[20] had wondered whether selenium would alleviate crib death disease. He suggested that since New Zealand cows are fed almost entirely on forage, often grown in seriously selenium deficient areas, selenium deficiency might be the cause. Despite results indicating selenium supplementation is not effective against this problem of the very young, their frequent incidence under conditions of less-than-adequate selenium supply leaves the door open to continuing interest and speculation.

The list of selenium functions increase with time. Results indicated that selenium is associated with pancreatic function of the chick,[21] hepatic heme metabolism,[22] aging,[23] immunity and immune cytotoxicity,[24] spermatogenesis,[25] heavy metal detoxication,[26] dental cavies,[27] rheumatoid arthritis and alcoholic cirrhosis,[28] ... and many more to come.

Indeed, selenium's schizopherenic chemical personality opened to scientists the door of an endless treasury.

REFERENCES

1. Mellor, J.W. (1956) A Comprehensive Treatise on Inorganic and Theoretical Chemistry. John Wiley and Sons, Inc.:New York, NY.

2. Berzelius, J.J. (1817) Annales de chimie et de physique, Paris, Serie 2, Tome 7, 199.

3. Franke, K.W. (1934) A new toxicant occuring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminaty feeding trials. J. Nutr. 8:597-608.

4. Schwarz, K. (1951) A hitherto unrecognized factor against dietary necrotic liver degeneration in American yeast (factor 3). Proc. Soc. Exp. Biol. Med. 78:852-856.

5. Scott, M.L. (1951) Studies on the enlarged hock disorder in turkeys. 3. Evidence of the detrimental effect of fish liver oil and the beneficial effect of dried brewers' yeast and other materials. Poul. Sci. 30:846-855.

6. Schwarz, K. and Foltz, C.M. (1957) Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79:3292-3283.

7. Schwarz, K., Bierl, J.G., Briggs, G.M. and Scott, M.L. (1957) Prevention of exudative diathesis in chicks by factor 3 and selenium. Proc. Soc. Exp. Biol. Med. 45:621-625.

8. Muth, O.H., Oldfield, J.E., Remmert, L.F., and Schubert, J.R. (1958) Effects of selenium and Vitamin E on white muscle disease. Science 128:1090.

9. Proctor, J.E., Hague, D.E., and Warner, R.G. (1958) Selenium, Vitamin E, and linseed oil meal as preventives of muscular dystrophy in lambs. J. Am. Sci. 17:1183-1187.

10. Andrews, E.D., Hartley, W.J., and Grant, A.B. (1968) Selenium-responsive diseases of animals in New Zealand. N.Z. Vel. J. 16:3-17.

11. Nelson, A.A., Fitzhugh, O.G., and Calvery, H.O. (1943) Liver tumors following cirrhosis caused by selenium in rats. Cancer Res. 3:230-236.

12. Tinsley, I.J., Harr, J.R., Weswig, P.H. and Yamamoto, R.S. (1967) Selenium toxicity in rats. I. Growth and longevity. In Symposium: Selenium in Biomedicine. O.H. Muth (editor). AVI Publishing Co.:Westport, CT.

13. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.C., and Hoekstra, W.G. (1973) Selenium, biochemical role as a component of glutathionine peroxidase. Science 179:588-590.

14. Noguchi, T., Langevin, M.L., Combs, G.F.Jr., and Scott, M.L. (1973) Biochemical and histochemical studies of the selenium deficient pancreas in chicks. J. Nutr. 103:44.

15. Noguchi, T. Contor, A.H., and Scott, M.L. (1973) Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. J. Nutr. 103:1502.

16. Frost, D.V. (1972) The two faces of selenium--can selenophobia be cured? CRC Crit. Rev. Toxicol. 1:467-514.

17. Shapiro, J.R. (1972) Selenium and carcinogenesis: a review. In Organic selenium and tellurium chemistry. Ann. N.Y. Acad. Sci. 192:215-219.

18. Greeder, G.A., Poiner, K.A., and Milner, J.A. (1980) Selenium and Cancer. Illinois Research (Winter issue) 8-9.

19. Chen, X., Chen, X., Yang, G., Wen, Z., Chen, J., and Ge, K. (1979) Observations on the effect of sodium selenite in prevention of Keshan disease. Chin. Med. J. 92:471-478.

20. Money, D.F.L. (1970) Vitamin E and selenium deficiencies and their possible actiological role in the sudden death in infants syndrome. N.Z. Med. J. 71:32-34.

21. Noguchi, T., Langerin, M.L., Combs, G.F.Jr., and Scott, M.L. (1973) Biochemical and histochemical studies of the selenium deficient pancreas in chicks. J. Nutr. 103:444-453.

22. Burk, R.F., Lawrence, R.A., and Correia, M.A. (1980) Sex differences in biochemical manifestations of selenium deficiency in rat liver with special reference to heme metabolism. Biochem. Pharmacol. 29:39-42.

23. Burch, R.E., Sullivan, J.F., Jetton, M.M., and Hahn, H.K.J. (1979) The effect of aging on trace element content of various rat tissues. I. Early stages of aging. Age 2:103-107.

24. Spallholz, J.E. (1981) Anti-inflammatory, immunologic, and carcinostatic attributes of selenium in experimental animals. In Diet and Resistance to Disease. American Chemical Society Symposium. Plenum Press. pp. 43-62.

25. Wu, S.H., Oldfield, J.E., Whanger, P.D., Weswig, P.H. (1973) Effects of selenium, Vitamin E, and antioxidants on testicular function in rats. Biol. Reprod. 8:625-629.

26. Whanger, P.D. (1981) Selenium and heavy metal toxicity. In Selenium in Biology and Medicine. Spallhols, J.E., Martin, J.L., and Ganther, H.E. (editors). AVI Publishing Company, Inc.:Westport, CT.

27. Curzon, M.E.J. and Crocker, D.C. (1978) Relationships of trace elements in human tooth enamel to dental cavies. Arch. Oral Biol. 23:647-653.

28. Aaseth, J., Munthe, E., Forre, O, and Steinnes, E. (1978) Trace elements in serum and urine of patients with rheumatoid arthritis. Scand. J. Rheumatol. 7:237-240.

CHAPTER II
INTRODUCTION

Despite the extensive data available on absorption, distribution, and route of excretion of selenium (Se) in laboratory and large animals,[1] information on the cellular metabolism of Se is incomplete. Administered Se has been found to be transported in plasma bound to proteins.[2] The mechanism of labelling plasma proteins with Se has been studied by several workers.[3-7] The labelling of plasma proteins with Se derived from selenite is dependent upon the presence of erythrocytes and this is influenced by glutathione (GSH). Similar to selenite, other studies have found that Se from selenomethionine (SEM) is transported in blood bound to plasma proteins.[8,9] The rate of binding Se from SEM to plasma proteins remains unchanged in animals with either subtotal or total hepatectomy.[10] Moreover, a small proportion of Se from SEM is bound to plasma proteins after *in vitro* incubation with whole blood. These results suggested that labelling of plasma proteins with Se from SEM is not dependent on the liver, and red blood cells appear to be involved. At present, neither the exact role of red blood cells in the metabolism of selenite is completely understood, nor is their role in labelling plasma proteins with Se from SEM known. The *in vitro* study in Chapter III was conducted to obtain more information on the mechanism by which Se^{75} derived from selenite or SEM is transported and metabolized by chick blood.

The role of Se as an essential trace element has been well established. Selenium deficiency has been associated with various

pathological conditions, namely liver necrosis in rats,[11] white muscle disease in lambs and calves,[12] hepatosis dietatica in pigs,[13] and exudative diathesis in chicks.[14] In all these disorders, subnormal activity of the enzyme, glutathione peroxidase (E.C. 1.11.1.9) was observed, suggesting that the Se role in alleviating these cases is mediated through this enzyme. Deficiency of Se also causes structural deformation of the sperm tail of rats[15] and pancreatic fibrosis in the chick.[16] The role of Se in preventing these symptoms does not appear to be through glutathione peroxidase. Moreover, SEM was found more potent in protecting chicks against pancreatic fibrosis than selenite,[17] suggesting a unique role, not only specific for Se, but also for certain chemical forms.

Also, Se has been advocated to have a variety of functions including the inhibition of tumor growth,[18] improving immune response,[19] preventing eye cataracts,[20] counteracting certain heavy metal toxicity,[21,22] and protecting against cardiac disorders in humans.[23] The variety of functions of Se suggest multiple modes of biochemical action probably as an integral part of many selenoproteins or as a cofactor for many enzymes. In an attempt to study the metabolic role of Se, another investigation was performed (Chapter IV). This was done by studying the metabolism of selenite and SEM as judged by incorporation into glutathione peroxidase, and by incorporation into the cytosolic Se binding proteins of various chick tissues.

The adverse effect of elevated temperature on chick growth and on

diet consumption is well known. The impaired growth is partly due to less feed intake but mostly by less efficient feed conversion.[24] In an attempt to overcome the adverse effect of heat on chick productivity and performance, many experiments were conducted seeking a nutritional solution.[25-28] In the literature, there is no evidence that the effect of Se and/or Vitamin E on alleviating heat stress was studied, despite the fact that there is a good indication that these two substances could play certain roles in overcoming this problem.

It was indicated that the erythrocytes count is lower for chicks raised at high environmental temperatures than if raised at recommended levels.[29] Meanwhile, oxygen consumption and respiratory rate increases and the hemoglobin level decreases in chicks as the environmental temperature rises. Such a situation subjects the red blood cells under extremely high stress that might affect their efficiency as well as their span of life. Se and Vitamin E has been shown to protect erythrocytes against oxidative damage.

As stated previously the depression in growth is mostly due to a less efficient feed conversion, mostly because of a decrease in amylolytic and proteolytic activity of the digestive enzymes. Since the pancreas is the organ responsible for secreting most of these important digestive enzymes, the condition of the pancreas would largely be reflected in the activity of digestion and, thus, feed utilization. The role of Se in pancreatic function has been documented[16] and a high level of Se in diets of chicks raised under

high temperatures could improve feed conversion.[30] Chapter V reports a study to investigate the effect of supplementing broiler diets with high levels of Se and/or Vitamin E on their performance under heat stress.

REFERENCES

1. Ganther, H.E. (1965) The fate of selenium in animals. World Review of Nutrition and Dietetics. 5:38.

2. Jenkins, K.J. and Hidiroglou (1969) Intravascular transport of selenium by chick serum proteins. Can J. Phys. Pharmacol. 47:459-467.

3. Lee, M., Dong, A., and Yano, J. (1969) Metabolism of Se^{75}-selenite by human whole blood *in vitro*. Can. J. Biochem. 47:791-797.

4. Sandholm, M. (1973) The metabolism of selenite in cow blood *in vitro*. Acta Pharmacol. et Toxicol. 33:6-16.

5. Sandholm, M. (1975) Function of erythrocytes in attaching selenite-Se onto specific plasma proteins. Acta Pharmacol. Toxicol. 36:321-327.

6. McMurray, C.H., and Davidson, W.B. (1979) *In vitro* metabolism of selenite in sheep blood, factors controlling the distribution of selenium and the labelling of plasma proteins. Biochem. Biophys. Acta. 583:332-343.

7. Proter, E.K., Karle, J.A., and Shrift, A. (1979) Uptake of selenium-75 by human lymphocytes *in vitro*. J. Nutr. 109:1901-1908.

8. Plenner, J.A. (1964) Selenomethionine incorporation into plasma proteins. Clin. Res. 12:277.

9. Awwad, H.K., Potchen, E.J., Adelstein, S.J., and Dealy, J.B. Jr. (1966) Se^{75}-selenomethionine incorporation into human plasma proteins and erythrocytes. Metabolism. 15:626-640.

10. Sternberg, J., and Inbach, A. (1967) Metabolic studies with seleniated compounds, II. Turnover studies with Se^{75}-methionine in rats. Int. J. Appl. Radiat. Isot. 18:557-568.

11. Schwarz, K. and Foltz, C.M. (1967) Selenium as an integral part of factor 3 against necrotic liver degeneration. J. Am. Chem. Soc. 79:3293-3300.

12. Muth, O.H., Oldfield, J.E., Remmert, L.F., and Schubert, J.R. (1958) Effect of selenium and Vitamin E on white muscle disease. Science. 128:1090.

13. Eggert, R.G., Patterson, E., Akers, W.J., and Stokstad, E.L.R. (1957) The role of Vitamin E and selenium in the nutrition of the pig. J. An. Sci. 16:1037.

14. Scott, M.L., Bieri, J.G., Briggs, G.M., and Schwarz, K. (1957) Prevention of exudative diathesis by factor 3 in chick on Vitamin E-deficient formula yeast diets. Poultry Sci. 36:1155.

15. Wu, S.H., Oldfield, J.E., Whanger, P.D., and Weswig, P.H. (1973) Effects of selenium, Vitamin E, and antioxidants on testicular function in rats. Biol. Reprod. 8:625-629.

16. Noguchi, T., Langevin, M.L., Combs, G.F.Jr. and Scott, M.L. (1973) Biochemical and histochemical studies of the selenium deficient pancreas in chicks. J. Nutr. 103:444-453.

17. Combs, G.F.Jr., and Bunk, M.J. (1981) In Selenium in Biology and Medicine. Spallholz, J.E., Martin, J.L., and Ganther, H.E. (editors). AVI Publishing Company, Inc.:Westport,CT. pp. 70-85.

18. Shapiro, J.R. (1972) Selenium and carcinogenesis, a review. In Organic selenium and tellurium chemistry. Ann. N.Y. Acad. Sci. 192:215-219.

19. Baumgartner, W.A. (1979) In Trace metals in health and disease. Kharasch, N. (editor). Raven Press:New York. pp. 287-305.

20. Bhuyan, K.C., Bhuyan, D.K., and Podos, S.M. (1981) In Selenium in Biology and Medicine. Spallholz, J.E., Martin, J.L., and Ganther, H.E. (editors). AVI Publishing Company, Inc.:Westport:CT. pp. 403-412.

21. Levander, O.A., Morris, V.C., and Ferretti, R.J. (1977) Comparative effects of selenium and Vitamin E in lead-poisoned rats. J. Nutr. 107:378-382.

22. Whanger, P.D. (1981) In Selenium in Biology and Medicine. Spallholz, J.E., Martin, J.L., and Ganther, H.E. (editors). AVI Publishing Company, Inc.:Westport,CT. pp. 230-255.

23. Thomson, C.D. and Robinson, M.F. (1980) Selenium in human health and disease with emphasis on those aspects peculiar to New Zealand. Am. J. Clin. Nutr. 33:303-323.

24. Adams, R.L., Andrews, F.N., Gardiner, E.E., Fontaine, W.E., and Carrick, C.W. (1962) The effect of environmental temperature on the growth and nutritional requirements of the chick. Poultry Sci. 41:588.

25. McCorrick, Garligh, J.D., and Eders, F.W. (1979) The effect of P and Ca nitrition on the tolerance of chicks to heat stress. Fed. Proc. 38:766.

26. Dale, N.M. and Fuller, H.L. (1979) Effects of diet composition on feed intake and growth of chicks under head stress. I. Dietary fat levels. Poultry Sci. 58:1529-1534.

27. Dale, N.M. and Fuller, H.L. (1980) Effect of diet composition on feed intake and growth of chicks under heat stress. II. Constant vs. cycling temperatures. Poultry Sci. 59:1434-1441.

28. Waldroup, P.W. (1982) Influence of environmental temperature on protein and amino acid needs by poultry. Fed. Proc. 41:2821-2823.

29. Deaton, J.W., Reece, F.N., McNally, E.H., and Torrer, W.J. (1969) Liver, heart, and acrenal weights of broilers reared under constant temperatures. Poultry Sci. 48:283.

30. Bunk, M.J., and Combs, G.F.Jr. (1980) Effect of selenium on appetite in the selenium-deficient chick. J. Nutr. 110:743-749.

CHAPTER III

In Vitro Metabolism of Se^{75}-Selenite and Se^{75}-Selenomethionine in Chick Blood

by

M.A. Alayan,[1] P.D. Whanger,[2] and M.A. Beilstein[2]

[1]Department of Biochemistry and Biophysics
Oregon State University
Corvallis, Oregon 97331

[2]Department of Agricultural Chemistry
Oregon State University
Corvallis, Oregon 97331

CONTRIBUTIONS

Mohammad A. Alayan participated in developing the experimental design, raising the chicks, taking blood samples, conducting all the experiments and biochemical analyses, analyzing the data and calculating the results, performing the statistical analyses and writing the article. Dr. P.D. Whanger participated in the experimental design and editing of the article. M.A. Beilstein assisted in training M.A. Alayan on the amino acid analyzer and discussing the experimental results.

In Vitro Metabolism of Se^{75}-Selenite and Se^{75}-Selenomethionine in Chick Blood

ABSTRACT

The present *in vitro* study was conducted to investigate the metabolism and distribution of Se^{75}-selenite and Se^{75}-selenomethionine (SEM) in chick blood. Se^{75}-selenite is taken up by RBC (13% within 20 minutes) and expelled into the plasma to become bound to proteins. In contrast, Se^{75}-SEM showed a more gradual and continuous buildup in RBC with time, according to a hyperbolic type function. Chromatography of RBC lysates on Sephadex G200 established that Se^{75} from selenite is more specifically incorporated into GSH-Px (MW 150,000), whereas Se^{75} from SEM is mostly incorporated into Hb (MW 66,000). The elution pattern of plasma Se^{75}-selenoproteins from Se^{75}-selenite or Se^{75}-SEM on Sephadex G200 showed that radioactivity is associated with two peaks corresponding to MW >440,000 and 89,000.

Binding of Se^{75}, from selenite or SEM, to plasma proteins was dependent on the presence of RBC. Addition of GSH and GSSG reductase to plasma gave the same effects as RBC on binding of Se^{75} from selenite, but not from SEM, to plasma proteins.

To study the nature of this bond, the resistance of plasma protein bound Se^{75} (from selenite or SEM) to extraction by TCA or exhaustive dialysis was studied and indicated that the element was tightly bound to plasma proteins. Treatment with β-mercaptoethanol

followed by TCA, however, caused release of most of the Se^{75} plasma selenoproteins (from both Se sources), suggesting that Se^{75} is present in proteins as a selenotrisulfide bond (Pr-S-Se^{75}-S-Pr). This was confirmed by chromatography of the released radioactivity on an amino acid analyzer. Based on these results, an *in vitro* model of selenium metabolism in chick blood is postulated.

IN VITRO METABOLISM OF Se^{75}-SELENITE AND Se^{75}-SELENOMETHIONINE IN CHICK BLOOD

INTRODUCTION

Absorbed selenium (Se) has been found to be transported in blood plasma bound to proteins, and studies have been conducted in various species to investigate the mechanism of Se transport in whole blood.[1-9] These studies have shown that binding of administered Se, as selenite, to plasma proteins is dependent on the presence of erythrocytes, suggesting that a transformation of selenite occurs within red blood cells (RBC). Reports indicated that 50-70% of the introduced radioactive sodium selenite ($Na_2Se^{75}O_3$) accumulated inside RBC within one minute.[3,5] Thereafter, there was a gradual expulsion of Se^{75} from the cell. The process of Se^{75} expulsion from RBC depends on adequate glutathione (GSH) levels in these cells.[8] Up to this date, knowledge concerning the metabolism of selenite in RBC has been incomplete.

Although selenomethionine (SEM) has been found to be transported in blood plasma associated to proteins,[10-12] information about the role of RBC in its metabolism is lacking. The present *in vitro* study was performed to obtain further information on the mechanism by which Se^{75}-selenite and Se^{75}-selenomethionine are transported and metabolized by chick blood.

MATERIALS AND METHODS

Animal Selection and Blood Collection

The animals used in this study were White Leghorn male pullets raised on the floor until they were six weeks old. The chicks were fed a practical corn-soybean meal ration containing 20% dietary crude protein, 2800 kcal/kg metabolizable energy and 0.15 ppm Se. The blood was collected in vacutainer evacuated glass tubes coated with heparin as an anticoagulant from the wing vein.

Experiment 1

1. Uptake and release of Se^{75} by chick RBC

The method of Wright and Bell[13] was used to study the uptake and release of Se^{75} as selenite and SEM by chick RBC with some modification.[3] Blood was incubated at 41.5°C in a water bath for 10 minutes to adjust its temperature to the normal deep body temperature of the chick. Exactly 3 mls of blood were taken and incubated with 100 µl of Se^{75} selenite (6.04 ng Se^{75}, specific activity 437.57 mCi/mg). Another 3 mls blood were incubated with 100 µl of Se^{75}-SEM (1.685 ng Se^{75}, specific activity 4.54mCi/mg). After mixing, duplicate 100 µl samples were removed at 0, 5, 10, 20, 40, 80, 120, 160, and 12 hours after both treatments, and rapidly expelled into test tubes containing 0.5 ml iced physiological saline (0.9% NaCl). Samples were centrifuged for 1 minute at 4000xg and the supernatant (plasma

fraction) was removed and replaced with 0.5 ml physiological saline. After gentle mixing, the tubes were centrifuged and the washing was repeated 3 times. After the third washing, the RBC were suspended in physiological saline and counted in Beckman scintillation counter (Model 8000). In addition, the plasma and washing were counted. The remaining blood samples, 12 hours after incubation with Se^{75}-selenite and Se^{75}SEM, were centrifuged at 4000xg for 1 minute, and the plasma was removed and stored at -8°C until further analyses. RBC, from both treatments, were washed as described earlier. The RBC lysate was prepared by suspending RBC in 2 mls double distilled water (DDH_2O), hand shaken, and centrifuged at 4000xg for 1 minute to separate the particulate fraction. The particulate fraction was washed twice with 2 mls DDH_2O and the washing added to the lysates to bring the volume to 5 mls. The RBC lysates were stored at -8°C for gel filtration studies.

2. Distribution of Se^{75} among plasma and RBC lysates

Five ml samples of RBC lysates were applied to 2 x 130 cm column containing Sephadex G200 (flow rate of 12 ml/hr) and chromatographed using 0.05 M phosphate buffer containing 0.01 EDTA and 0.003 N NaN_3. Six ml fractions were collected and assayed for radioactivity, glutathione peroxidase activity[14,15] and optical density at 280 nm. The Sephadex G200 was prepared according to the method described by Cooper[16] and details concerning its calibration are described in chapter IV, Materials and Methods section.

Plasma samples were first chromatographed on Sephadex G25

superfine, particle diameter (10-40 μ) as a quick technique to test if Se^{75} was bound to protein, using the buffer described earlier. Fifty 1 ml fractions were collected and assayed for radioactivity and optical density at 280 nm. The percentage distribution of Se^{75} as protein bound and non-protein bound was calculated. Se^{75}-plasma protein bound fractions from each treatment were pooled and then applied to columns containing Sephadex G200 and eluted with the buffer described above. Seven ml fractions were collected and assayed for radioactivity and optical density at 280 nm.

Sephadex G25 column [bed volume (V_t) 29 ml] was calibrated prior to usage. Blue dextran (Pharmacia) with an average molecular weight (MW) of 2 x 10^6 dalton (d) was used to determine the void volume (V_o). Se^{75}-labelled compounds (selenite MW 129, SEM MW 196, and Selenocysteine MW 334) were used to calibrate the column and the distribution coefficient was calculated (Table III.1).

Experiment 2

In this experiment, the role of RBC in labelling plasma proteins with Se^{75} from both selenite and SEM was investigated. The following treatments were used:

1. 3 ml blood + 100 μl Se^{75}-selenite solution
2. 3 ml blood + 100 μl Se^{75}-SEM solution
3. 0.5 ml plasma (without RBC) + 17 μl Se^{75}-selenite solution
4. 0.5 ml plasma (without RBC) + 17 μl Se^{75}-SEM solution

All treatments were incubated at 41.5°C for 12 hours. After the incubation period, treatments 3 and 4 were stored at -8°C and treatment 1 and 2 were centrifuged at 4000xg for 1 minute to separate plasma from RBC. The plasma was aspirated and stored at -8°C. RBC of each treatment were washed and RBC lysate was prepared as described before.

RBC lysates and plasma samples of the various treatments were applied to a column containing Sephadex G25 and eluted using the buffer described earlier. Fifty 1 ml fractions were collected and assayed for radioactivity and O.D. at 280 nm. The flow rate was maintained at 1 ml/4.5 min., using a peristaltic pump.

From the chromatogram, the percentage of distribution of Se^{75} as protein bound and non-protein bound, as evidenced by optical density scans at 280 nm and 20% trichloroacetic acid (TCA) treatments, was calculated. Afterward, Se^{75} protein bound fractions from RBC lysates and plasma samples of various treatments were pooled and subjected to dialysis.

Dialysis was performed in stirred 2L baths at 4°C for 24 hours using dialysis tubing with a pore radius permeability up to 3500 d. The samples were counted before dialysis, after dialysis against DDH_2O, and after dialysis against 0.05 M Tris base at pH 11. The counts were corrected for decay and percentage of loss was calculated.

Experiment 3

In this experiment the effect of GSH, NADPH and the enzyme glutathione reductase (GSSG reductase, E.C. 1.6.4.2) on the binding of

Se^{75} from selenite and SEM was studied. The following treatments were prepared.

1. 0.05 ml plasma + 17 µl Se^{75} (as selenite or SEM)
2. treatment 1 + 100 µl 0.01 GSH solution
3. treatment 2 + 10 µl GSSG reductase, 9.2 mg protein/ml, 225 units/mg (one unit will reduce 1.0 µmole of GSSG/min at pH 7.6 at 25°C
4. treatment 3 + 100 µl 0.01 M NADPH solution

All treatments were incubated at 41.5°C for 12 hours, then were chromatographed on Sephadex G25 as described before, and the fractions were assayed for radioactivity and O.D. as 280 nm. The percentage distribution of Se^{75} as protein and non-protein bound, as evidenced by optical density scans at 280 nm and 20% TCA treatment, was calculated. The Se^{75} protein bound fractions of the selenite treatments were pooled, and the Se^{75} non-protein bound peak corresponding to K_{av} 1.73 of various selenite treatments were pooled also and stored at -8°C for further investigation. The Se^{75} protein bound fractions of SEM treatments were combined to form one sample and stored at -8°C.

To study the nature of bonding of Se^{75} to plasma proteins, Se^{75} selenoprotein samples of selenite and SEM were divided into three portions. The first portion was dialyzed against DDH_2O, followed by 0.05 M Tris base (pH 11) as mentioned in the previous experiment. The second portion was treated with 20% TCA and the denatured proteins were separated by centrifugation at 1000xg for 5 minutes. The precipitated protein samples were washed twice with

DDH_2O. Both supernatants and precipitated protein samples were assayed for radioactivity.

Finally, the third portion was treated with 1 ml 0.013 M beta-mercaptoethanol (βME) and incubated for 2 hours at 25°C, then treated with 20% TCA and centrifuged to separate denatured proteins. The supernatants were aspirated and the denatured protein samples were washed twice with DDH_2O. All fractions were assayed for radioactivity. An aliquot (0.5 ml) of each supernatant was chromatographed on a Beckman amino acid analyzer (Model 120B) with a column of Dionex DC6A ion exchange resin using sodium citrate buffers of pH 3.22, 4.12, and 6.17.[17] This procedure gave satisfactory separation of the standards used to calibrate the amino acid analyzer. Eighty 1 ml fractions were collected for radioactivity measurement.

The amino acid analyzer was calibrated prior to use. The standards used were, Se^{75}-selenite solution, Se^{75}-selenite solution + βME, Se^{75}-selenocystine solution, Se^{75}-selenocystine solution + βME, Se^{75}-selenomethionine solution, and Se^{75}-selenomethionine solution + βME.

Exactly 0.5 ml of the samples and standards was injected into the amino acid analyzer and eighty 1 ml fractions were collected and assayed for radioactivity.

In an attempt to study the nature of Se^{75} non-protein bound peak corresponding to K_{av}=1.73 (Figure III.5), aliquots were treated with concentrated HCl according to Diplock _et al_.[18] using an apparatus similar to that described by the author. The released gases were

trapped in 3 ml 0.1 M $AgNO_3$, and the apparatus was operated under nitrogen.

Experiment 4

This experiment was conducted to investigate if the binding of Se from selenite to plasma proteins is accompanied by the binding of glutathione, in the form of plasma protein-Se-S-G. The following treatments were used.

a. 1 ml plasma + 200 µl 0.06M GSH + 10 µl GSSG-reductase

b. a + 200µ l 0.05M $NaSeO_3$

c. 1.5 ml blood + 200 µl 0.125M phosphate buffer pH 6.3

d. c + 200 µl 0.05M $NaSeO_3$

The various treatments were incubated at 41.5°C for 12 hours. After the incubation period, treatments a and b were stored at -8°C and treatments c and d were centrifuged at 1000xg and plasma was aspirated. The plasma samples from all treatments were chromatographed on Sephadex G25 to separate plasma proteins from low molecular weight compounds. Then plasma protein samples were hydrolyzed for amino acid analysis according to the procedure described by Beilstein et al.[19]

Amino acid analysis was performed under N_2 atmosphere with a Beckman amino acid analyzer (Model 120B) on a Dionex DC6A ion exchange resin using sodium citrate buffers of pH 3.22, 4.12, and 6.17 with ninhydrin. From the charts, residual percentage distribution of amino acids in plasma proteins were calculated.

RESULTS

The time course study in Experiment 1, indicated a noticable difference in the manner in which chick RBC metabolize Se as selenite or SEM.

Figure III.1 represents the pattern of uptake and release of radioactivity by RBC when Se^{75}-selenite or Se^{75}-SEM is incubated with whole blood in vitro at 41.5°C. Results indicate that Se^{75}-selenite accumulated in RBC faster than Se^{75}-SEM under the experimental conditions. Maximum uptake of Se^{75}-selenite is reached in about 20 minutes, whereby 13% of the dose was present in RBC. Then the Se^{75}-selenite pool in RBC started to decline by expelling radioactivity in the medium with a continuous build up of the plasma pool. The inverse linear relationship between time and log CPM in RBC indicated that the release follows a first order reaction.

On the other hand, the uptake and "release" of Se^{75}-SEM is distinct from that of Se^{75}-selenite (Figure III.1). A continuous buildup of Se^{75}-SEM in RBC according to a hyperbolic type function is observed.

Gel filtration chromatography of RBC lysates on the Sephadex G200 revealed that Se^{75} (from selenite) is distributed among three peaks in a 32:21:51 ratio (Figure III.2). The first peak of radioactivity and first peak of GSH Px activity occurred at K_{av} of 0.27 (MW 150,000). The second peak of radioactivity and hemoglobin co-eluted at K_{av} of 0.43 (MW 66,000). The third peak of radioactivity was not

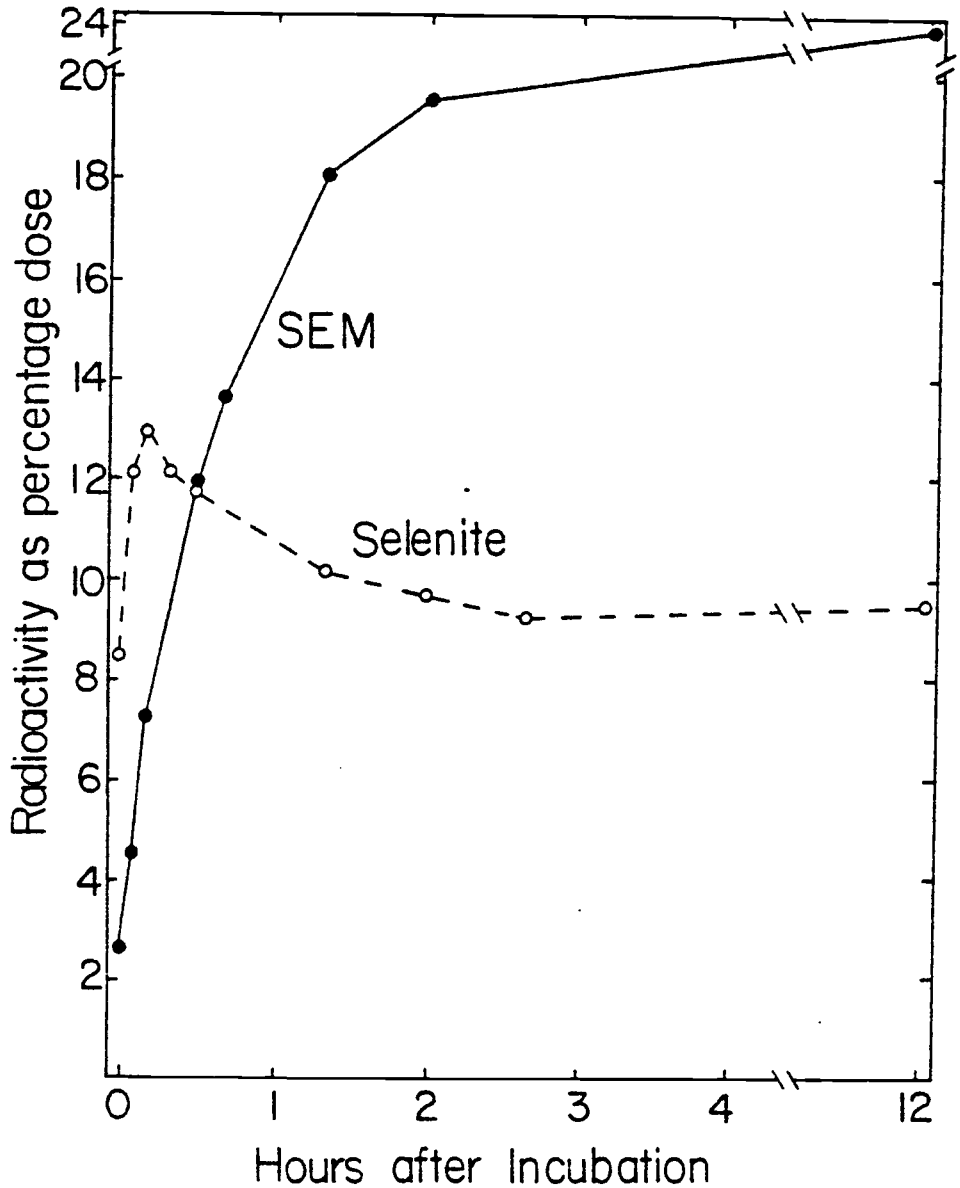


Figure III.1. Absorption of Se^{75} from selenite (0-0), empty, and SEM ●-●, solid, by chick erythrocytes at various incubation intervals (values represent the mean of two observations).

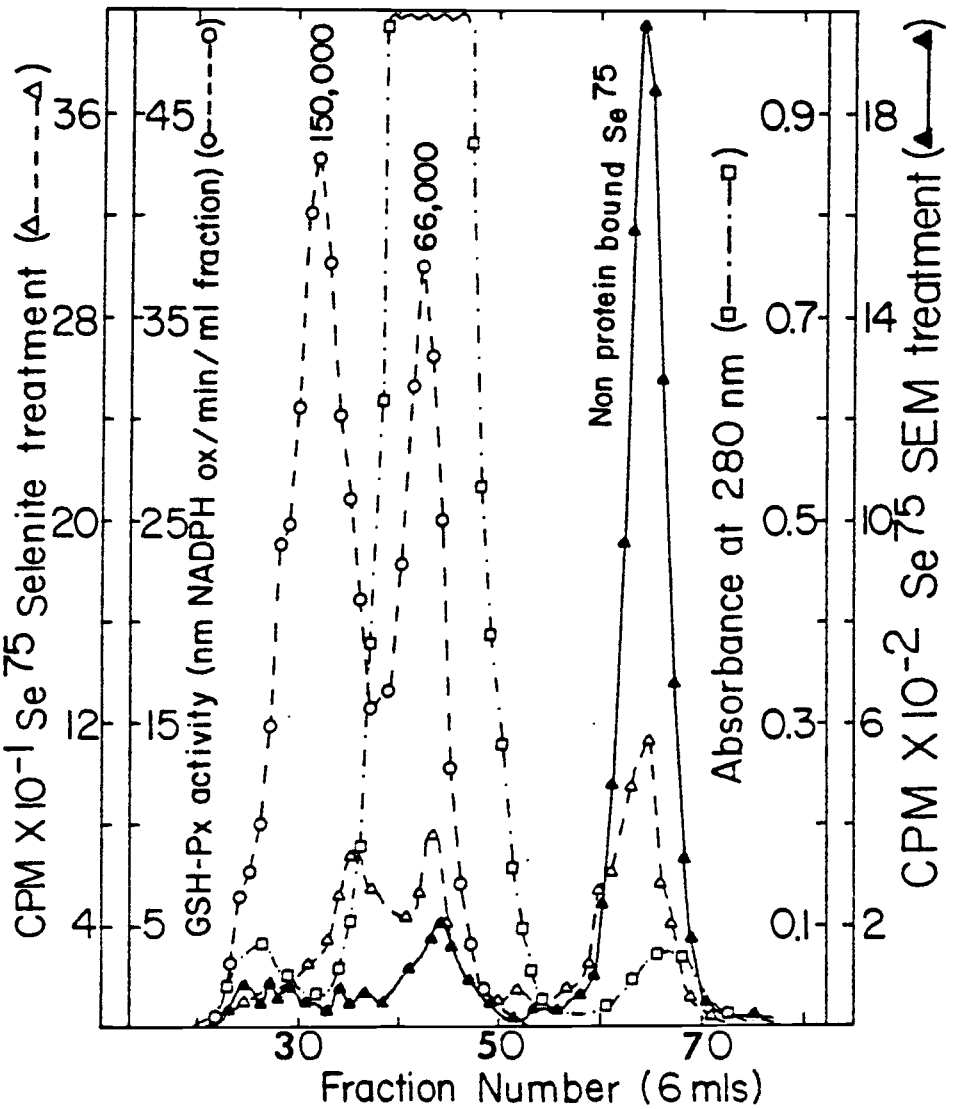


Figure III.2. Elution pattern of RBC lysates from whole blood incubated for 12 hours with Se^{75}-selenite or Se^{75}-SEM when applied to columns of Sephadex G200 and eluted with 0.05 M phosphate buffer pH 6.3.

associated with proteins as evidenced by optical density scan at 280 nm and 20% TCA treatment.

Sephadex chromatography of RBC lysate of Se^{75}-SEM on G200 column (Figure III.2) revealed that radioactivity is distributed between two peaks in a ratio of 12:88. The first peak of radioactivity was associated with hemoglobin and the second peak corresponding to Se^{75} non-protein bound as evidenced by absorbance at 280 nm and 20% TCA treatment. Finally, GSH Px activity scans of RBC lysates on Sephadex G200 (Figure III.2) revealed that chick hemoglobin possesses glutathione peroxidase activity.

Chromatography of plasma samples from whole blood incubated with Se^{75} (as selenite or SEM) on Sephadex G25 indicated that 53% and 33% of Se^{75} in plasma from Se^{75}-selenite and Se^{75}-SEM respectively is associated with proteins. The rest is Se^{75} non-protein bound.

Gel filtration chromatography of plasma Se^{75}-selenoproteins on Sephadex G200 (Figure III.3) showed that radioactivity is distributed between two peaks. About 40% and 44% of the radioactivity in plasma Se^{75} selenoproteins from Se^{75}-selenite and Se^{75}-SEM respectively is associated with the first peak (MW >440,000). The second peak (MW 89,000) contained the rest.

Results of the second experiment clearly demonstrate that chick plasma proteins do not bind Se^{75} (from selenite or SEM) to an appreciable extent in the absence of RBC. This conclusion is derived from data presented in Table III.2. These results indicate that 48.8% and 21.3% of the dose as Se^{75}-selenite and Se^{75}-SEM respectively is

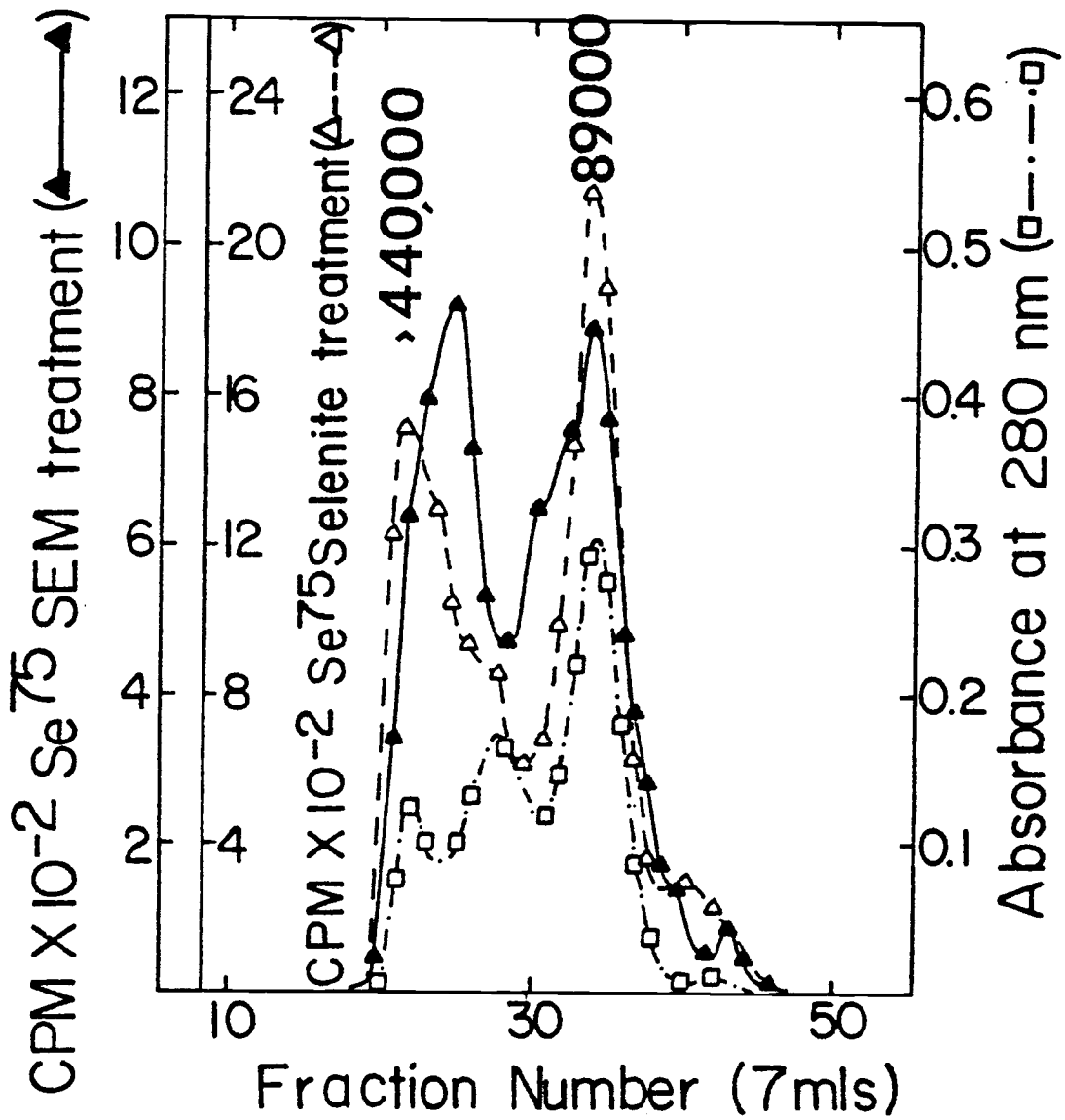


Figure III.3. Elution pattern of Se^{75}-labelled plasma proteins from whole blood incubated for 12 hours with either Se^{75}-selenite or Se^{75}-SEM when applied to Sephadex G200 columns and eluted with 0.05 M phosphate buffer pH 6.3.

Table III.1 The distribution coefficient of the standard compounds used to calibrate Sephadex G25 column.

Substance	Molecular Weight (MW)	Distribution Coefficient (K_{av})
Se^{75}-selenious acid	129	0.87
Se^{75}-selenomethionine	196	0.80
Se^{75}-selenocystine	334	0.67

Table III.2. The results of Se^{75} distribution and dialysis of RBC lysate and plasma samples of various treatments after applying on the G25 column.

Sample	Distribution of Se^{75} as Percentage of the dose		Percentage of Se^{75} protein bound lost during dialysis; dialysis baths	
	Protein bound	Non protein bound	DDH_2O	0.05M Tris base pH 11
RBC lysate-selenite	5.9*	2.6	9.4	17.8
RBC lysate-SEM	9.9	14.1	0.0	15.2
Plasma (whole blood) selenite	48.8	30.1	1.6	19.2
Plasma (whole blood) SEM	21.3	43.6	0.0	19.2
Plasma (without RBC) selenite	8.9	91.2	0.0	11.5
Plasma (without RBC) SEM	7.1	92.9	0.0	17.3

*Each value is an average of two observations.

associated with plasma proteins in the presence of RBC, however, only 8.9% and 7.1% of the dose of Se^{75}-selenite and Se^{75}-SEM is bound to plasma proteins in the absence of RBC. In red blood cells, 5.9% and 9.9% of Se^{75}-selenite and Se^{75}-SEM doses respectively, was associated with proteins. Larger levels (14% of the dose) of non-protein bound Se^{75} from SEM accumulated in RBC if compared to the level (2.6) of Se^{75} from selenite.

Results of dialyzing the Se^{75} protein bound samples from plasma samples and RBC lysates is also listed in Table III.2. Very little radioactivity (9.4%) was released from RBC lysate of the Se^{75}-selenite treatment and nc radioactivity was released from RBC lysate of Se^{75}-SEM or other plasma samples when dialyzed against DDH_2O. The alkaline bath (0.05M Tris base, pH 11), however, removed some of the radioactivity (11.6-19.2%) associated with proteins in these samples.

Figure III.4 shows the elution pattern of RBC lysates of Se^{75}-selenite and Se^{75}-SEM treatments on Sephadex G25 column. Results indicate that Se^{75} not associated with proteins of Se^{75}-selenite treatment as evidenced by optical density scans at 280 nm and 20% TCA treatment, is distributed between two peaks with K_{av} of 0.6 and 0.87 in the ratio of 3:1. By comparing the K_{av} of these two peaks with the standards (Table III.1) it is observed that only one-fourth of the non protein Se^{75} is selenite and three-fourths is other forms with an estimated MW of >334. On the other hand, the majority (92%) of Se^{75} non protein bound in RBC lysate of Se^{75}-SEM

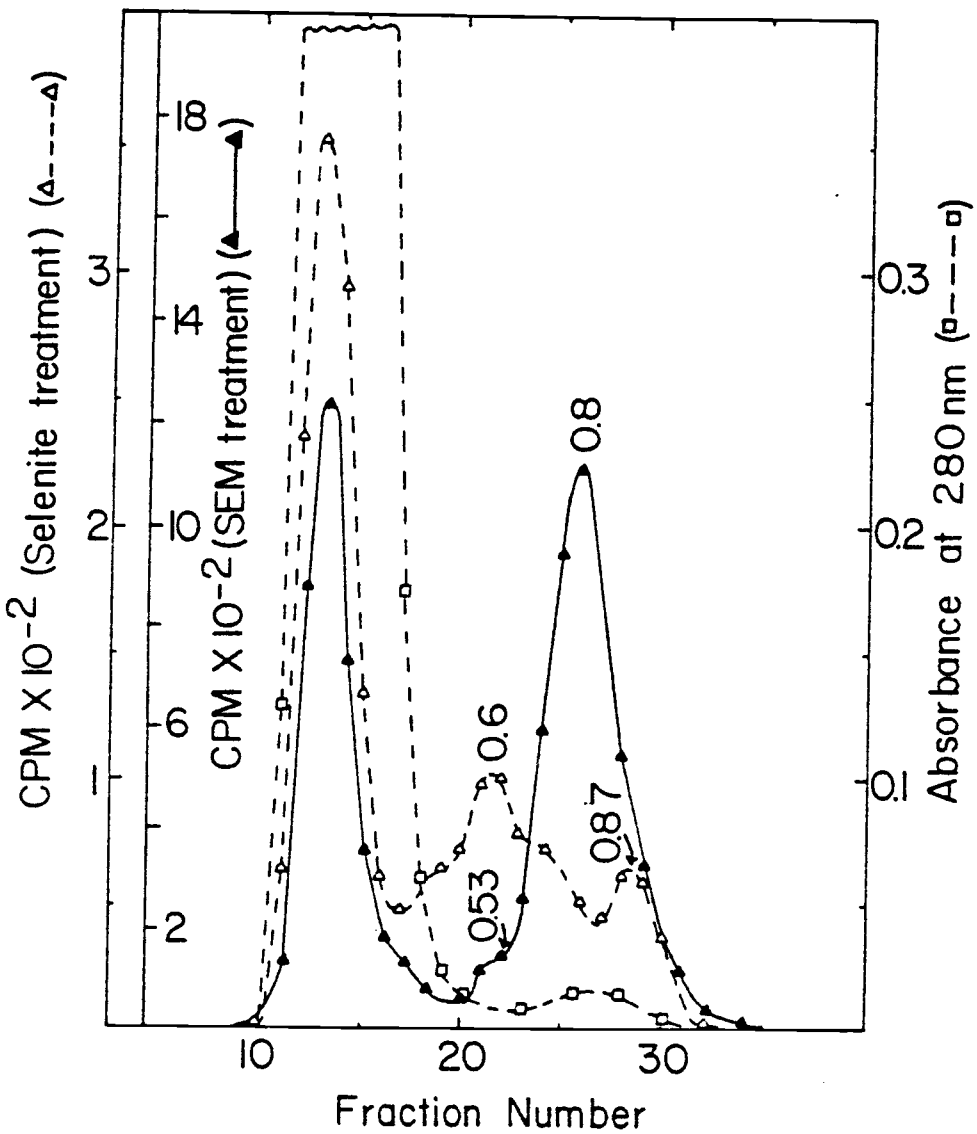


Figure III.4. Elution pattern of RBC lysates from whole blood incubated for 12 hours with Se^{75}-selenite or Se^{75}-SEM when applied on Sephadex G25 column and eluted with 0.05 M phosphate buffer pH 6.3.

treatment (Figure III.4) is associated with a peak having K_{av} of 0.8, and very little radioactivity (8%) is associated with a peak having a K_{av} of 0.53. Such results indicate that most of the Se^{75} inside RBC is free Se^{75}-SEM. Finally, only one Se^{75} non protein peak in Se^{75}-selenite and Se^{75}-SEM plasma samples corresponding to K_{av} of 0.87 and 0.8, respectively, suggesting that Se^{75} occurs as free Se^{75}-selenite and Se^{75}-SEM in plasma.

The effect of GSH, GSSG reductase and NADPH on the binding of Se^{75} from selenite or SEM to plasma proteins in the absence of RBC is shown in Table III.3. Results indicate a significant increase in the binding of Se^{75} to plasma proteins if GSH is added to plasma incubated with Se^{75}-selenite. The maximum binding of Se^{75} to plasma proteins was attained by adding GSH and GSSG reductase to plasma incubated with Se^{75}-selenite. Addition of NADPH together with GSH and GSSG reductase decreased the binding level if compared to adding GSH and GSSG reductase alone. The addition of GSH, GSSG reductase, and NADPH to plasma incubated with Se^{75}-SEM did not improve the binding of Se^{75} to plasma proteins significantly.

Sephadex chromatography on G25 of plasma samples incubated with Se^{75}-selenite and GSH (treatment 2), Se^{75}-selenite and GSH and GSSG reductase (treatment 3), or Se^{75}-selenite and GSH and GSSG reductase and NADPH (treatment 4), indicated that Se^{75} not associated with protein is distributed among three peaks corresponding to K_{av} of 0.6, 0.87, and 1.73 (Figure III.5). Only one Se^{75} non-protein bound

Table III.3. Effect of GSH, GSSG reductase, and NADPH on the binding of Se^{75} (from selenite and SEM) to plasma proteins.

Treatment	Percentage Distribution of Se^{75} in Plasma				
	Se^{75}-selenite			Se^{75}-SEM	
	Protein bound peak	Non-Protein bound		Protein bound	Non-protein bound
		Peak II & III K_{av} (0.6,0.87)	Peak IV K_{av} (1.73)		K_{av} (0.8)
1. Plasma + Se^{75}	8.7±1.5 a*	92 ±1.0 a	-	7.1**	92.9
2. 1 + GSH	38.6±1.0 bc	43.1±1.0 b	18.3±1.4 b	11.4	88.5
3. 2 + GSSG reductase	45.9±1.4 c	41.8±4.6 b	12.3±0.5 a	11.0	89.0
4. 3 + NADPH	34.2±2.7 b	41.9±5.6 b	23.9±3.1 c	11.5	88.5

*Each mean ± σ_{n-1} is the average of three observations and means within the column followed by different letters are statistically significant at $P<0.05$ using the Duncan range test.[20]

**Each mean is the average of two observations.

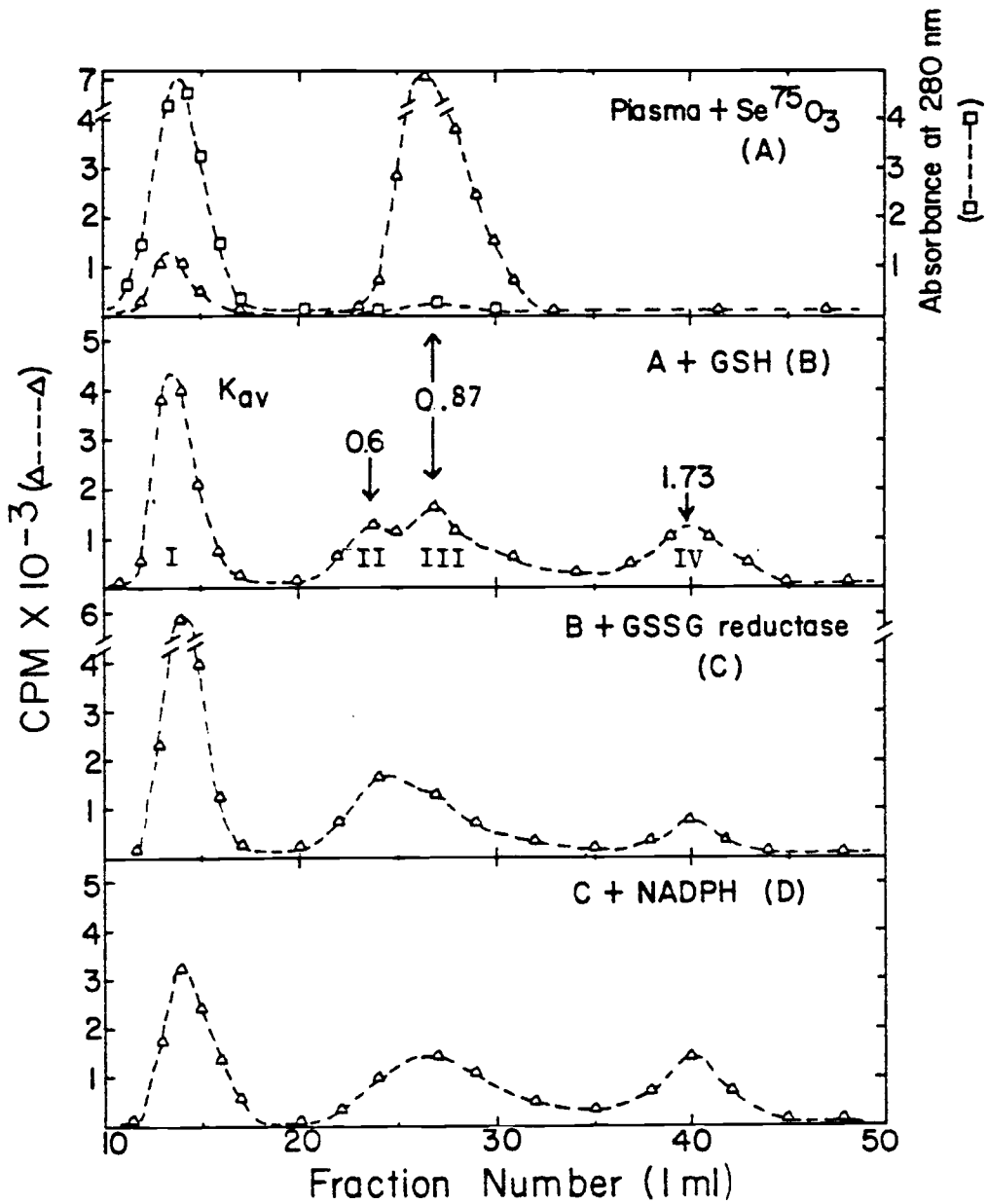


Figure III.5. The effect of GSH, NADPH, and GSSG reductase on labelling plasma proteins with Se^{75} from Se^{75}-selenite as evidenced from the elution pattern of plasma when applied to Sephadex G25 columns and eluted with 0.05 M phosphate buffer at pH 6.7.

peak was detected in plasma incubated with Se^{75}-selenite (treatment 1), as shown in Figure III.5, upper portion.

Results in Table III.3 indicated that the percentage distribution of Se^{75} non-protein bound, associated with peak number II and III (K_{av} 0.6 and 0.87) in treatment 2, 3, and 4 is not significantly different at $P < 0.05$. On the contrary, Se^{75} non-protein bound in peak IV (K_{av} 1.73) in treatment 2, 3, and 4 is significantly different at $P < 0.05$.The addition of GSSG reductase (treatment 3) resulted in a significant lowering of the level of Se^{75} non-protein bound associated with peak IV, however, adding NADPH (treatment 4) causes a significant increase if both treatments (3 and 4) are compared to treatment 2. Only one Se^{75} non-protein bound peak was noticed in plasma treatments incubated with Se^{75}-SEM.

The nature of the bond holding Se^{75} from both selenite or SEM to plasma proteins was investigated (Table III.4). Exhaustive dialysis against DDH_2O did not release any Se^{75} activity, however, dialysis against 0.05 M Tris base pH 11 resulted in the release of 19%, regardless of the Se^{75} source. Most of the Se^{75} activity was precipitated after treating Se^{75} selenoprotein samples with 20% TCA. In contrast, the majority of Se^{75} activity was released from Se^{75} selenoprotein samples if treated first with βME followed by 20% TCA treatment. No difference was found in the behavior of Se^{75} selenoproteins from either selenite or SEM treatments.

To further investigate the nature of Se^{75} bound to plasma proteins from both Se^{75}-selenite and Se^{75}-SEM, aliquots (0.5 ml) of

Table III.4. The effect of dialysis, TCA treatment, and βME + TCA treatment on binding of Se^{75} (from selenite and SEM) to plasma proteins.

Sample	Percentage Se^{75} lost during dialysis dialysis baths		Distribution of Se^{75} after 20% TCA treatment		Distribution of Se^{75} after βME + 20% TCA treatment	
	DDH_2O	0.05 M Tris base, pH 11	Supernatant (%)	Precipitate (%)	Supernatant (%)	Precipitate (%)
Plasma Se^{75}-selenoproteins (selenite)	0.0	19.0	7.1	92.9	88.6	11.4
Plasma Se^{75}-selenoproteins (SEM)	0.0	19.5	2.8	97.2	94.3	5.7

the supernatants, after treating plasma Se^{75}-selenoproteins with βME and 20% TCA, were chromatographed on a Dionex DC6A ion exchange (Figure III.6A) as mentioned in the materials and methods section. No selenite, SEM, selenocytine, or selenocysteine were detected. Instead a chromatogram comparable to selenite treatment with βME was observed in both selenite and SEM treatments, suggesting the form of Se^{75} attached to plasma proteins is H_2Se.

The nature of Se^{75} non-protein bound associated with peak IV (K_{av} 1.73) was also investigated. Results indicated that Se^{75} is not present as H_2Se since only 5% of Se^{75} activity was trapped in 0.1 M $AgNO_3$ when treated with concentrated HCl.

Experiment 4 was conducted to investigate whether Se^{75} binding to plasma protein is accompanied with binding of glutathione in the following manner (Pr-Se-SG). If this is the case, one would expect higher levels of glycine, glutamic acid, and cysteine in the amino acid hydrolysate of plasma selenoproteins if compared to amino acid profiles of these proteins prior to Se binding. Results in Table III.5 did not show such a trend, suggesting that Se binding to plasma proteins is not accompanied with glutathione.

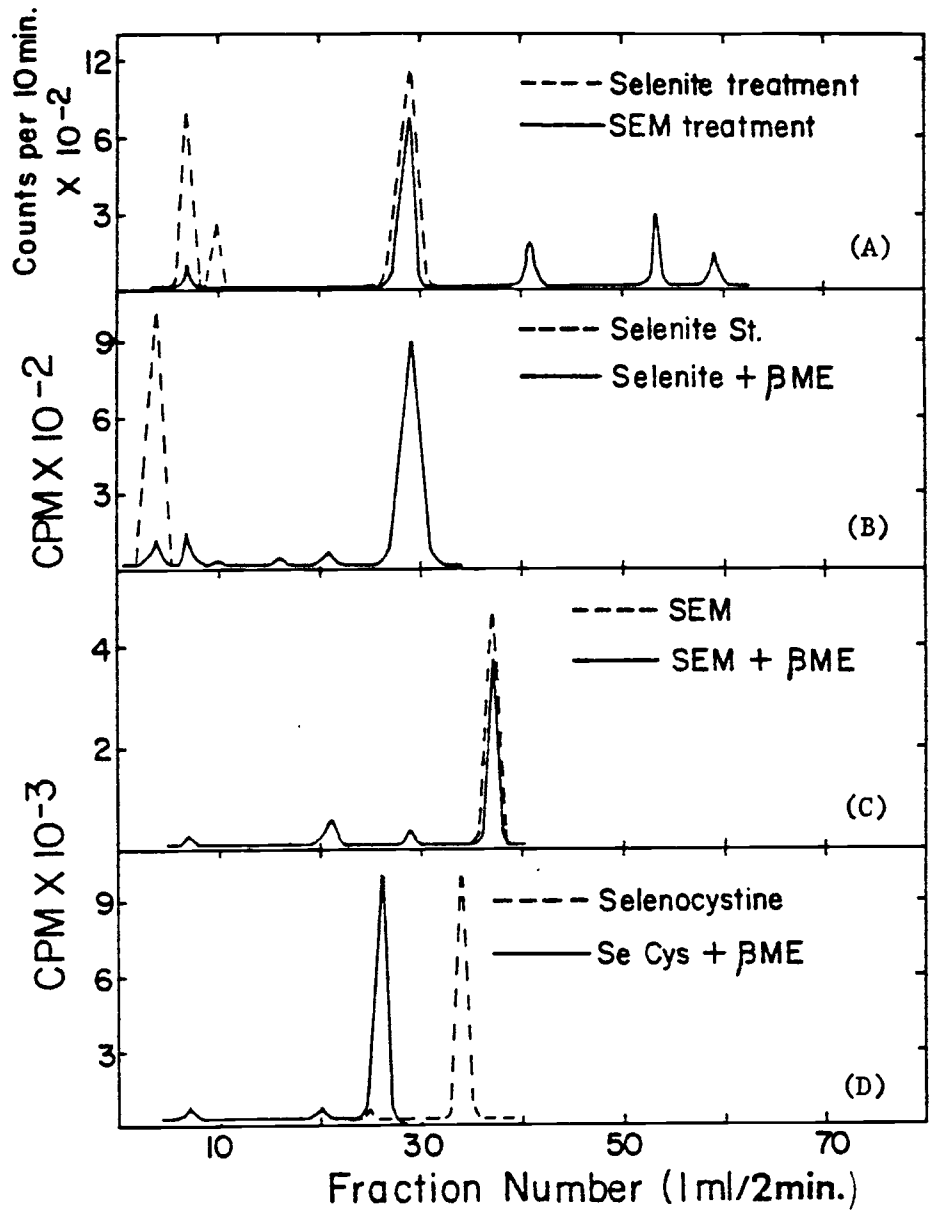


Figure III.6. Elution pattern on Dionex DC6A ion exchange column for Se^{75} standards (B, C, and D) and Se^{75} supernatants (A) from Se^{75} plasma selenoproteins of Se^{75}-selenite and Se^{75}-SEM after treatment with βME followed by 20% TCA.

Table III.5. Amino acid profile of plasma protein hydrolysates from various treatments in Experiment 4.

Treatment	Cysteic acid	Aspartic acid	Threonine	Serine	Glutamic acid	Glycine	Alanine	Cysteine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine	Proline
A. Plasma + GSH + GSSG reductase	0.243	10.16	5.34	7.41	13.10	5.3	7.88	3.12	5.53	1.80	4.29	7.75	2.86	3.76	2.29	6.83	5.31	5.00
B. A + Na_2SeO_3	0.132	9.64	5.45	7.17	13.03	5.08	7.19	3.55	5.25	2.25	4.21	7.56	3.24	3.99	2.19	7.18	5.32	5.56
C. Blood + Phosphate buffer	0.558	9.86	5.86	7.14	12.51	5.52	5.86	2.58	5.93	2.02	4.13	7.95	2.61	3.45	2.26	6.57	4.55	9.58
D. C + Na_2SeO_3	0.327	9.54	5.63	7.14	12.86	6.09	7.45	3.74	5.50	2.07	4.15	7.58	2.83	3.56	2.15	7.51	5.07	5.01

DISCUSSION

The metabolism of trace quantities of Se^{75}-selenite by chick RBC involved the two processes shown in Figure III.1. Within the first twenty minutes, accumulation of Se^{75}-selenite continued to build up. This was followed by extrusion of Se^{75} in a form bound to plasma proteins. These results agree with those of Lee *et al.*[3] and Sandholm,[5] however, the rate of uptake and release of Se^{75}-selenite by chick RBC seems slower than in human[3] or cow[5] erythrocytes as indicated earlier by Edwardly.[21]

It is evident from Figure III.1 and Table III.3 that the chemical form of Se^{75} can markedly affect its metabolism (*in vitro*) in chick erythrocytes. Uptake of Se^{75} from SEM showed a more gradual and consistent increase with time if compared to Se^{75}-selenite (Figure III.1). This pattern of uptake and release of Se^{75} from selenite and SEM by chick RBC is similar to that described by White et al.[22] for the mouse fibroblasts grown in tissue cultures.

Results of chromatographing RBC lysates on Sephadex G200 (Figure III.2) indicated that Se^{75} from selenite is more specifically incorporated into GSH-Px and Se^{75} from SEM is more specifically incorporated into hemoglobin. The nature of Se^{75} incorporation in these proteins, however, is not clear. Since chick erythrocytes have been shown to synthesize proteins,[23] it is possible that Se^{75} was incorporated in the polypeptide backbone of GSH-Px and hemoglobin. Results of GSH-Px scans (Figure III.2) indicated that chick hemoglobin

possesses peroxidase activity. This observation is consistent with human and monkey hemoglobins which have been shown to possess GSH-Px activity,[24] and a mechanism to explain this observation is suggested in the discussion section of Chapter IV.

Gel filtration chromatography of Se^{75}-labelled plasma samples (Figure III.3) showed that radioactivity is distributed between two peaks corresponding to molecular weights of >440,000 and 89,000, regardless of Se^{75} source. This result indicates that both Se^{75}-selenite and Se^{75}-SEM absorbed by chick RBC may be metabolized to a common form that permits bounding to plasma proteins.

Results of the second experiment (Table III.2) demonstrate that chick plasma proteins bind very little Se^{75} from selenite and SEM in the absence of RBC. These results not only confirm the finding of several workers[1-9] that binding of Se as selenite to plasma proteins is dependent upon the presence of erythrocytes, but also indicates that labelling of plasma proteins with Se^{75} from SEM also requires RBC. The small loss of the plasma protein-bound Se^{75} (from selenite or SEM) by dialysis against DDH_2O followed by 0.05 M Tris base (pH 11) suggests that the Se^{75} was not loosely bound to the proteins, but instead, was tightly bound.

Several studies have stressed the role of GSH in the metabolism of Se^{75}-selenite in RBC. Sandholm,[6] in an _in vitro_ study using cow blood, concluded that the release of Se^{75} from erythrocytes is dependent on GSH. This confirms results reported by Lee _et al._[3] that the extrusion of radioactivity from human erythrocytes is inhibited by

10^{-3} M para-chloro mercuribenzoate probably by its oxidative effects on GSH. Results in this study (Table III.3) regarding the ability of added GSH and GSSG reductase to plasma in duplicating the effect of erythrocytes on binding Se^{75} to plasma proteins, clearly demonstrate the importance of GSH and GSSG reductase in the metabolism of Se^{75}-selenite by chick erythrocytes. The biochemical effect of GSH and GSSG reductase on Se^{75}-selenite was recognized by Ganther.[25,26] The present results appear to confirm the operation of the GSH, GSSG reductase pathway (Figure III.7) for selenite metabolism in chick erythrocytes, but not for Se^{75}-SEM (Table III.3).

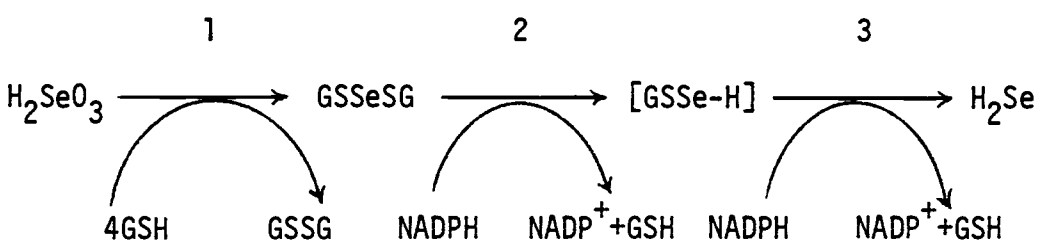


Figure III.7. Reduction of selenite by GSH, NADPH, and GSSG reductase. Reaction 1 is a non enzymic complex reaction with known stoichiometry[27], reaction 2 and 3 require NADPH and the enzyme GSSG reductase.

The observation of a Se^{75} non-protein bound peak with a K_{av} of 0.60 (Figure III.5) may suggest the presence of GSSeSG. This is supported by the data showing this peak in RBC lysate of Se^{75}-selenite treatment (Figure III.4). Although formed inside the RBC, selenodiglutathione (GSSeSG) is apparently not expelled into the medium as evidenced by the absence of a peak corresponding to K_{av} of

0.60 in plasma from whole blood. This observation is consistent with earlier work by Gasiewicz and Smith.[28] Moreover, these investigators indicated that Se is bound to plasma proteins in the form of selenotrisulfide. Such indication is evident in Figure III.6 where most of the Se^{75} released from Se^{75}-selenoproteins formed from Se^{75}-selenite or Se^{75}-SEM after the treatment with βME followed by TCA corresponds to a form similar to the form of Se after treating selenite with βME. According to the present study, Se^{75} in selenite and SEM was metabolized via different metabolic routes (Table III.3) to a similar form (most probably H_2Se) as evidenced by results in Table III.4 and Figures III.3 and III.6.

Although the form of Se^{75} associated with peak IV (K_{av} 1.73) in Figure III.5 proved not to be H_2Se, as evidenced by the degree of radioactivity trapped in 0.1 M $AgNO_3$ when treated with concentrated HCl, it is believed that the form of Se^{75} in peak IV is elemental selenium (Se^0) which originated from the oxidation of "released H_2Se" by oxygen. Results in Table III.3 and Figure III.4 and III.5 suggest that the "released H_2Se" is produced in side reactions because of the less than perfect coupled reaction of labelling plasma proteins with Se^{75} from H_2Se (Step 10 in Figure III.8).

In contrast to Sandholm's findings[24], results in Table III.5 indicate that binding of Se^{75} to plasma proteins is not accompanied by concomitant binding of GSH. These results preclude the possibility that Se^{75} is bound to plasma proteins in the form of Pr-Se^{75}-SG, i.e., selenopersulfide (HSe^{75}-SG) is not expelled in plasma.

The present study, demonstrated that labelling of plasma proteins with Se^{75}-derived from SEM is dependent on the presence of RBC (Table III.2). This finding could explain the observations made by Sternberg and Imbach[11] that the binding rate of Se from SEM to plasma proteins is not dependent on the liver and a small proportion of Se^{75} from SEM is bound to plasma proteins after in vitro incubation with whole rat blood. In addition, results in Table III.4 and Figures III.3 and III.6 indicated that the forms of Se^{75} in plasma selenoproteins of SEM and selenite treatments are similar, and suggested that Se^{75}-SEM and Se^{75}-selenite are metabolized to a common product, but via different metabolic pathways (Table III.3), and become bound to plasma proteins in the selenotrisulfide form (R-S-Se^{75}-S-R).

The results described may best be summarized by the following model in Figure III.8. The first steps (1 and 2) are the uptake of Se^{75}-selenite and Se^{75}SEM by RBC. Absorption of Se^{75}-selenite is faster than Se^{75}-SEM by erythrocytes (Figure III.1). The uptake of Se^{75}-selenite was found to be energy independent.[29] However, the mode of Se^{75}-SEM absorption by RBC is not clear. Subsequently, Se^{75}-selenite is converted (probably non-enzymatically) to selenodiglutathione (GS-Se^{75}-SG) which in turn is reduced to selenide (Steps 4 and 5) by NADPH and glutathione reductase.

On the other hand, Se^{75}-SEM is also metabolized to selenide (HSe^{-}). It seems that there are two possible pathways for the conversion of Se^{75}-SEM to selenide, either analogous to the methione transamination pathway[30] (Step 6) or according to the pathway

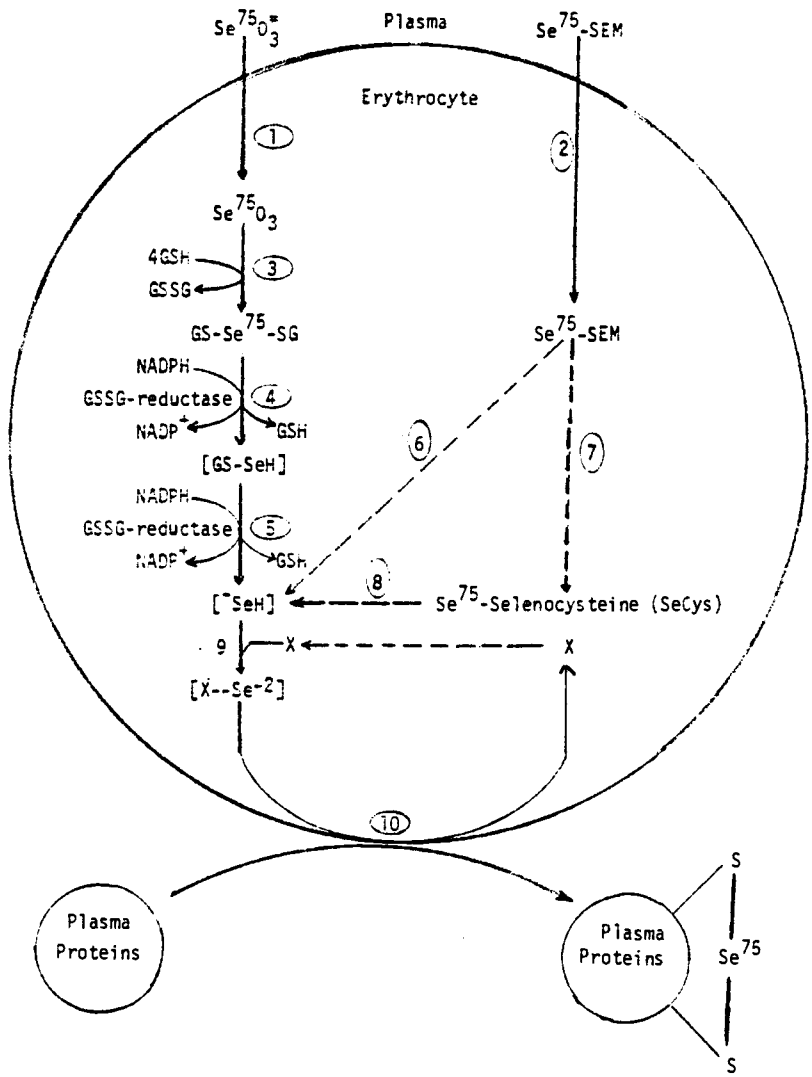


Figure III.8. Proposed model of <u>in vitro</u> $Se^{75}O_3^=$ and Se^{75}-SEM metabolism. Steps 1 and 2: entry of $Se^{75}O_3^=$ and Se^{75}-SEM into RBC. Step 3: non-enzymic reaction of 1 mole of $Se^{75}O_3^=$ with 4 moles of GSH to form the selenodiglutathione ($GSSe^{75}SG$). Steps 4 and 5: sequential reduction of $GSSe^{75}SG$ to GSH and selenide by NADPH and the enzyme glutathione reductase. Two possible routes for the conversion of Se^{75} in SEM to H_2Se, either via Step 6, analogous to the methionine transamination pathway, or via Step 7, conversion of Se^{75}-SEM to Se^{75}-SeCys followed by Step 8: degradation of Se^{75}-SeCys to selenide and alanine by selenocysteine lyase. Step 9: binding of selenide to a specific carrier (X). Step 10: release of ^{75}Se from erythrocytes dependent on plasma proteins to which ^{75}Se is bound as a selenotrisulfide.

described by Esaki et al.[31] for the synthesis of selenocysteine from SEM (Step 7) then cleaving of SeCys to selenide and alanine (Step 8) by the enzyme selenocysteine lyase.[32] Step 9 describes the stabiliation of released selenide through binding to compound X (the carrier compound, probably hemoglobin). Finally plasma protein becomes labelled with Se^{75} (from selenite or SEM) as a result of the coupled reaction with $X\text{-}Se^{-2}$. Step 10 is believed to be a coupled reaction as evidenced by the observations made by McMurray and Davidson[9] that accumulated radioactivity is not expelled from RBC if suspended in phosphate buffered saline, however upon incubating these labelled erythrocytes with unlabelled plasma there was a transfer of radioactivity to the plasma. Moreover, Gasiewicz and Smith[28] found that the form of Se expelled by RBC is probably H_2Se, rather than GSSeSG, as suggested by Jenkins and Hidiroglou.[12] Results of the present study are in accordance with Gasiewicz and Smith[28] and no evidence of expelling GSSeSG in the plasma was observed. The present results provide an integrated and clear picture of the role of RBC in metabolizing Se^{75}-selenite and Se^{75}-SEM. Moreover, this is the first report to indicate the role of RBC in the metabolism of Se^{75}-SEM and further research is required to reveal the biochemical pathway of the metabolism of Se^{75}-SEM to selenide in RBC. Finally, the metabolic pathway of labelling plasma proteins with Se^{75} from Se^{75}-selenite in chick whole blood is confirmed.

REFERENCES

1. Imbach, A. and Sternberg, J. (1967) Metabolic studies with seleniated compounds. I. Kinetic studies with $Se^{75}O_3$ in rats. Int. J. Appl. Radiat. Isot. 18:545-556.

2. Jenkins, K.J., Hidiroglou, M., and Ryan, J.F. (1969) Intravascular transport of selenium by chick serum proteins. Can. J. Physiol. Pharmacol. 47:459-467.

3. Lee, M., Doug, A., and Yano, J. (1969) Metabolism of Se^{75}-selenite by human whole blood in vitro. Can. J. Biochem. 47:791-797.

4. Burk, R.F. (1973) Effect of dietary selenium level on ^{75}Se binding to rat plasma proteins. Proc. Soc. Exp. Biol. Med. 143:719-722.

5. Sandholm, M. (1973) The initial fate of a trace amount of intravenously administered selenite. Acta Pharmacol. Toxicol. 33:1-5.

6. Sandholm, M. (1973) The metabolism of selenite in cow blood in vitro. Acta Pharmacol. Toxicol. 33:6-16.

7. Sandholm, M. (1974) Selenium carrier proteins in mouse plasma. Acta Pharmacol. Toxicol. 35:424-428.

8. Sandholm, M. (1975) Function of erythrocytes in attaching selenite-Se onto specific plasma proteins. Acta Pharmacol. Toxicol. 365:321-327.

9. McMurray, C.H., and Davidson, W.B. (1979) In vitro metabolism of selenite in sheep blood. Factors controlling the distribution of selenium and the labelling of plasma proteins. Biochem. Biophys. Acta. 583:332-343.

10. Awwad, H.K., Potchen, E.J., Adelstein, S.J., and Dealy Jr., J.B. (1966) Se^{75}-selenomethionine incorporation into human plasma proteins and erythrocytes. Metabolism 15:626-640.

11. Sternberg, J., and Imbach, A. (1967) Metabolic studies with seleniated compounds. II. Turnover studies with Se^{75}-methionine in rats. Int. J. Appl. Radiat. Isot. 18:557-568.

12. Jenkins, K.J., and Hidiroglou, M. (1972) Comparative metabolism of Se^{75}-selenite, Se^{75}-selenate, and Se^{75}-selenomethionine in bovine erythrocytes. Can. J. Physiol. Pharmacol. 50:927-935.

13. Wright, P.L., and Bell, M.C. (1963) Selenium and vitamin E influence upon the in vitro uptake of Se^{75} by ovine blood cells. Proc. Soc. Exp. Biol. Med. 14:379-382.

14. Paglia, D.E., and Valentine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.

15. Gunzler, W.A., Kremers, H., and Flohe, L. (1974) An improved coupled test procedure for glutathione peroxidase (E.C. 1.11.1.9) in blood. 2 Klin. Chem. Klin Biochem. 12:444-448.

16. Cooper, T.G. (1977) In The Tools of Biochemistry. John Wiley and Sons, Inc.:New York, NY. pp. 169-193.

17. Moore, S., and Stein, W.H. (1963) Chromatographic determination of amino acids by the use of automatic recording equipment. Method. Enzymol. 6:819-820.

18. Diplock, A.T., Caygin, C.P.T., Jeffery, E.H., and Thomas, C. (1973) The nature of the acid volatile selenium in the liver of the male rat. Biochem. J. 134:283-293.

19. Beilstein, M.A., Tripp, M.J., and Whanger, P.D. (1981) Evidence for selenocysteine in ovine tissue organelles. J. Inorg. Biochem. 15:339-347.

20. Duncan, D.B. (1955) Multiple range and multiple F tests. Biometrics. 11:1-42.

21. Edwardly, J.S. (1981) In Effect of Supplemental Selenium on the Performance and Se^{75} Metabolism in Chicks. TEMA-4, Howell, J. McC., Gawthorne, M.J., and White, C.L. (editors). Australian Academy of Science, Canberra. pp. 226-229.

22. White, C.L., and Hockstra, W.G. (1979) The metabolism of selenite and selenomethionine in mouse fibroblasts grown in tissue culture. Biol. Trace. Elem. Res. 1:243-257.

23. Bell, D.J., and Freeman, B.M. (1971) In Physiology and Biochemistry of the Domestic Fowl, Vol. 2. Academic Press:New York, NY. pp. 858.

24. Butler, J.A., Whanger, P.D., and Tripp, M.J. (1982) Blood selenium and glutathione peroxidase activity in pregnant women:

comparative assays in primates and other animals. Am. J. Clin. Nutr. 36:15-23.

25. Ganther, H.E. (1968) Selenotrisulfides formation by the reaction of thiols with selenious acid. Biochemistry 8:2898-2905.

26. Ganther, H.E. (1971) Reduction of the selenotrisulfide derivative of glutathione to a persulfide analog by glutathione reductase. Biochemistry 10:4089-4098.

27. Ganther, H.E., and Corcoran, C. (1969) Selenotrisulfides. II. Cross-linking of reduced pancreatic ribonuclease with selenium. Biochemistry 8:2557.

28. Gasiewicz, T.A., and Smith, J.C. (1977) Similar properties of cadmium and selenium complex formed in rat plasma in vivo and in vitro. Fed. Proc. 36:1152.

29. Proter, E.K., Karle, J.A., and Shrift, A. (1979) Uptake of selenium^{75} by human lymphocytes in vitro. J. Nutr. 109:1901-1908.

30. Steele, R.D., and Benevenga, N.J. (1979) The metabolism of 3-methyl thio propionate in rat liver homogenates. J. Biol. Chem. 254:8885-8890.

31. Esaki, N., Nakamura, T., Tanaka, H., Suzuki, T., Morino, Y., and Soda, K. (1981) Enzymatic synthesis of selenocysteine in rat liver. Biochemistry 20:4492-4496.

32. Esaki, N., Nakamura, T., Tanaka, H., and Soda, K. (1982) Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. J. Biol. Chem. 257:4386-4391.

CHAPTER IV

A Comparative Study of Se^{75}-Selenite and Se^{75}-Selenomethionine Metabolism in Broiler Chicks

by

M.A. Alayan,[1] P.D. Whanger,[2] and G. Arscott[3]

[1]Department of Biochemistry and Biophysics
Oregon State University
Corvallis, Oregon 97331

[2]Department of Agricultural Chemistry
Oregon State University
Corvallis, Oregon 97331

[3]Department of Poultry Sciences
Oregon State University
Corvallis, Oregon 97331

CONTRIBUTIONS

Mohammad A. Alayan participated in planning the experiment, raising the chicks, conducting all the experimental steps, performing all the biochemical analyses, data analyses and results calculations, and writing the article. P.D. Whanger participated in designing the experiment, supervising the lab work, and editing the article. G.H. Arscott helped in providing the chicks, and designing the experiment.

A Comparative Study of Se^{75}-Selenite and Se^{75}-Selenomethionine Metabolism in Broiler chicks

ABSTRACT

After chicks were intubated with Se^{75}-selenite or Se^{75}-selenomethionine (SEM), the distribution and metabolism of radioactivity, as judged by incorporation into cytosolic proteins of various tissues, was studied. Gel filtration studies indicated that Se^{75} from selenite is more specifically incorporated into erythrocyte GSH-Px (MW 150,000) than Se^{75}-SEM. It was observed that chick hemoglobin (Hb, MW 66,000) bound Se^{75} from both sources, and possessed peroxidase activity. A mechanism was postulated to explain Hb peroxidase activity. Results of chromatographing plasma on Sephadex G200 indicated that proteins corresponding to MW of 338,000 are the major carriers of Se^{75} from selenite or SEM 6 hours after dosing, however proteins possessing a MW of 89,000 are the important Se^{75} carriers 96 hours after intubation.

With other tissues, the liver and kidney contained the highest concentrations of Se^{75}. Gel filtration chromatography of tissue cytosols on Sephadex G200 indicated that the majority (34-80%) of Se^{75}, from selenite or SEM, is associated with the enzyme GSH-Px 24 hours after dosing. Pancreatic cytosol of chicks dosed with Se^{75}-SEM had the majority of radioactivity associated with a peak corresponding to MW 44,500. Evidence for non-glutathione peroxidase

selenoproteins were detected in various tissues. These included high molecular weight (>440,000) selenoproteins in cytosols of all tissues examined except in bone marrow, a peak (MW 44,500) specific to selenite but not SEM in liver cytosols, a low molecular weight (MW 25,100) protein in kidney and spleen cytosols from both Se^{75} treatments, and two distinct testicular selenoproteins (MW 35,000 and 263,000) from both Se^{75} treatments. A hypothetical pathway for the incorporation of Se^{75} into selenocysteine-containing proteins (i.e., GSH-Px) is presented to account for these observations.

A COMPARATIVE STUDY OF Se^{75}-SELENITE AND Se^{75}-SELENOMETHIONINE METABOLISM IN BROILER CHICKS

INTRODUCTION

The essentiality of selenium as a dietary requirement for animals has been well documented. Selenium deficiency results in the development of various pathological conditions such as, liver necrosis in rats[1], white muscle disease in lambs[2] and calves[3], hepatosis dietatica in pigs[4], and exudative diathesis in chicks[5]. All these disorders are biochemically characterized by subnormal concentrations of glutathione peroxidase (GSH-Px) [E.C. 1.11.1.9], the only known mammalian Se-containing enzyme.[6] In 1970, severe Se deficiency was shown to result in pancreatic atrophy in the chick, in addition to poor growth and feathering, even in the presence of high levels of vitamin E.[7] A very interesting feature of pancreatic fibrosis was that no relationship was found between pancreatic and plasma GSH-Px and the protection against the disease.[7] This observation led Cantor et al.[8,9] to conclude that the biochemical role of Se in protecting against pancreatic fibrosis is distinct from GSH-Px activity.

On the other hand, selenium is generally administered as selenite to protect animals from selenium deficiency syndromes. However, the dietary source of Se is mostly the organic forms such as selenomethionine (SEM) and selenocystine. Therefore, the aim of the present study is to investigate the metabolism of selenite and selenomethione (as Se sources) as judged by incorporation into GSH-Px and by incorporation into the cytosolic selenium binding proteins of various chicken tissues.

MATERIALS AND METHODS

Animal Selections

A total of 30 one-day-old broiler males were obtained from a commercial hatchery. The chicks were housed on the floor in an electrically-heated pen. They were fed a broiler starter-type diet (Table IV.1), containing 0.15 ppm selenium, and water *ad libitum*. When the chicks were 41 days old, feed was removed and fasted for 18 hours. On Day 42, the chicks were weighed and 18 broiler males were selected on the basis of body size uniformity (1726±50 gm). Nine chicks were intubated with 1.0 ml of an aqueous solution of Se^{75}-selenite (8.5μCi/ml, specific activity 437mCi/mg), into the crop via a stainless steel intubation tube attached to a syringe. The remaining nine males were intubated with 1.0 ml of an aqueous solution of Se^{75}-SEM (11.4μCi/ml, specific activity 4.54mCi/mg). Chicks of each treatment were housed in a wire-floored brooder pen where they had access to drinking water. Feed was supplied 3 hours after dosing. Three chicks from each treatment were picked at 6, 24, and 96 hours after dosing, weighed, and blood samples collected (6-7 mls) from the wing vein into vacutainer evacuated glass tubes coated with heparin as an anticoagulant. The chicks were killed by chloroform suffocation and liver, heart, gizzard, pancreas, kidney, spleen, testes, part of the

Table IV.1. Composition of the basal diet.

Ingredient	(%)
Yellow corn (ground	64.59
Soybean meal (dehulled)	29.0
Alfalfa (dehydrated, 17%)	2.0
Safflower oil	1.0
Calcium phosphate (defluorinated)	1.9
Limestone (ground)	0.8
Salt (NaCl)	0.3
Vitamin premix and Mineral premix*	0.25
Dl-methionine (98%)	0.06
Calculated analysis	
Metabolizable energy (kcal/kg)	3012
Crude protein (%)	20.05
Calcium (%)	1.02
Phosphorous, total (%)	0.71
Lysine (%)	1.08
Methionine + cysteine (%)	0.71
Selenium (ppm)	0.15

* Vitamin and mineral premixes supplied the following perkg of diet: Vit A, 8872 IU; Vit D, 1100 ICU; Vit E, 25.5 IU; Riboflavin, 5.11mg; D-pant. acid, 12.5mg; niacin, 43.5mg; choline, 1375mg; Betain, 70.4mg; Vit B_{12}, 5.5 MCG; Vit K, 0.7mg; pyrodixine 4.3mg; folacin, 1.52mg; biotin (total), 0.15mg, thiamine, 3.1mg; Mn, 83mg; Fe, 140.8mg; Cu, 414mg; I, 31mg.

breast muscle, and bone marrow from the tibia were removed, weighed, and placed in an ice bath. One ml blood from each chick was centrifuged at 1000xg for five minutes in a clinical centrifuge to separate plasma from red blood cells (RBC). The RBC were washed three times with physiological saline (0.9% NaCl) and suspended in 1 ml saline solution. Plasma, washed RBC, and samples of organs were counted in a Beckman Model 8000 gamma scintillation counter to determine the isotope distribution in blood constituents and the percentage dose distribution in these organs was calculated. The organs, plasma, and RBC lysates were stored at -8°C until gel filtrations were performed.

Preparation of Cytosols and RBC Lysates

About 5 gm frozen sample from each organ (of the 24 hr treatment), except 1 gm testes and 3 gm bone marrow, were sliced and homogenized in 25 ml 10% buffered sucrose (0.05 M phosphate pH 6.3) solution using a Sorvall Omnimixer (2 minutes at maximum speed). The homogenizer container was immersed in ice during homogenization. The homogenate and successive supernatant fractions were centrifuged at 1500 xg for 10 minutes, at 10,000xg for 10 minutes, and at 113,000xg for 90 minutes to obtain respectively crude nuclear, mitochondrial, microsomal, and soluble (cytosol) fractions. All centrifugations were performed at 4°C. The percentage distribution of Se^{75} among the various cellular

fractions were calculated by dividing the sum of counts of all fractions into those of the individual fractions (Table IV.3).

Five ml of blood from 6 and 96 hr time point treatments were centrifuged in a clinical centrifuge at 1000xg for 5 minutes to separate plasma from blood cells. The plasma was separated and kept in plastic vials in the freezer at -8°C until gel filtration analysis. The red blood cells were washed with saline solution (0.9% NaCl) twice, and then resuspended in 7 ml double distilled H_2O, hand shaken and centrifuged (1000xg) for 5 minutes to separate the particulate fraction. The lysate was separated and the pellet was washed twice with 4 ml DDH_2O. The 8 ml washing was added to the 7 ml lysate to bring the total volume to 15 ml. The RBC lysates were stored also in the freezer at -8°C until gel filtration was done.

Gel Filtration Chromatography and Molecular Weight Determination

Sephadex G200, particle size (40-120 μ) was used. The gel was allowed to swell in 0.15 M phosphate buffer containing 0.031 EDTA and 0.01 NaN_3 for 8 hours in a boiling water bath. After the swollen gel was allowed to cool to room temperature, the supernatant was decanted and replaced with fresh buffer kept at 4°C. The slurry was placed in the refrigerator at 4°C for a few hours before pouring into a 2 x 130 cm glass column fitted with a 500 ml reservoir. The gel was allowed to settle for about 24 hours and was washed with about 1 L buffer at a flow rate of 12 ml/hr, using a peristaltic pump. Cytosolic, RBC lysate, and plasma samples were applied to the column, and eluted with

the buffer used for equilibrating the column. The flow rate was maintained at 12 ml/hr and about 6 ml fractions were collected. All gel filtration studies were conducted at 4°C. At the end of the run, the fractions were assayed for GSH-Px activity according to the procedure of Paglia and Valentine[10] with some modifications[11]. The enzyme unit used in this study was an nmol NADPH oxidized/min/ml fraction. The column fractions were counted in the Beckman gamma counter and the optical density of the fractions recorded at 280 nm.

The column was calibrated for molecular weight determination and homogenity of the gel before running any samples. Blue Dextran (Pharmacia) with an average molecular weight (MW) of 2×10^6 dalton was used to determine the void volume and to determine whether the columns were packed correctly. Proteins used to calibrate the column were ferritin (440,000), catalase (232,000), aldolase (158,000), bovine serum albumin (67,000), ovalbumin (43,000), chymotrypsinogen A (25,000) and horse myoglobin (16,700) and the distribution coefficient K_{av} was calculated using the formula $K_{av} = (Ve - Vo)/Vx$ where Ve equals elution volume, Vo equals void volume, and Vx is the internal volume (Vt - Ve).

A plot of K_{ar} versus the log molecular weights of the standard proteins is shown in Figure IV.1.

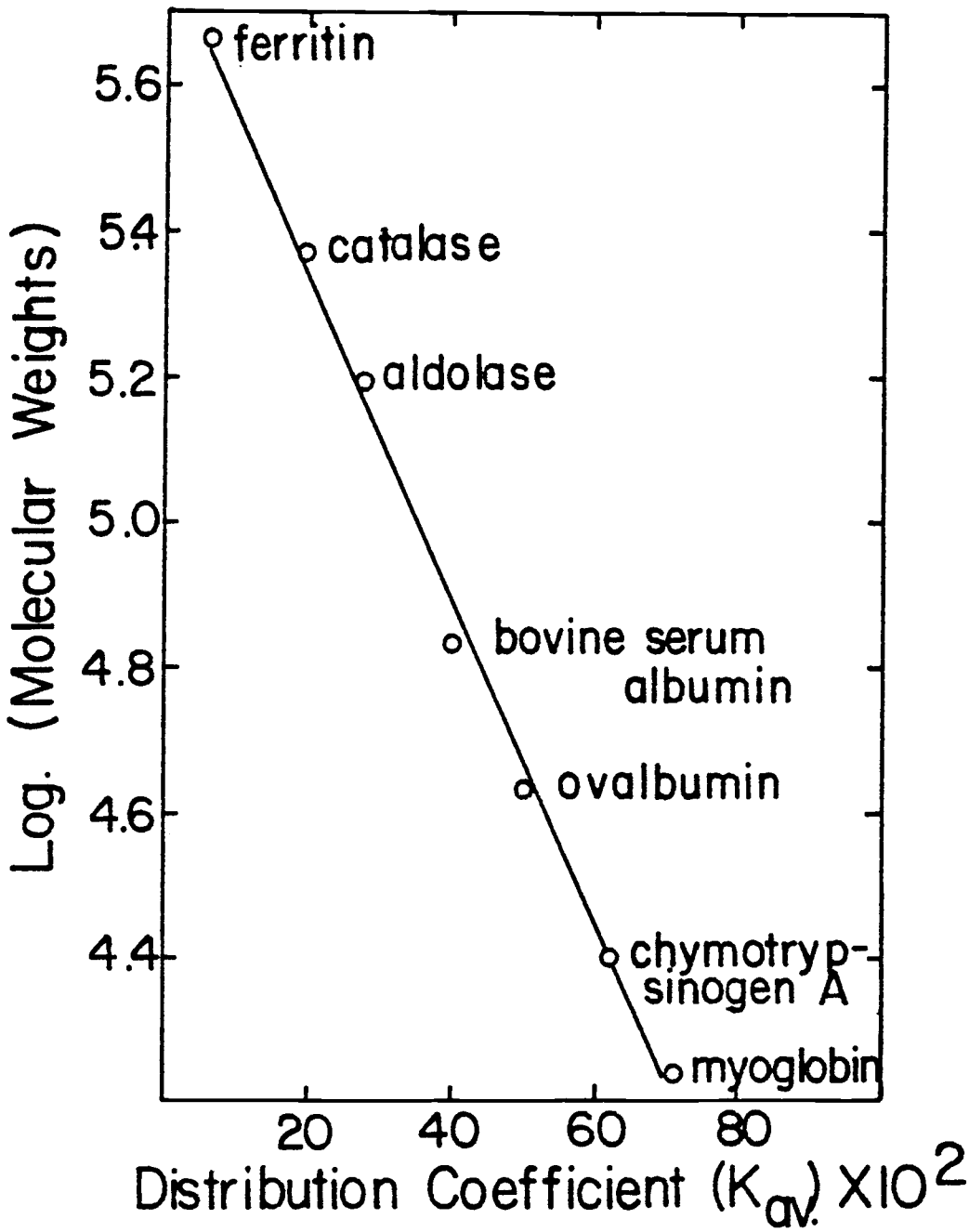


Figure IV.1 Protein standards chromatographed on Sephadex G200. Details of buffers and flow rate are described in the materials and methods section.

RESULTS

The distribution of Se^{75} from selenite and SEM in chick blood at 6, 24, and 96 hours after crop intubation is presented in Figure IV.2. Results indicate gradual decline in Se^{75} pool after 6 hours in both treatments. The disappearance rate of Se^{75} from SEM was found to be slower than Se^{75}-selenite as indicated by 29% and 44% radioactivity from SEM present at 6 hours was removed from the blood after 24 and 96 hours respectively, whereas 47% and 78% of Se^{75} from selenite treatment disappeared after 24 and 96 hours, respectively.

The distribution of Se^{75} between blood cells and plasma was also studied. After 6 hours from intubation, 90% of the Se^{75} from SEM and 80% of the Se^{75}-selenite in blood was associated with plasma. However, 86% of Se^{75}-SEM and 87% of Se^{75}-selenite was detected in plasma after 24 hours. Finally, 67% of Se^{75}-SEM and 83% of Se^{75}-selenite was present in plasma after 96 hours after dosing. The result of plotting log (% CPM) associated with plasma versus time after incubation indicates that plasma Se^{75}-SEM pool dropped in accordance to a first order reaction, however Se^{75}-selenite pool continued to build up and reached maximum at 24 hours, then started to decline, suggesting distinct metabolic routes for SEM and selenite.[12,13]

The level of Se^{75} in blood cells, from chicks dosed with Se^{75}-selenite dropped 65% and 81% after 24 and 96 hours if compared to the level detected after 6 hours after intubation. On the other hand, the level of Se^{75}-SEM in blood cells dropped only 12% after 24 hours then increased 67% after 96 hours in comparison with the level of

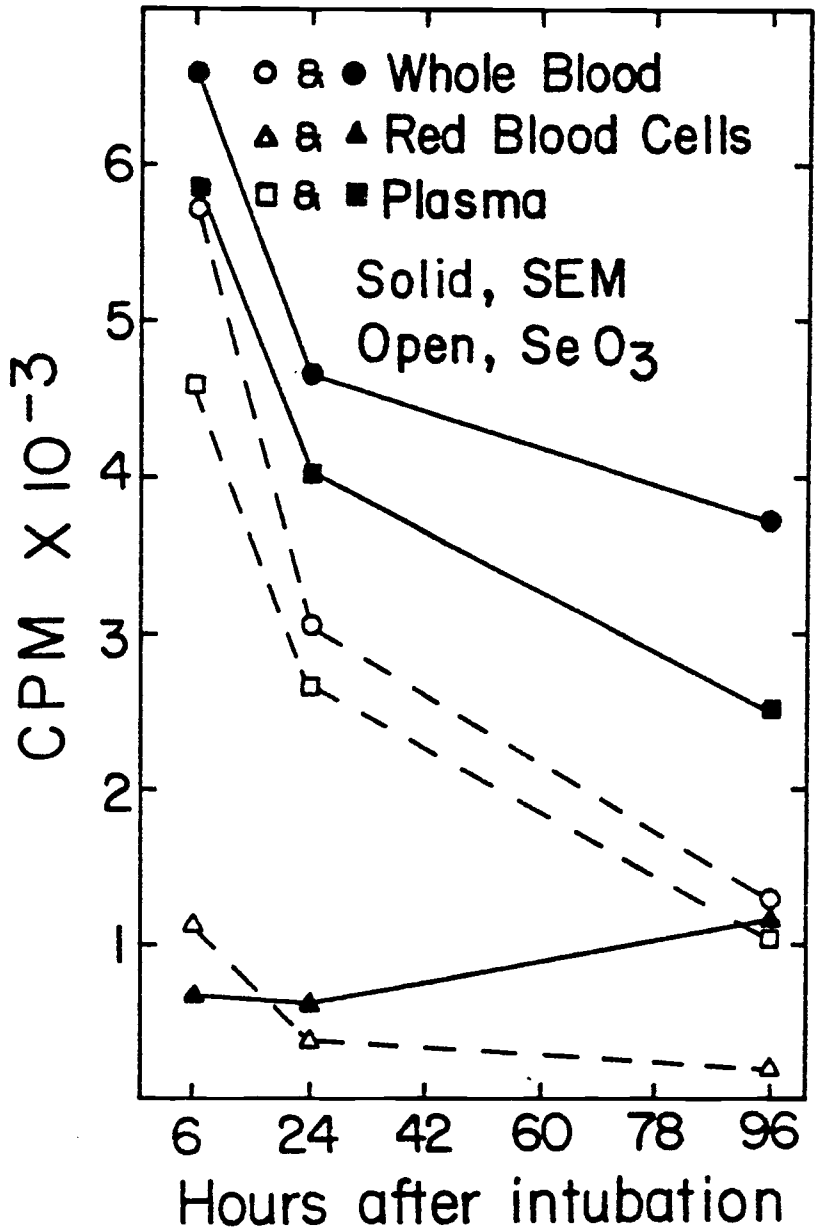


Figure IV.2. The distribution of crop intubated trace amounts of Se^{75}-selenomethionine (solid) and Se^{75}-selenite (open) in chick blood and blood constituents, with time. These results are averaged from three observations.

Se^{75}-SEM in blood cells at 6 hours after dosing.

The gel filtration pattern of blood cell lysates of selenite and SEM treatments at 6 and 96 hours after dosing are shown in Figures IV.3 and IV.4. Five Se^{75} protein-bound peaks corresponding to molecular weights >440,000, 440,000, 150,000, 66,000, and 17,000 were detected in RBC lysate of Se^{75}-SEM treatment, 6 hours after dosing (Figure IV.3, upper portion) in the ratio 11:10:22:37:20, respectively (Table IV.2). The sixth peak is eluted at the same position (360 ml) as that of free Se^{75}SEM in the standard chromatogram and was shown to be non-protein bound by 20% TCA treatment. After 96 hours (Figure IV.3, lower portion), the majority of radioactivity (76%) was associated with one protein peak (MW 66,000) and the Se^{75} non-protein peak disappeared completely.

On the other hand, two Se^{75} protein-bound peaks corresponding to molecular weights 150,000 and 66,000 were detected after 6 and 96 hours in blood cells lysates of chicks dosed with Se^{75}-selenite (Figure IV.4). The majority of radioactivity (61%) was associated with the second peak (66,000) after 6 hours but with the first peak (61%) after 96 hours (Table IV.2). The Se^{75} non-protein bound peak of the selenite treatment is only detected 6 hours after dosing (third peak in Figure IV.4, upper portion).

The first GSH-Px peak (Figures IV.3 and IV.4, upper portion) and the radioactivity peak (MW 150,000) elute together. The hemoglobin peak (MW 66,000), as evidenced by the absorption at 280 nm and red color of the fractions, co-elute with the second GSH-Px peak. These

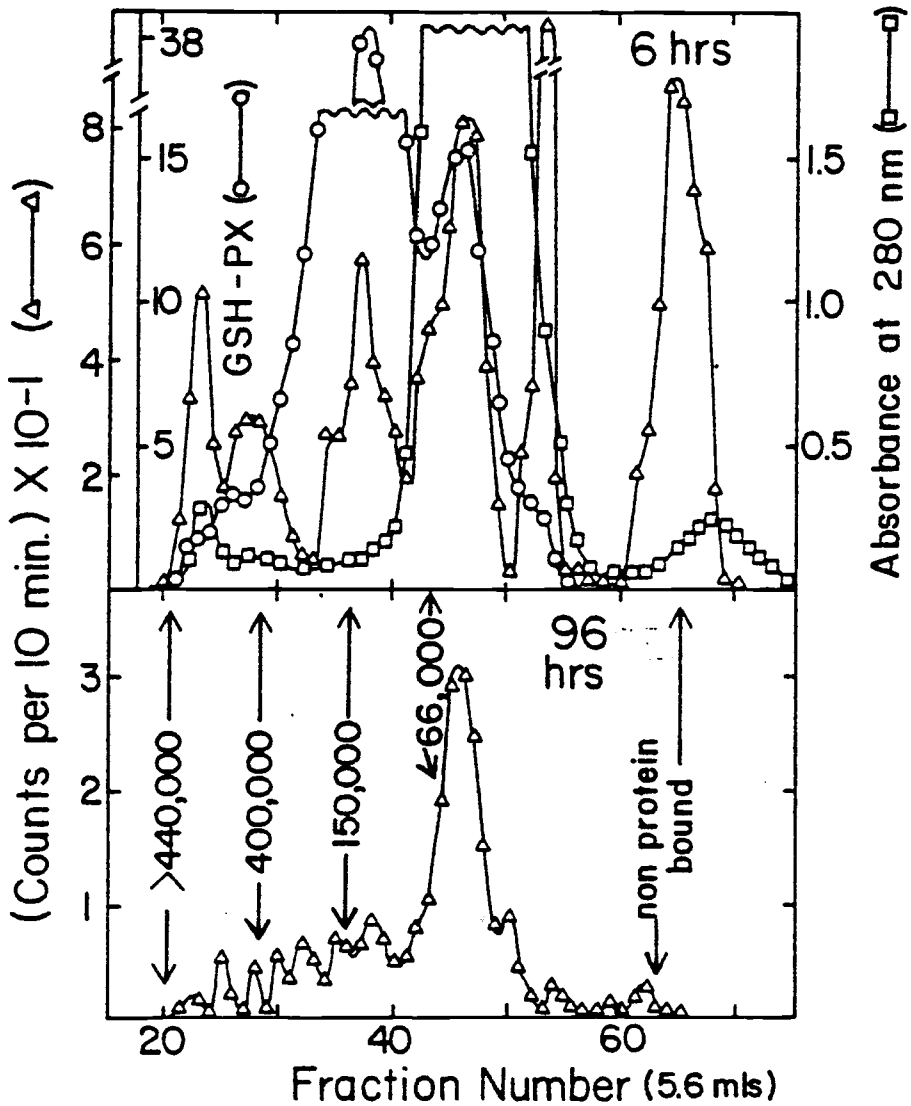


Figure IV.3. Sephadex G200 chromatography of RBC lysate of chicks treated with Se^{75}-SEM 6 hours (upper portion) and 96 hours (lower portion) after intubation.

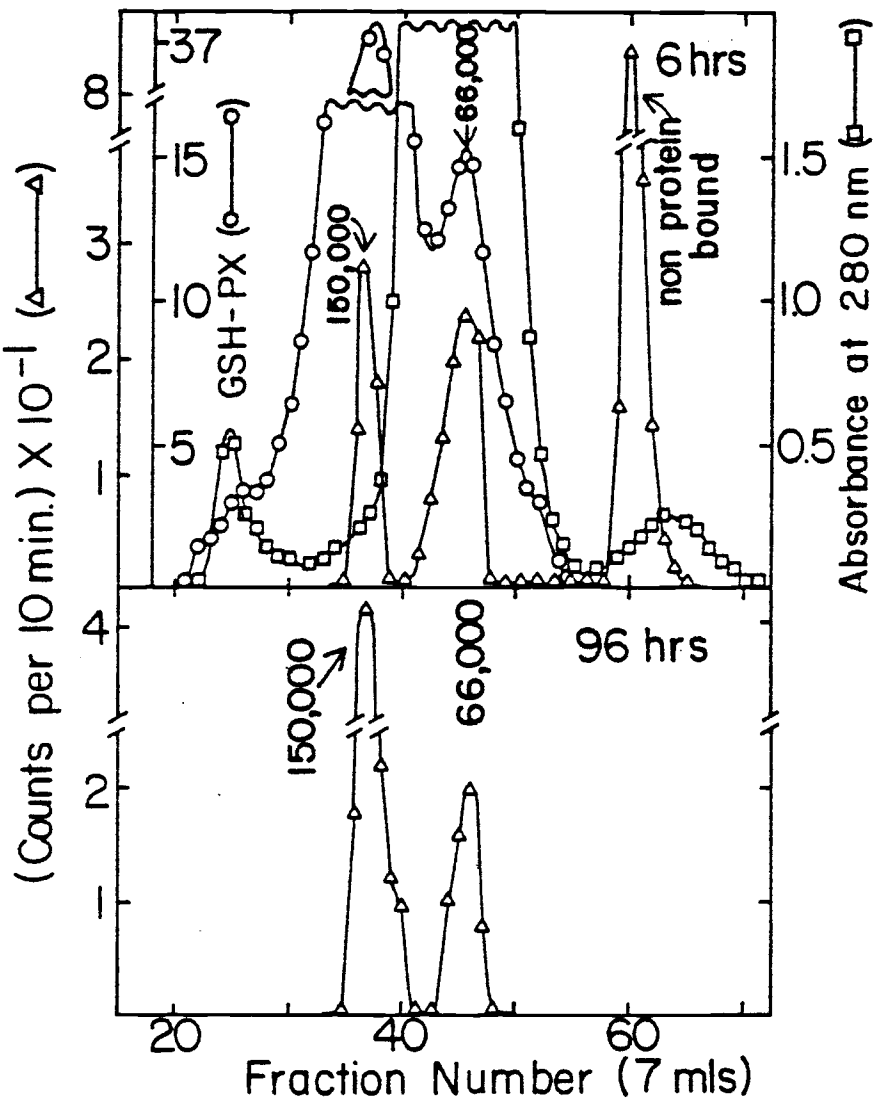


Figure IV.4. Sephadex G200 chromatography of RBC lysate of chicks treated with Se^{75}-selenite 6 hours (upper portion) and 96 hours (lower portion) after intubation.

Table IV.2. Percentage distribution of Se^{75} containing proteins in chick blood cells and plasma at 6 and 96 hours after crop intubation.

Sample	Percentage of Se^{75} Proteins							
	above 440,000	440,000	338,000	150,000 (GSH Px)	89,000	66,000 (Hb)	30,000	17,000
RBC lysate-SEM (6 hrs)	11	10	ND	22	ND	37	ND	20
RBC lysate-SEM (96 hrs)	D	D	ND	D	ND	76	ND	ND
RBC lysate-selenite (6 hrs)	ND	ND	ND	39	ND	61	ND	ND
RBC lysate-selenite (96 hrs)	ND	ND	ND	61	ND	39	ND	ND
Plasma-SEM (6 hrs)	5	ND	58	ND	37	ND	ND	ND
Plasma-SEM (96 hrs)	14	ND	19	10	56	ND	D	ND
Plasma-selenite (6 hrs)	ND	ND	92	ND	ND	ND	ND	ND
Plasma-selenite (96hrs)	21	ND	34	11	21	ND	12	ND

ND = None detected
D = detected

results suggest that chick GSH-Px enzyme has a MW of 150,000 and chick hemoglobin binds Se^{75} (from selenite or SEM) and possesses GSH-Px activity.

Gel filtration chromatography of plasma 6 and 96 hours after intubating chicks with Se^{75} (as selenite or SEM) is shown in Figures IV.5 and IV.6. Results in Figure IV.5, upper portion, indicate that Se^{75}-SEM is incorporated into three protein-bound peaks after 6 hours, as evidenced by the absorption at 280 nm and 20% TCA treatment, having molecular weights (>440,000, 338,000, and 89,000). Another Se^{75}-protein bound peak (MW 150,000) was detected 96 hours after dosing (Figure IV.5, lower portion).

Only one Se^{75}-protein bound peak (MW 338,000) was detected in chick plasma 6 hours after dosing with Se^{75}-selenite (Figure IV.6, upper portion), however, five Se^{75}-protein bound peaks corresponding to MW >440,000, 338,000, 150,000, 89,000, and 30,000 were observed after 96 hours (Figure IV.6, lower portion).

The percentage distribution of Se^{75} among plasma proteins is shown in Table IV.2. Results indicate that 58% and 92% of Se^{75} in plasma of SEM and selenite treatments, respectively, is associated with proteins having MW 338,000 after 6 hours. However, proteins with MW >440,000 and 89,000 seem to be the principal carriers of radioactivity after 96 hours in both treatments. The appearance of radioactivity (11%) in proteins with MW 150,000 after 96 hours, in both treatments, suggests the incorporation of Se^{75} in newly synthesized plasma GSH-Px. Finally, Se^{75} protein-bound peak having a MW of 30,000 is

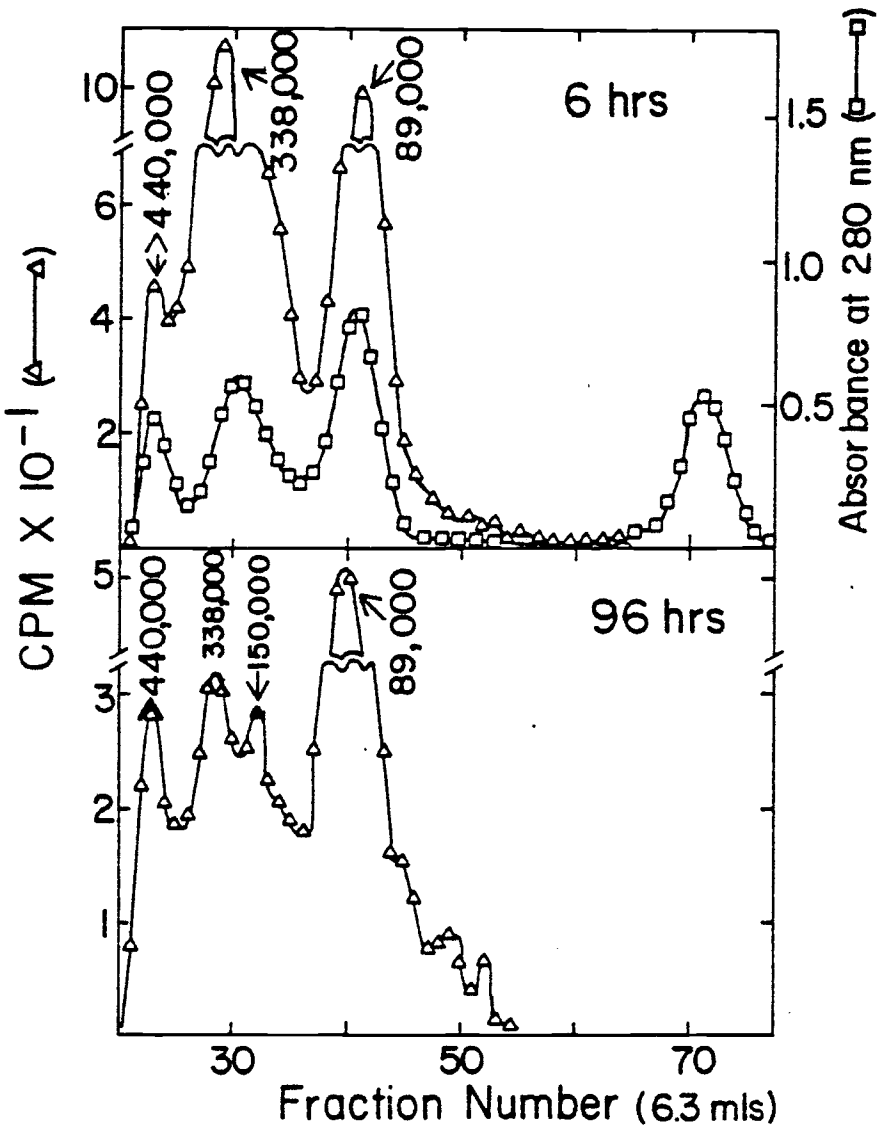


Figure IV.5. Sephadex G200 chromatography of plasma from whole blood of chicks treated with Se^{75}-SEM 6 hours (upper portion) and 96 hours (lower portion) after intubation.

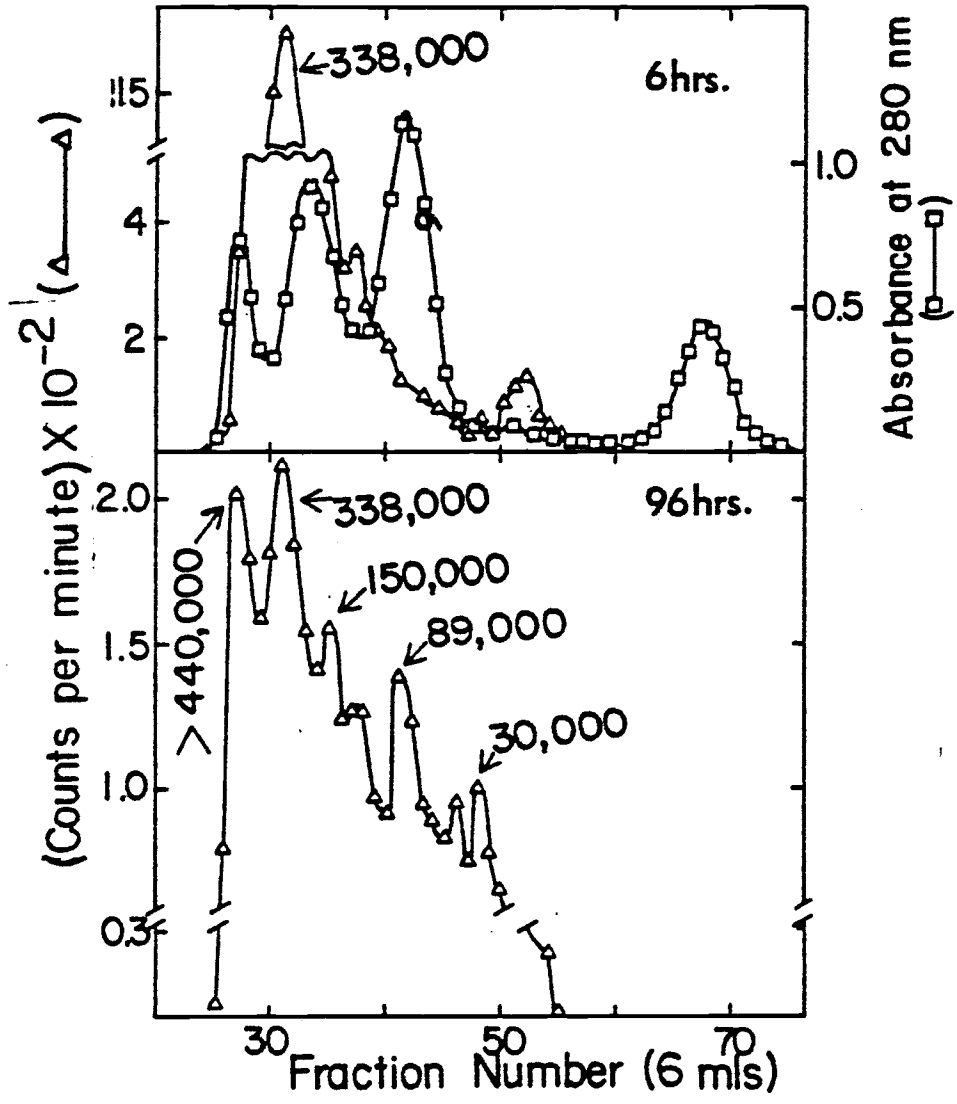


Figure IV.6. Sephadex G200 chromatography of plasma from whole blood of chicks treated with Se^{75}-selenite 6 hours (upper portion) and 96 hours (lower portion) from intubation.

noticed in selenite treatment plasma 96 hours after dosing only.

Figure IV.7 shows the distribution of Se^{75} from selenite or SEM among several chick tissues various times after intubation. The highest Se^{75} concentration (as selenite or SEM) was found in the liver and kidney, followed by the spleen. The testes from the Se^{75}-selenite treatment contained a larger dose than bone marrow, pancreas, heart, gizzard, and breast muscle but less than the spleen. Also, similar levels of Se^{75}-selenite were detected in bone marrow, pancreas, heart muscle, and gizzard muscle. The breast muscle was found to contain the lowest Se^{75} concentration in both Se^{75}-selenite and Se^{75}-SEM treatments.

Interestingly, the pancreas was found to accumulate larger Se^{75}-SEM amounts than the testes, gizzard muscle, heart muscle, and bone marrow. Also, most of the tissues contained comparable levels (as percentage of the dose) of Se^{75}-selenite and Se^{75}-SEM except for the pancreas where the level of Se^{75} from SEM was five times higher than that from Se^{75}-selenite.

Similar levels of Se^{75}-selenite were observed in the liver and kidney 6 and 24 hours after dosing. At 96 hours, these levels dropped about 47%. Similar trends were noticed for the testes, heart muscle, and bone marrow where 42, 43, and 55% decrease in the radioactivity occurred after 96 hours, respectively.

The uptake of Se^{75}-selenite continued to increase in the spleen, reaching a maximum level at 24 hours. Subsequently, it started to decline until 43% of radioactivity remained 96 hours after intubation.

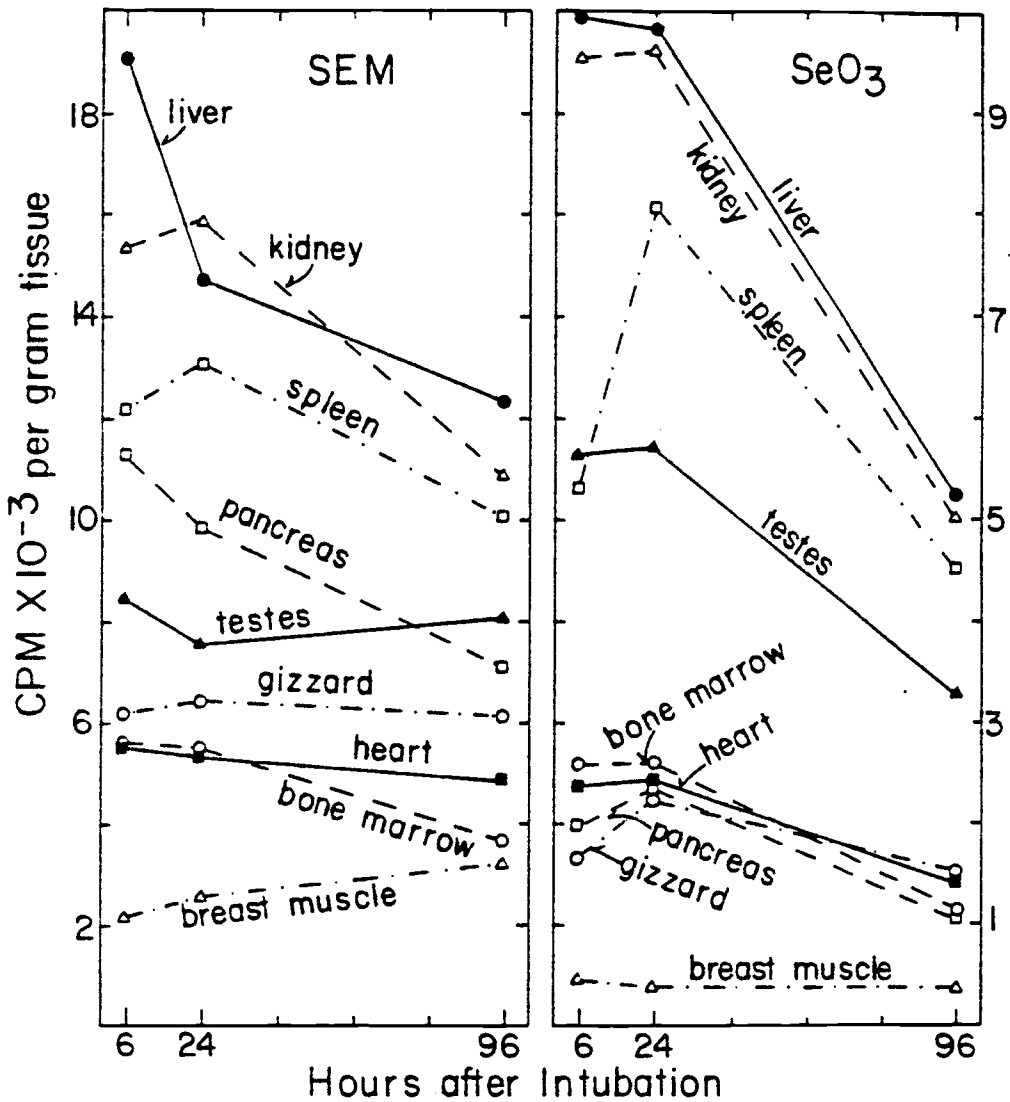


Figure IV.7. The distribution of crop intubated trace amounts of Se^{75}-SEM (left portion) and Se^{75}-selenite (right portion) in chick tissues. These results are the average of three observations.

The same observation was made for gizzard muscle and pancreas in chicks dosed with Se^{75}-selenite. However, the Se^{75}-selenite content in breast muscle remained relatively unchanged at 6, 24, and 96 hours after dosing.

On the other hand, the Se^{75}-SEM content continued to decline in the liver and pancreas in which 35% of the radioactivity, detected at 6 hours, was lost 96 hours after intubation. Maximum levels of Se^{75}-SEM were reached at 24 hours in kidney and spleen (under our experimental conditions), then started to drop afterward. However, Se^{75}-SEM levels remained relatively constant in heart muscle, gizzard muscle, and testes at the various time intervals studied. A gradual drop in Se^{75}-SEM level was noticed in bone marrow, however, gradual increases of Se^{75}-SEM concentration were observed for breast muscle.

The distribution of Se^{75} (from selenite or SEM) in various cellular fractions of various tissues is presented in Table IV.3. The nuclear and cytosolic fractions were found to contain the highest Se^{75} concentrations (as Se^{75}-selenite or Se^{75}-SEM) with lower levels in mitochondrial and microsomal fractions. The distribution of Se^{75}-selenite and Se^{75}-SEM in various cellular fractions was noticed to be similar in most of the tissues. The highest Se^{75} concentrations in the nuclear fractions were associated with the muscular tissues such as the gizzard and heart. Lower levels were observed for the soft tissues such as pancreas, liver, bone marrow, and kidney. The mitochondrial fraction of the pancreas of chicks dosed with Se^{75}-SEM was found to be exceptionally high if compared to the

Table IV.3. Percentage distribution of Se^{75} as selenite or selenomethionine among various cellular fractions in various tissues from chicks killed after 24 hours from intubation.

	Cellular Fractions							
	Nuclear		Mitochondrial		Microsomal		Cytosolic	
Tissue	SeO_3	SEM	SeO_3	SEM	SeO_3	SEM	SeO_3	SEM
Liver	50.37	54.06	7.4	3.5	4.43	5.28	37.8	37.14
Kidney	58.07	51.96	6.98	9.32	4.83	8.06	20.12	30.66
Spleen	73.72	66.15	3.69	3.59	4.06	3.83	18.53	25.42
Pancreas	46.47	42.59	16.29	23.32	10.35	7.81	25.89	25.28
Gizzard	83.58	84.5	1.1	2.6	2.18	1.56	13.14	11.33
Heart	77.97	79.44	3.25	2.56	1.82	2.53	16.95	14.46
Bone Marrow	58.9	49.73	1.54	0.96	0.95	0.82	38.61	48.74

pancreas of Se^{75}-selenite treatment or other tissues. The lowest level of radioactivity in the mitochondrial fraction was associated with tissues from gizzard muscle and bone marrow.

The pancreas microsomal fraction is noticed to contain higher Se^{75} concentration, in both treatments, than other microsomal fractions. The lowest Se^{75} concentration is associated with the microsomal fraction of bone marrow in both treatments. The lowest Se^{75} concentrations in the cytosolic fractions were found in the muscular organs such as gizzard and heart followed by the spleen. However, the highest level is detected in bone marrow cytosol in both treatments, followed by the liver then pancreas where similar levels of distribution were noticed for both Se^{75}-selenite and Se^{75}-SEM treatments.

Gel filtration chromatography of liver cytosol (Figure IV.8) indicated that 55% of Se^{75} (from SEM) is associated with GSH-Px and little (7.5%) is associated with MW >440,000 proteins. The third peak was shown to be unassociated with protein, as evidenced by 20% TCA treatment. The same Se peaks were also seen in liver cytosol of the selenite treatment (Figure IV.8, lower portion) together with a fourth peak of radioactivity associated with protein with a MW of 44,500. Also, two peaks of GSH-Px activity are noticed having MW of 150,000 and 21,400 in liver cytosol of both treatments. The 280 nm scans of liver cytosol fractions of both treatments were similar.

The elution patterns of kidney cytosols from both SEM (upper portion) and selenite (lower portion) are shown in Figure IV.9. Three

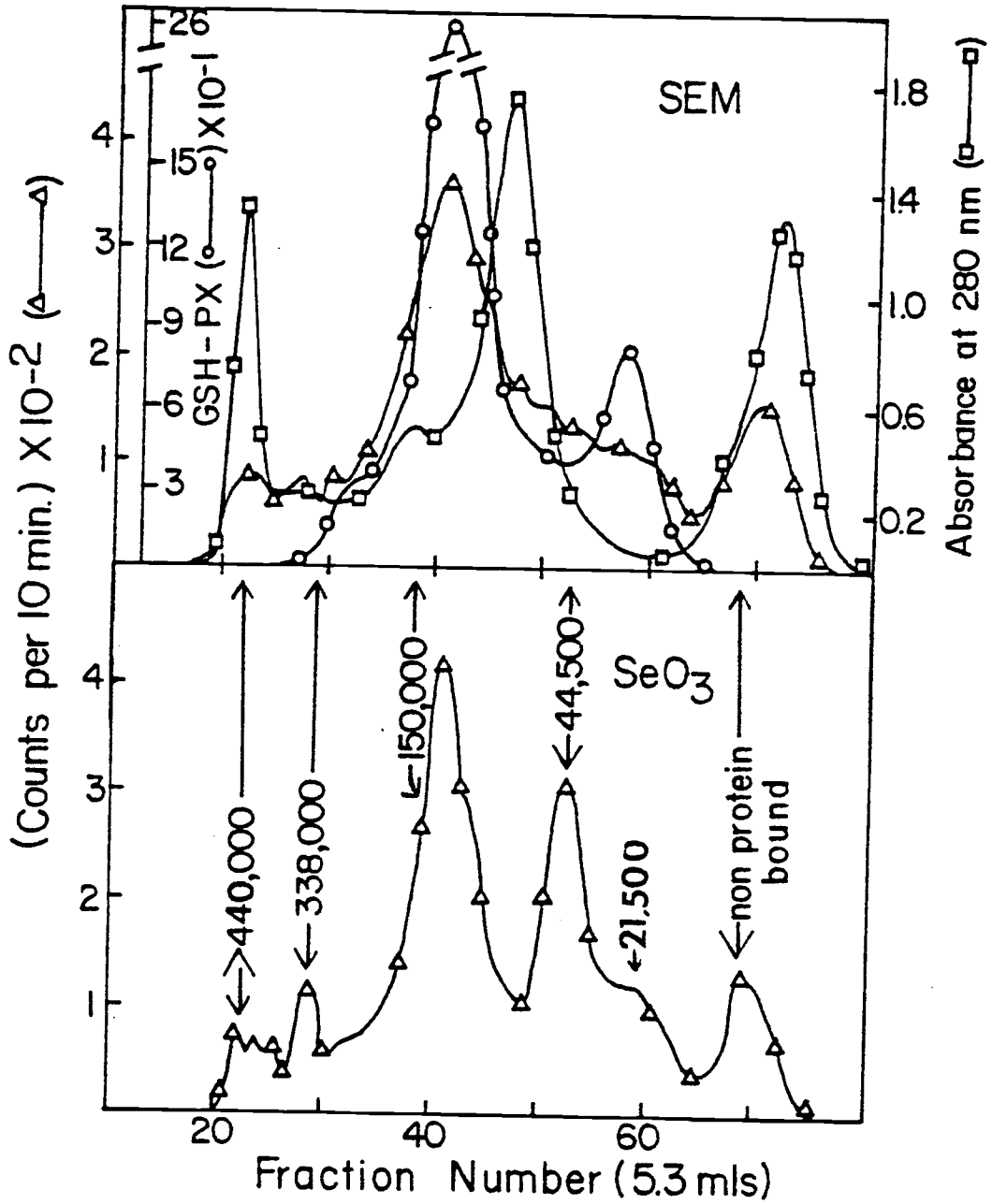


Figure IV.8. Sephadex G200 chromatography of liver cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after intubation.

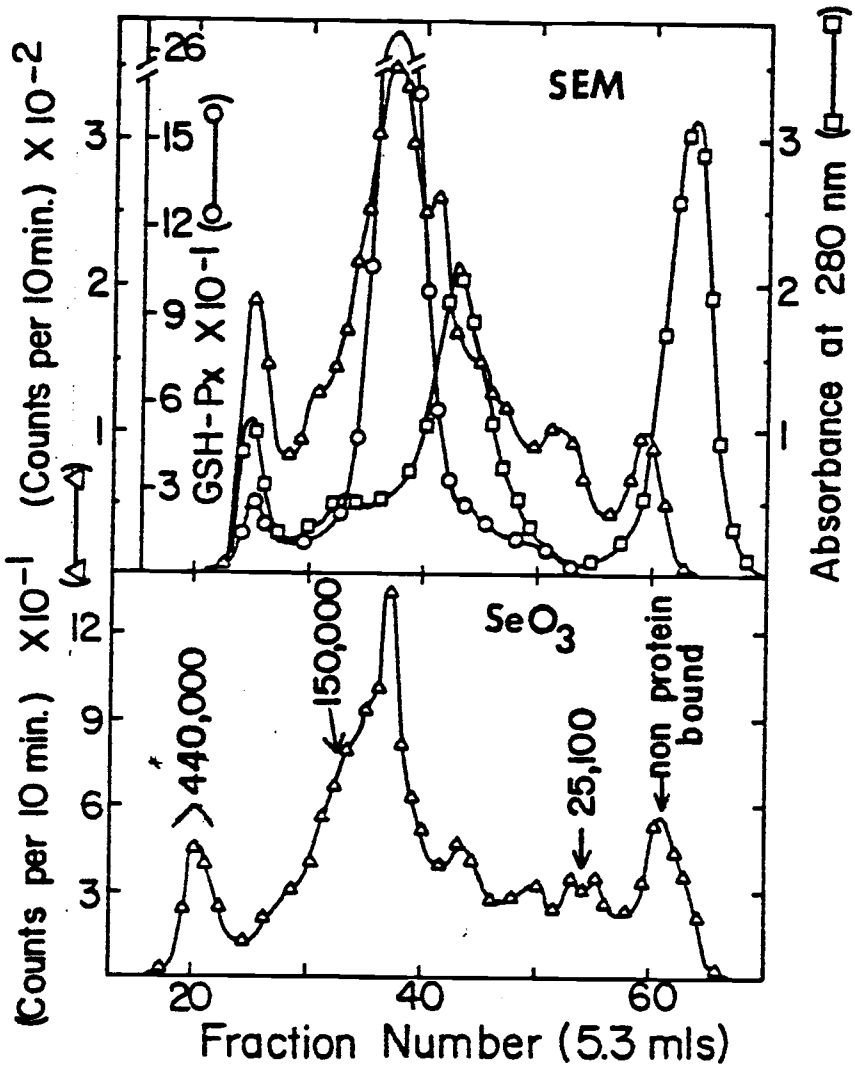


Figure IV.9. Sephadex G200 chromatography of kidney cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after intubation.

Se^{75} protein-bound peaks corresponding to molecular weights >440,000, 150,000, and 25,100 were detected in both treatments. The fourth peak (in both chromatograms) is not associated with proteins and could be free Se^{75}-SEM or Se^{75}-selenite, respectively. According to Table IV.4, most of the Se^{75} (53% and 60% from SEM and selenite, respectively) is associated with GSH-Px activity, as evidenced by GSH-Px assay. Little GSH-Px activity is found to be associated with proteins having more than 440,000 molecular weights. The optical density scans at 280 nm in both treatments are similar.

Figure IV.10 represents the elution pattern of spleen cytosol from SEM (upper portion) and selenite (lower portion) dosed birds. Se^{75}-protein bound is distributed among three peaks corresponding to M.W. >440,000, 150,000 and 16,700 and rudimentary amounts of Se^{75} is associated with proteins with MW of 338,000, 67,000, and 25,100. The majority of Se^{75} (53% and 60% from SEM and selenite, respectively) present in spleen cytosol is associated with the enzyme GSH-Px as evidenced by GSH-Px scans in Figure IV.10. High molecular weight proteins (>440,000) seem to play some role in Se metabolism in spleen. The third peak (MW 16,700) contained 8% and 13% of Se^{75} from SEM and selenite treatments. Also, Se^{75} non protein-bound peak was observed in both treatments. Finally, little GSH-Px activity is associated with low MW proteins (25,100) and absorbance scans at 280 nm were similar in both treatments.

The elution patterns from pancreatic cytosols from both treatments are shown in Figure IV.11. The upper portion, the SEM treatment, shows

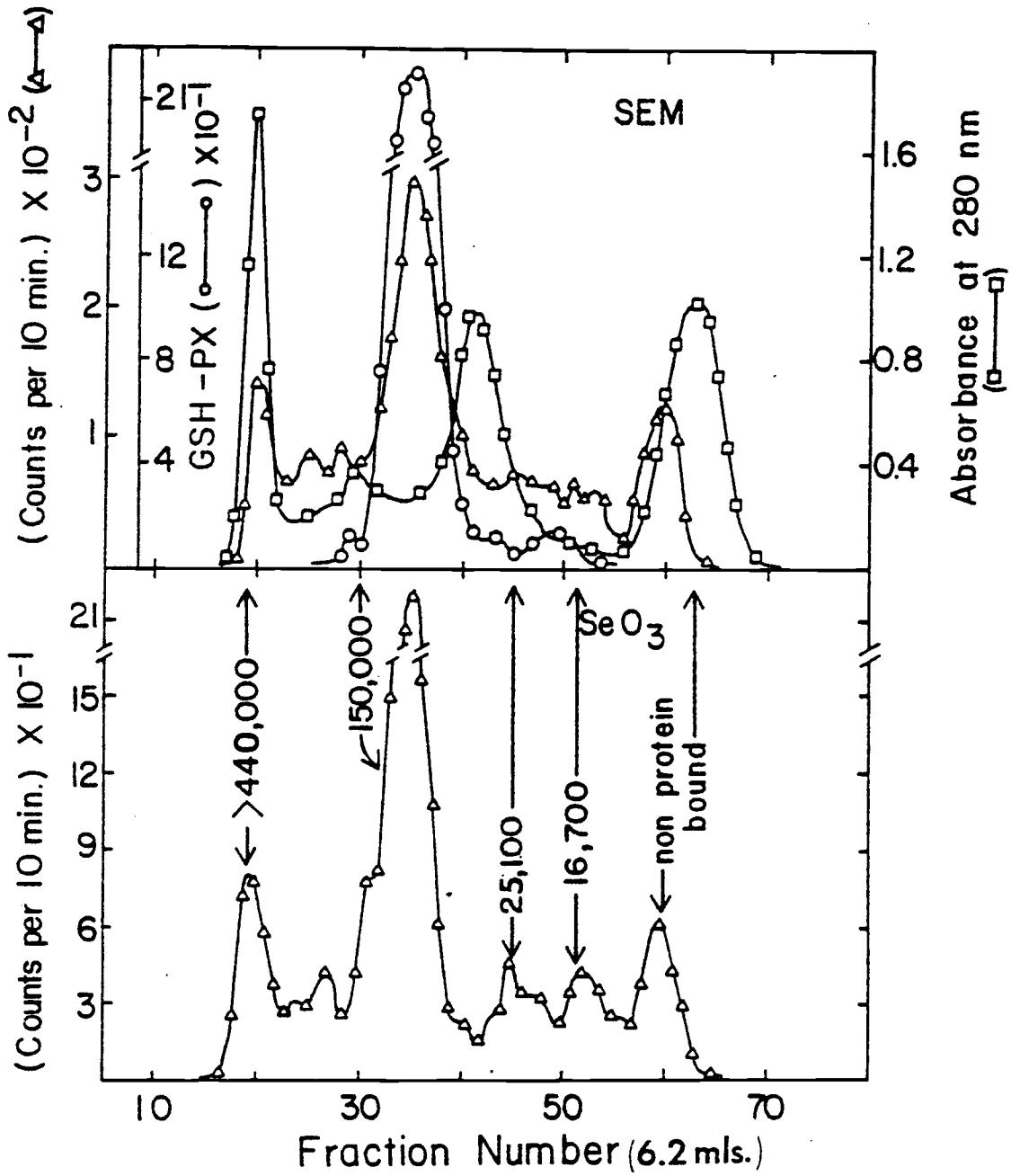


Figure IV.10. Sephadex G200 chromatography of spleen cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after intubation.

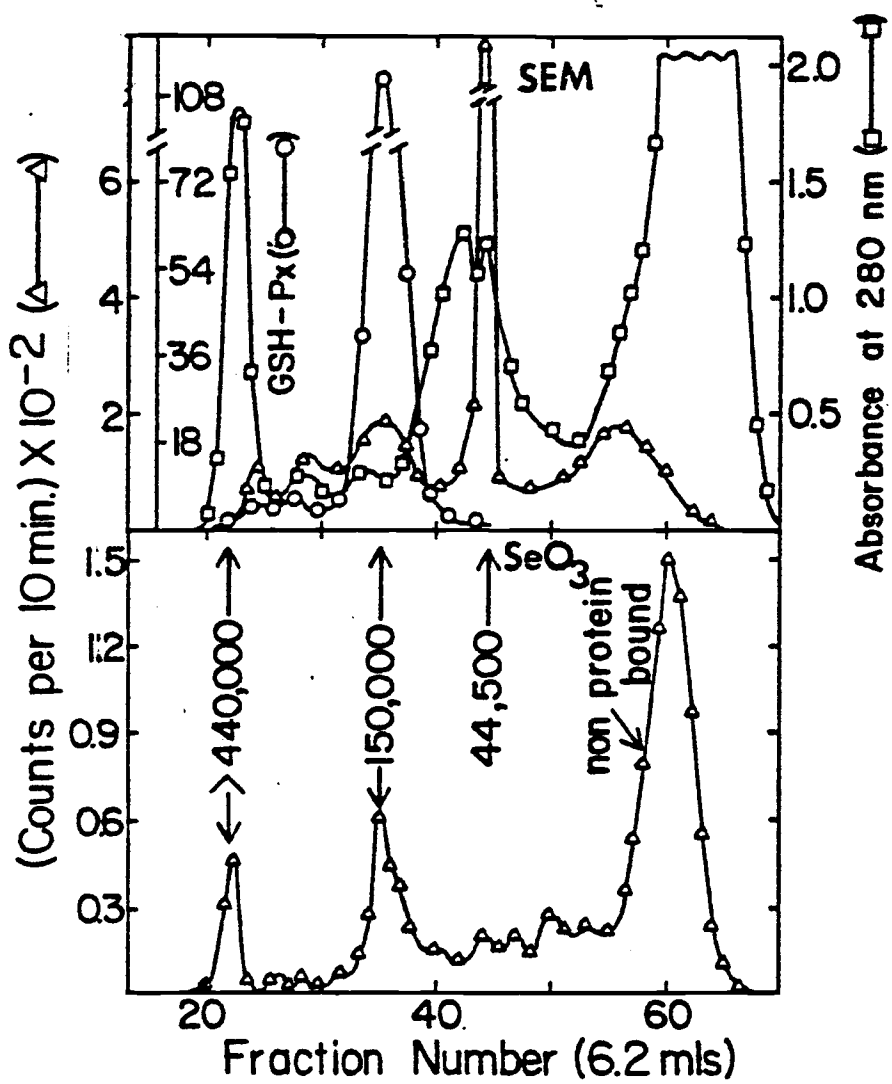


Figure IV.11. Sephadex G200 chromatography of pancreatic cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after intubation.

three Se^{75} protein-bound peaks corresponding to molecular weights of >440,000, 150,000, and 44,500 and one Se^{75} non-protein-bound peak. Only 28% of Se^{75} in pancreatic cytosol is bound to the enzyme GSH-Px and the majority (49%) of Se^{75}, from SEM treatment, is bound to protsins corresponding to a MW of 44,500. Little radioactivity (8%) is found in high molecular weights (>440,000) proteins.

The lower portion of Figure IV.11 shows two Se^{75}-protein bound peaks corresponding to molecular weights of >440,000 and 150,000. Rudimentary amounts of Se^{75} from selenite are bound to the third peak (44,500) noticed in the SEM treatment. Absorbance measurements at 280 nm and GSH-Px scans are similar in both treatments.

The chromatogram of testes cytosols on Sephadex G 200 is shown in Figure IV.12. Three Se^{75} protein-bound peaks were observed in both treatments corresponding to molecular weights >440,000, 150,000, and 35,500. In addition, another peak (263,000) is observed in the Se^{75}-selenite treatment (lower portion). Se^{75} non protein-bound was detected in both treatments. Finally, all the GSH-Px activity is associated with one peak (150,000) and similar optical density scans were observed for both Se^{75} treatments.

Gel filtration chromatography of bone marrow cytosols on the G200 column is shown in Figure IV.13. The radioactivity is distributed between two peaks corresponding to 150,000 and 66,000 in SEM treatment but only associated with 150,000 in the selenite treatment. Results of GSH-Px assay showed two peaks corresponding to 150,000 (major peak) and 25,100 (minor peak) in both treatments. Radioactivity was detected in

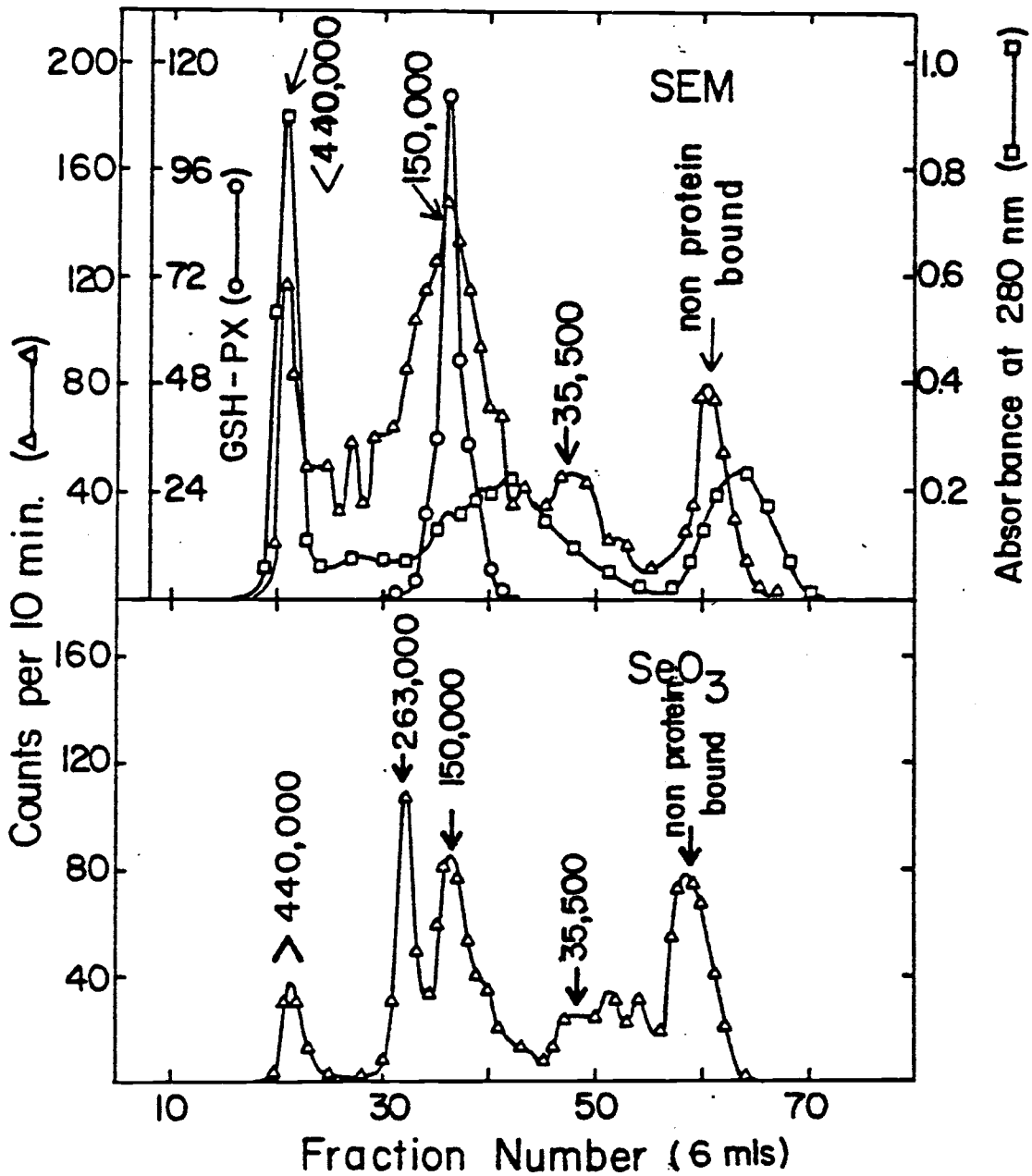


Figure IV.12. Sephadex G200 chromatography of testes cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after dosing.

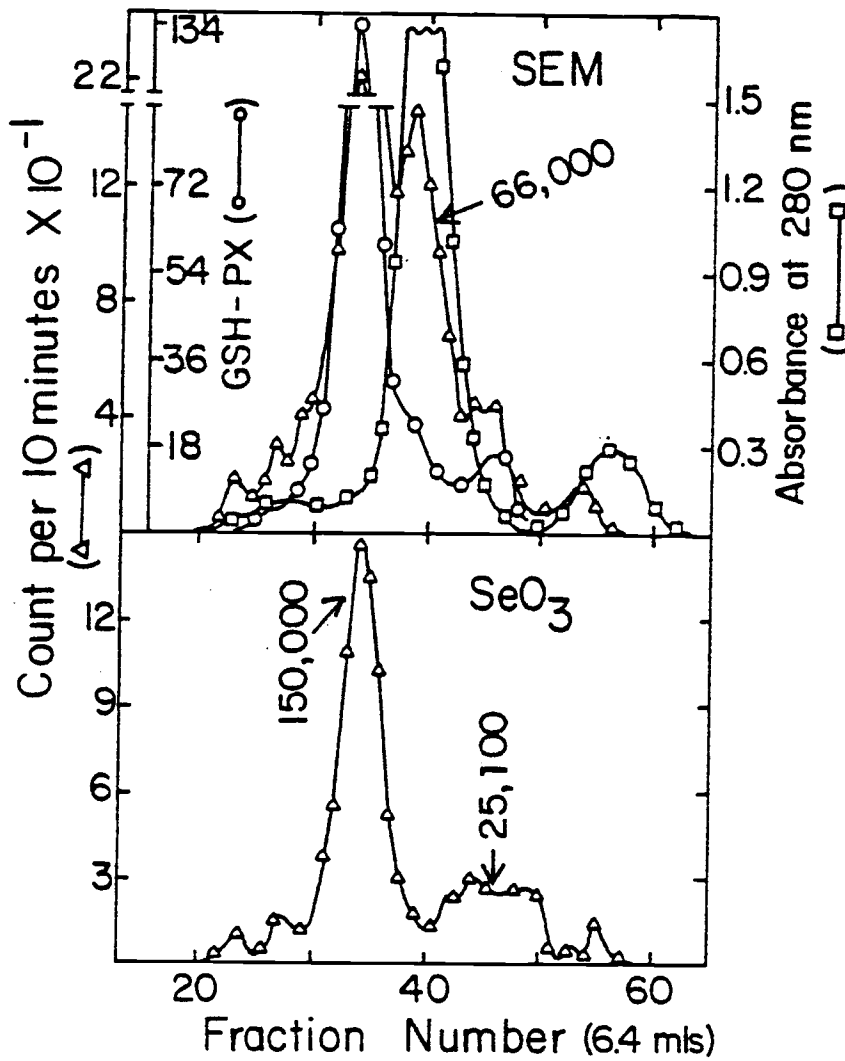


Figure IV.13. Sephadex G200 chromatography of bone marrow cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after intubation.

both GSH-Px peaks, and similar optical density scans were found in both treatments.

The elution pattern of heart cytosols on Sephadex G200 is shown in Figure IV.14. Se^{75} protein bound is observed in high molecular weight (>440,000) proteins and the enzyme GSH-Px in both Se^{75} treatments. A Se^{75} non protein-bound peak is detected in both treatments as well. Also, two peaks having GSH-Px corresponding to molecular weights 150,000 and 52,480 were observed in heart cytosols of both treatments.

Finally, Figure IV.15 represents the elution profile of gizzard cytosols from Sephadex G200 column. Five peaks of radioactivity which were associated with protein as evidenced by the absorption of 280 nm were observed in both treatments. Se^{75} (from selenite, upper portion, or SEM, lower portion), not bound to proteins, was detected in the last peak. The majority of GSH-Px activity is localized in the peak corresponding to MW 150,000, however, little enzymic activity (GSH-Px) is observed in low molecular weight (17,000) proteins which corresponds to myoglobin.

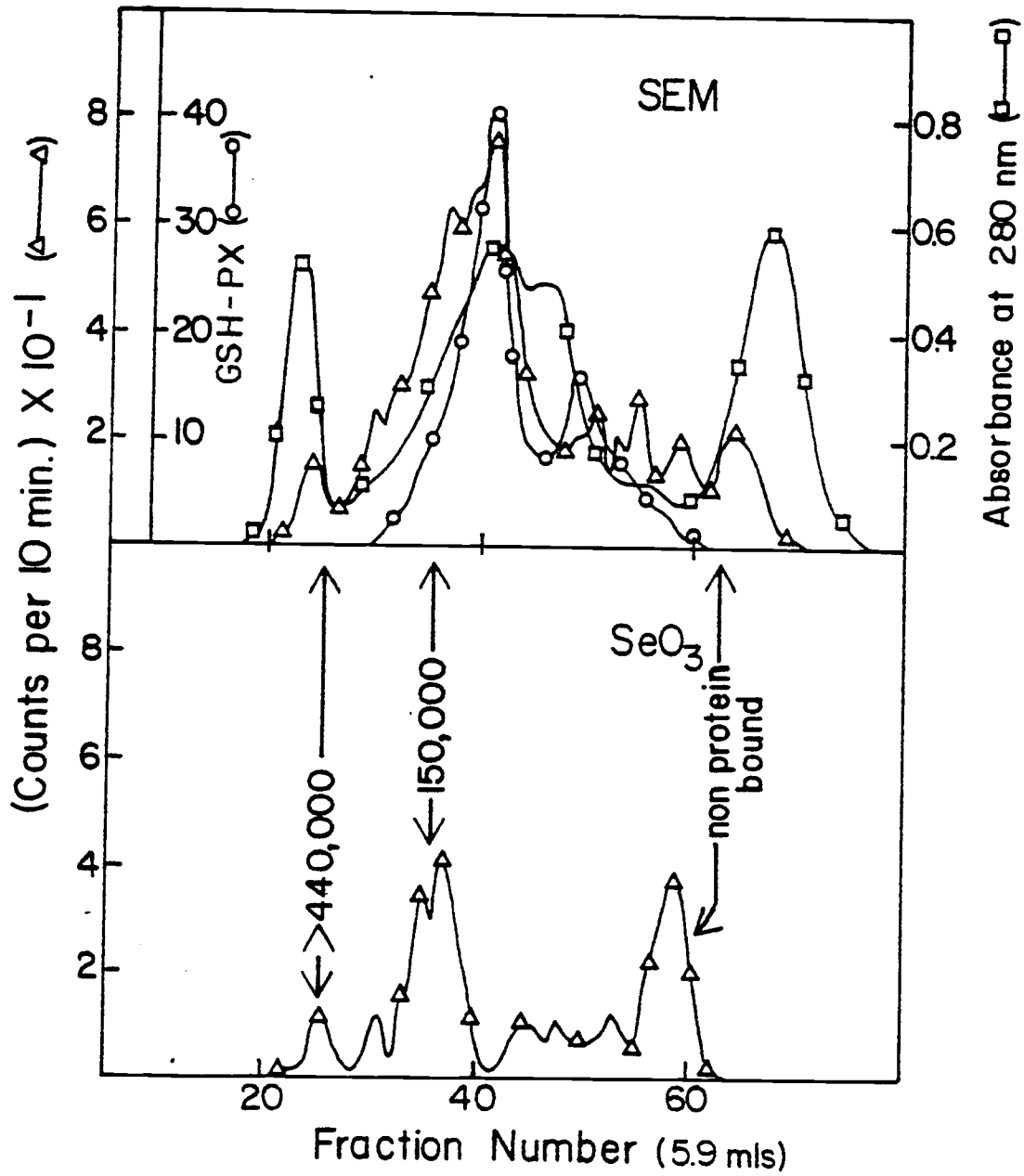


Figure IV.14. Sephadex G200 chromatography of heart cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after intubation.

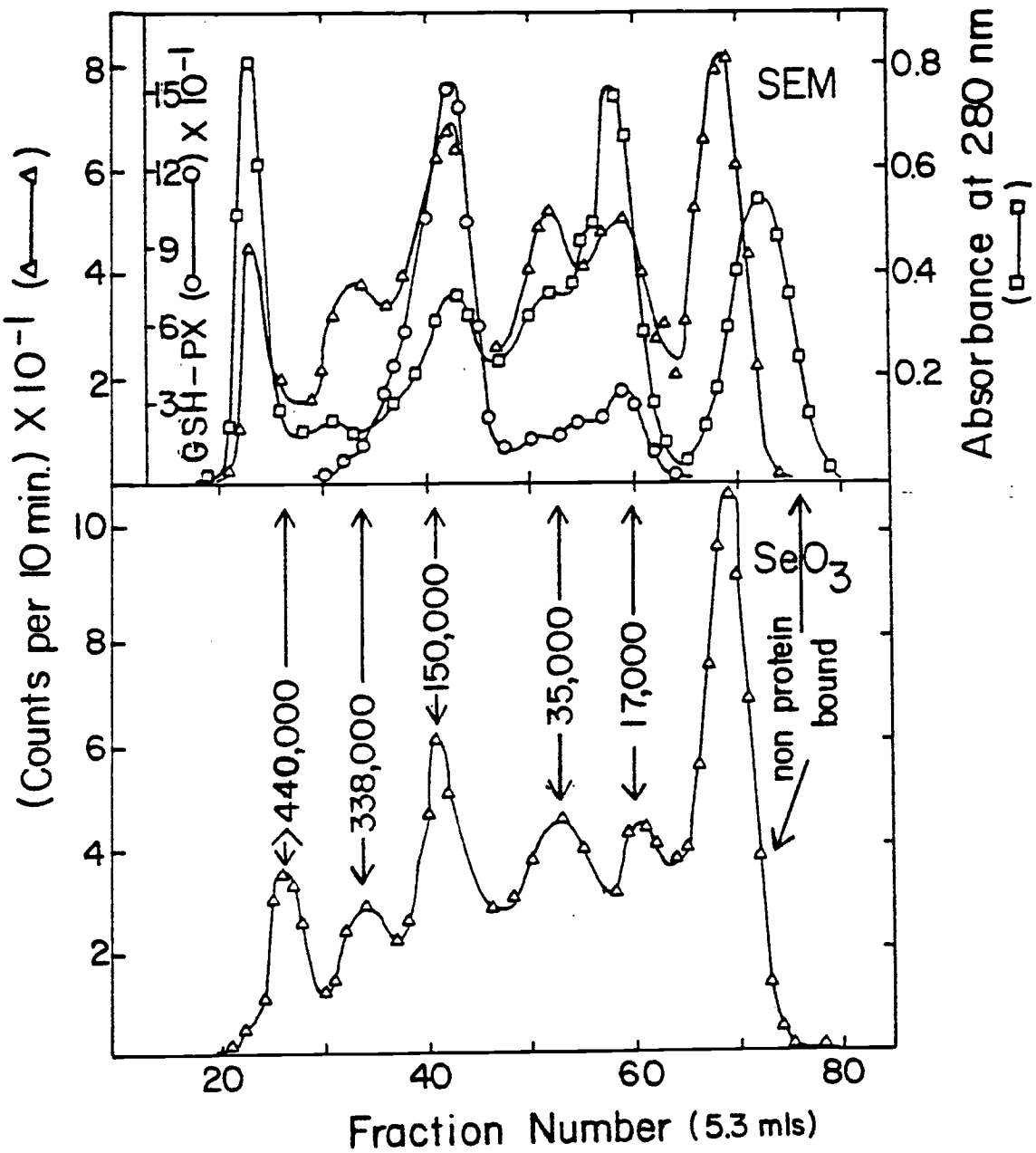


Figure IV.15. Sephadex G200 chromatography of gizzard cytosols of chicks treated with Se^{75}-SEM (upper portion) and chicks treated with Se^{75}-selenite (lower portion) 24 hours after intubation.

Table IV.4. Percentage distribution of Se^{75} protein bound peaks in various chick tissue cytosols after 24 hours from crop intubation.

Tissue cytosol	Se^{75} binding protein (MW)									
	above 440,000	338,000	263,000	150,000 (GSH Px)	67,000 (Hb)	44,500	35,500	25,100	21,000	17,000
Liver (SEM)	7.5	D	D	55	D	35	ND	ND	D*	ND
Liver (selenite)	5.6	D	D	52	D	ND	ND	ND	D*	ND
Kidney (SEM)	11.1	ND	ND	53	D	ND	ND	D	ND	ND
Kidney (selenite)	7.6	ND	ND	60	D	ND	ND	D	ND	ND
Spleen (SEM)	14.0	ND	ND	54	D	ND	ND	D	ND	8
Spleen (selenite)	17.0	ND	ND	69	ND	ND	ND	D	ND	13
Pancreas (SEM)	8.0	ND	ND	28	ND	49	ND	ND	ND	ND
Pancreas (selenite)	16.0	ND	ND	43	ND	D	ND	ND	ND	ND
Testes (SEM)	17.0	ND	D	66	ND	ND	16	ND	ND	ND
Testes (selenite)	10.0	ND	27	37	ND	ND	D	ND	ND	ND
Bone Marrow (SEM)	ND	ND	ND	55	38	ND	ND	D*	ND	ND
Bone Marrow (selenite)	ND	ND	ND	80	D	ND	ND	D*	ND	ND
Heart (SEM)	D	ND	ND	D	ND	ND	ND	D	ND	ND
Heart (selenite)	D	ND	ND	D	ND	ND	ND	D	ND	ND
Gizzard (SEM)	13.0	13	ND	41	ND	ND	31	ND	ND	21*
Gizzard (selenite)	12.0	11	ND	34	ND	ND	29	ND	ND	12*

* possesses GSH Px activity
D = detected
ND = none detected

DISCUSSION

It is evident from this study that the chemical form of selenium can markedly affect its metabolism in chick blood (Figures IV.2, IV.3, IV.4, IV.5, and IV.6). These results are in accordance with Awwad *et al.*[12] and Jenkins and Hidiroglou[13].

Gel filtration chromatography of RBC lysates (Figures IV.3 and IV.4) indicated that Se^{75}-selenite is more specifically incorporated into the enzyme, glutathione peroxidase, than Se^{75}-SEM. This result could explain the observation that Se from SEM is less effective than Se from sodium selenite in preventing exudative diathesis in chicks.[11]

There was a Se^{75} non-protein bound peak in blood (Figures IV.3 and IV.4, upper portions) 6 hours after dosing but not 96 hours after dosing (lower portions). This demonstrates the unique role of blood cells in metabolizing selenium from selenite or SEM so can bind to plasma proteins.[12,13]

Se^{75} from both sources was found to be bound to hemoglobin (Hb)[14]. However the mode of incorporation of Se^{75} from selenite seems to be distinct from that of Se^{75} from SEM as shown in Figure IV.13. Such results suggest that Se^{75} from SEM was incorporated into the polypeptide structure of Hb during erythropiosis[12], and Se^{75}-selenite is bound to Hb in the mature RBC probably in the selenotrisulfide form (R-S-Se-S-R). Also, it was noticed that chick hemoglobin possesses peroxidase activity as evidenced by GSH-Px scans

(Figures IV.3 and IV.4). Similar enzymic activity was detected for Hb from both monkey and human species.[15]

The biochemical principle of the assay system used to determine GSH-Px activity[10,11] is based on the oxidation of NADPH. This oxidation is due to the enzyme glutathione reductase [GSSG reductase, E.C. 1.6.4.2] in converting oxidized glutathione to reduced glutathione, which is utilized by the enzyme glutathione peroxidase to convert peroxides to alcohols (Figure IV.16). Hence, the detection of any GSH-Px activity using this assay system, is essentially dependent on the generation of $NADP^+$.

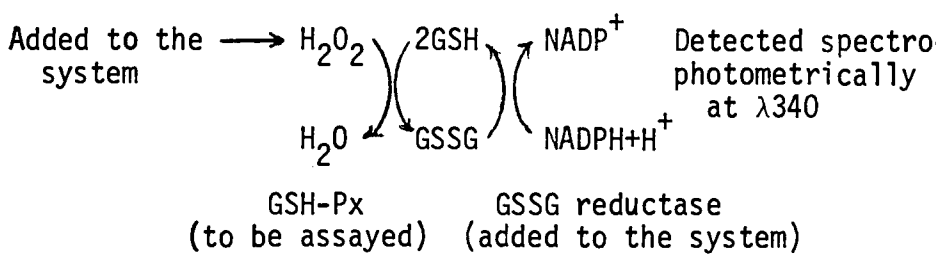


Figure IV.16. Reaction scheme of GSH-Px assay system.

On the other hand, several researchers have reported that Hb can destroy H_2O_2[15,16] according to the following reaction.

$$Fe^{II} + H_2O_2 \longrightarrow Fe^{III} + {}^{-}OH + {}^{\cdot}OH$$

Also, Levander et al.[17] reported that cytochrome C could be reduced by GSH in the presence of selenium as a catalyst.

$$2\ \text{Cyt C}\ (Fe^{III}) + 2\ \text{GSH} \xrightarrow{\text{Se}} 2\ \text{Cyt C}\ (Fe^{II}) + \text{GSSG}$$

These reactions led us to postulate a mechanism to explain hemoglobin peroxidase activity (Figure IV.17).

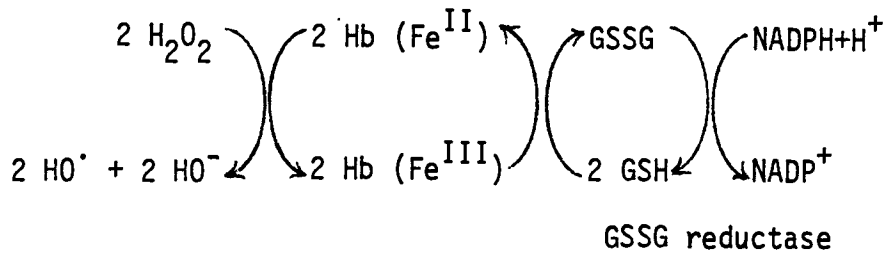


Figure IV.17. Proposed metabolic mechanism of hemoglobin peroxidase activity.

According to Figure IV.17, the destruction of hydrogen peroxide by hemoglobin will generate hydroxyl radicals (·OH) with consequent widespread damage to molecules of biological importance.[18]

Under normal conditions, adequate levels of the enzyme glutathione peroxidase will convert peroxides (potentially toxic substances) to alcohols (harmless substances) as shown in Figure IV.18 and prevent the formation of further damaging species (·OH).

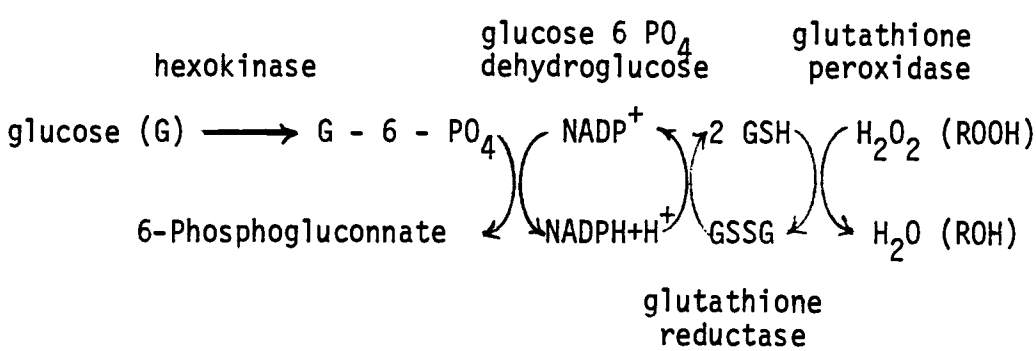


Figure IV.18. Metabolism of peroxides in RBC.

However, under abnormal conditions, i.e., selenium deficiency, defects in pentose phosphate pathway, or lack of any of the enzymes in Figure IV.18, peroxides will react with hemoglobin and generate ·OH causing widespread damage in the cell. Hence the production of hydroxyl radicals (·OH) could explain the increased susceptability of Se-deficient erythrocytes to oxidative damage,[19,20] and could furnish a possible mechanism of RBC self-destruction.

In this study, chick glutathione peroxidase was found to have a molecular weight of 150,000 d. In other animal species, values reported for the molecular weight of GSH-Px vary between 76,000 and 92,000.[21,22,23] According to these workers, the enzyme is composed of four apparently identical 19,000-23,000 dalton subunits, each of which contains selenium in the form of a single selenocyteine residue.[24,25]

The correct estimation of chick hemoglobin molecular weight as 66,000 d[26] and the recent observation that human milk peroxidase eluted at a position which corresponded to a molecular weight of approximately 150,000 on the Ultrogel AC A22 column[27], adds more evidences that the estimated molecular weight of chick GSH-Px enzyme is 150,000.

If this is the case, this difference in molecular weight of chick GSH-Px (150,000) and other species GSH-Px (76,000-92,000) is due to either a) a difference in the molecular weight of subunit structure (if existing) or b) differences in the quaternary structure (number of subunits).

The detection of Se^{75}-protein bound peak in liver and bone marrow cytosols (Figures IV.8 and IV.13) of both selenium treatments corresponding to MWs 21,500-25,000 d and posessing peroxidase activity as evidenced by GSH-Px scans, may suggest that chick glutathione peroxidase enzyme is composed of six subunits.

The incorporation of Se^{75} (from SEM BUT NOT from selenite) in protein(s) corresponding to a molecular weight of 17,000 d (in Figure IV.3) is an interesting observation. It is suggested that these proteins are hemoglobin subunits being synthesized by chick RBC. This is in accordance with results indicating that the mature avian erythrocyte synthesizes hemoglobin.[28]

Results of chromatographing plasma on Sephadex G 200 (Figure IV.5 and IV.6, upper portion) indicated that protein(s) corresponding to molecular weight of 338,000 d may be important in transporting Se^{75} from selenite and SEM 6 hours after dosing. However, proteins possessing an MW of 89,000 are the important Se^{75} carriers 96 hours after intubation. These results indicate that the type of carrier proteins influenced by the form of Se given.

Several groups of workers have conducted experiments in various species to study the mechanism of selenium transport in whole blood, using either _in vivo_ or _in vitro_ experiments. Jenkins _et al._,[29] in a study on intravascular transport of selenium by chick serum proteins, reported that α_2 and α_3 globulins are important in transporting Se from selenite after three hours. However, γ-globulins are important

Se carriers 24 hours after intubation with Se^{75}-selenite. It is very difficult to compare their observations and the present ones because of the difference in experimental techniques and conditions. However, both studies concluded that several plasma proteins are involved in the metabolism of Se^{75} in the chick. Recently, data presented by Motsenbocker and Tappel[30] also show a selenoprotein corresponding to molecular weight 80,000 d in rat and monkey plasma.

The detection of rudiment amounts of Se^{75} (from both treatments) associated with protein having a molecular weight of 150,000 d 96 hours after dosing in plasma might indicate the incorporation of Se^{75} (from selenite and SEM) into newly synthesized GSH-Px enzyme (probably in the liver).

Several investigator groups have carried out studies on the kinetics of selenium disappearance after intravenous injection[31,32] while others have carried out preliminary studies to characterize the selenium binding proteins in rat and sheep.[33,34,11] However, no work has been done to characterize Se-binding proteins in chick tissues.

Here we report a study on the kinetics of Se^{75} (from selenite, or SEM) disappearance and selenium binding proteins in various chick tissues 24 hours after crop intubation. Figure IV.7 indicates that Se (from selenite or SEM) is present in all chick tissues studied in concentrations that vary with the tissue and the chemical form of Se. The liver and kidney were found to contain the highest Se concentrations with varying lower levels in spleen, testes, bone marrow, pancreas, heart, gizzard, and skeletal muscles. Cardiac and gizzard muscles are

consistently higher in Se (from both sources) than skeletal muscle.[35,36]

Most cellular Se^{75} was found in the crude nuclear fraction (Table IV.3) regardless of Se source. This result indicates that disruption of cells by the technique adopted in this study is not efficient, especially in muscular organs (i.e., gizzard and heart).

Results in Table IV.4 indicate that most Se^{75}, 34-80% in various tissues, is associated with the enzyme GSH-Px after 24 hours from dosing, except in pancreas from chicks dosed with SEM. A variety of non glutathione peroxidase selenoproteins were also detected in various tissues. Another finding is the detection of non-selenium glutathione peroxidase in heart and spleen.

High molecular weight (>440,000) selenoproteins were detected in cytosols of all tissues examined except bone marrow. These proteins are believed to play certain roles in storage and transport of Se^{75} into and out of the cell.

The elution pattern of liver cytosols showed a peak corresponding to molecular weight of 44,500 in selenite treatment but not SEM, indicating that this protein(s) is important in selenite metabolism.

A non glutathione peroxidase selenoprotein, having MW of 25,100, was detected in kidney and spleen cytosols from both Se treatments (Table IV.4). This selenoprotein(s) may mediate the biological functions in those tissues.[37]

The low MW (16,700) selenoproteins, devoted of GSH-Px activity, in spleen cytosols from both treatments (Figure IV.10), may result from

breakdown of labeled hemoglobin in spleen.

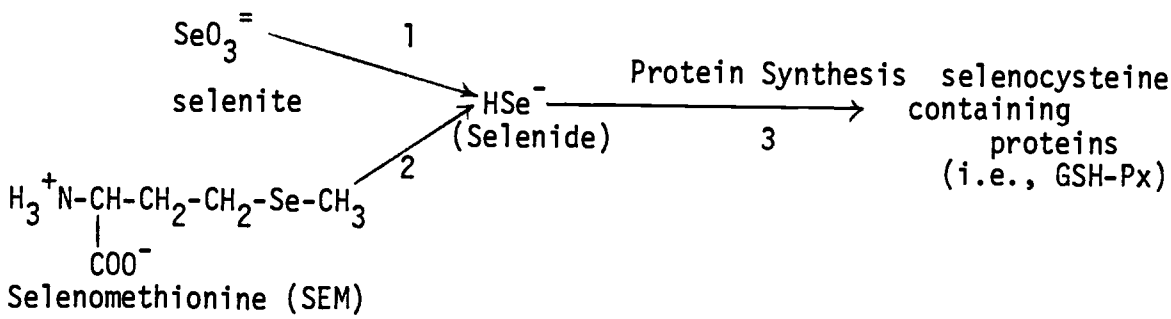
The role of Se (especially SEM) in protecting chicks from pancreatic fibrosis is very well documented.[38,39] In this study a selenoprotein peak corresponding to MW 44,500 was found in cytosols of SEM treatment only (Figure IV.11, upper portion). This non glutathione peroxidase selenoprotein may be important in protecting pancreatic acinar cells from atrophy and fibrosis.

Another function of Se is its requirement for normal spermatogenesis.[40] Several workers reported specific seleno proteins, distinct from GSH-x enzyme, in rat and bull testes.[41,42] Our studies showed two distinct testicular selenoproteins corresponding to MW of 35,500 and 263,000 d (Figure IV.12) in both treatments.

The gizzard muscle was found rich in myoglobin (17,000) which in turn contained Se^{75} and possessed GSH-Px activity (Figure IV.15). The mechanism postulated to explain hemoglobin peroxidase activity (Figure IV.17) is applicable for myoglobin.

In all tissue cytosols studied, Se^{75} non protein bound peak (from selenite and SEM) was observed. This observation indicates the capability of these tissues to incorporate Se from both sources into the enzyme GSH-Px and other seleno proteins according to the tissue needs. The form of Se^{75} non-protein bound in these cytosols remains unknown and whether these tissues are all able to utilize selenite and SEM as is, or require modified forms of these Se sources prior to utilization, is also not known and requires more investigation.

The results described may best be reconciled by the following model (Figure IV.19). In this model, selenite and selenomethionine are metabolized to hydrogen selenide, and the details of reactions 1 and 2 are mentioned in the discussion section of Chapter III. This postulate is supported by the efficient incorporation of selenite and SEM into GSH-Px in this study and others.[45]



Reaction 1: reductive pathway of selenite to selenide.[43]

Reaction 2: selenomethionine may be degraded with release of HSe^-, analogous to the methionine transamination pathway.[44]

Reaction 3: hypothetical tRNA selenocysteine synthesis.

Figure IV.19. Proposed pathway of selenium metabolism.

The selenium moiety of glutathione peroxidase from rat liver and bovine erythrocytes has been identified as selenocysteine and evidence has been obtained for the participation of selenocysteine residue (SeCys) in the catalytic processes.[46,47] By sequencing a partially purified selenopeptide from rat liver glutathione peroxidase, selenocysteine was shown to be within the polypeptide chain.[48] The presence of selenocysteine moiety specifically attached to a protein

posed the question of the mechanism by which this attachment is made.

There are two possible pathways for synthesis of the selenocysteine residue of glutathione peroxidase. 1) Post translational incorporation of selenium to the residue as suggested by Sunde and Hockstra.[49] These authors found that selenide (H_2Se) and selenite (H_2SeO_3) are more readily metabolized than is SeCys to the immediate precursor used for glutathione peroxidase synthesis. 2) Direct incorporation of SeCys during protein synthesis according to Hawkes et al.[50] and Tappel.[51] The isolation of Se^{75}-selenocysteyl tRNA distinct from ^{35}S-cysteyl-tRNAs supports the view that selenocysteyl-tRNA is the immediate precursor of glutathione peroxidase and that selenocysteine is incorporated into the enzyme during protein synthesis.

Although both possibilities are supported by experimental evidence, they do not fully account for existing information. Here we believe that the experimental evidences of both views are correct, and though seemingly contradictory, they are complimentary.

In our mechanism, selenium is incorporated into selenocysteine residue of GSH-Px according to, what we will call "Pre-translational modification".

Here, the precursor of selenocysteine [SeCys(P)] probably alanine or serine, is first attached to its specific tRNA, and while attached to tRNA is modified to selenocysteine by a selenocysteine synthase enzyme. Since codon recognition does not depend on the amino acid that is attached to tRNA, but depends on the tRNA anticodon,[52] the amino

acid (SeCys) will be incorporated in the polypeptide chain though the codon dectates the incorporational SeCys(P). Such a mechanism would account for all the existing information[50,51,52] in the framework of

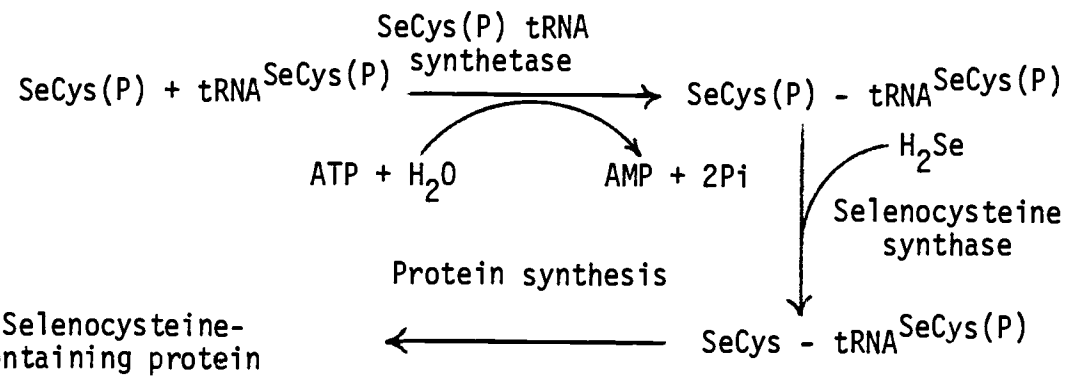


Figure IV.20. Proposed metabolic pathway for synthesis of selenocysteine residue in selenocysteine containing proteins, i.e., GSH-Px

current molecular biology principles. The inescapable question about the postulated mechanism in Figure IV.20 is the specificity of SeCys(P) to be modified to SeCys at the required position only and not anywhere else, especially since we are speculating that the precursor of SeCys is alanine or serine and GSH-Px enzyme monomer contains 11 and 8 residues of alanine and serine, respectively.[53] The answer to this question is the degeneracy of the genetic code (i.e., many amino acids are designated by more than one triplet) and the structure of the tRNA molecule. For example, serine is coded by six codons, namely ACU, ACC, ACA, ACG, AGU, and AGC. These codons are not likely to be recognized by one tRNA and, most probably, will require more than one $tRNA^{Ser}$. According to our mechanism, one of these $tRNA^{Ser}$, as determined by

one of the six codons, is specific for transferring SeCys after being synthesized from serine and H_2Se while being attached to $tRNA^{Ser}$. In turn, the structure of such tRNA will enable the enzyme selenocysteine synthase to recognize it and perform the transformation of the selenocysteine precursor to selenocysteine. Finally, SeCys will be incorporated in its specific position in the polypeptide backbone.

The characterization of selenocysteine lyase, a novel enzyme that specifically decomposes L-selenocysteine into L-alanine and H_2Se in mammalian tissues,[54] adds to our understanding of selenium metabolism. This enzyme was found to contain pyridoxal 5'-phosphate (PLP), a coenzyme with remarkable catalytic versatility, i.e., transamination, decarboxylations, deaminations, racemizations, and aldol cleavage of the α-C atom. In addition, PLP enzymes catalyze elimination and replacement reactions at the β-C-atom (i.e., tryptophan synthetase). This elimination and replacement reaction makes us question the possibility that selenocysteine lyase also catalyzes selenocysteine synthesis, as described earlier (Figure IV.20).

In summary, our studies reveal that further work will be necessary to characterize the non-glutathione peroxidase selenoproteins described in this study. Furthermore, experimental evidence is required to prove the validity of the proposed mechanisms in Figures IV.17 and IV.20.

REFERENCES

1. Schwarz, K. and Foltz, C.M. (1957) Selenium as an integral part of factor 3 against necrotic liver degeneration. J. Am. Chem. Soc. 79:3292-3300.

2. Muth, O.H., Oldfield, J.E., Remmert, L.F. and Schubert, J.R. (1958) Effect of Selenium and vitamin E on while muscle disease. Science 128:1090.

3. Hutcheson, L.M., Hill, D.C. and Jenkins, K.J. (1963) Influence of dietary fat on the effecacy of agents protecting against muscular dystrophy in the chick. Poultry Sci. 42:846-855.

4. Eggert, R.G., Patterson, E., Akers, W.J. and Stokstad, E.L.R. (1957) The role of vitamin E and selenium in the nutrition of the pig. J. Animal Sci. 16:1037.

5. Scott, M.L., Bieri, J.G., Briggs, G.M. and Schwarz, K. (1957) Prevention of exudative diathesis by factor 3 in chick on vitamin E-deficient torula yeast diets. Poultry Sci. 36:1155.

6. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafman, D.G., and Hockstra, W.G. (1973) Selenium; biochemical role as a component of glutathione peroxidase. Science 179:588

7. Thompson, J.N. and Scott, M.L. (1970) Impaired lipid and vitamin E absorption related to atrophy of the pancreas in selenium-deficient chicks. J. Nutr. 100:797.

8. Cantor, A.H., Scott, M.L. and Noguchi, T. (1975) Biological availability of selenium in feed stuffs and selenium compounds for prevention of exudative diathesis in chicks. J. Nutr. 105:96.

9. Cantor, A.H., Langerin, M.L., Noguchi, T. and Scott, M.L. (1975) Efficiency of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. J. Nutr. 105:106.

10. Paglia, D.E. and Valentine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.

11. Black, R.S., Tripp, M.J., Whanger, P.D. and Weswig, P.H. (1978) Selenium proteins in ovine tissues: III. Distribution of selenium and glutathione peroxidase in tissue cytosols. Bioinorg. Chem. 8:161-172.

12. Awwad, H.K., Potchen, E.J., Adelstein, S.J. and Dealy, Jr., J.B. (1966) Se^{75}selenomethionine incorporation into human plasma proteins and erythrocytes. Metabolism 15:626-640.

13. Jenkins, K.J. and Hidiroglou, M. (1972) Comparative metabolism of Se^{75}-selenate and Se^{75}-selenomethionine in bovine erythrocytes. Can J. Phys. Pharm. 50:927-935.

14. McConnel, K.P. (1963) Metabolism of selenium in the mammalian organism. J. Agr. Food Chem. 11:385.

15. Butler, J.A., Whanger, P.D. and Tripp, M.J. (1982) Blood selenium and glutathione peroxidase activity in pregnant women: comparative assays in primates and other animals. Am. J. Clin. Nutr. 36:15-23.

16. Halliwell, B. (1978) Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. Is it a mechanism for hydroxyl radical production in biochemical systems? FEBS lett. 92:321-326.

17. Levander, O.A., Morris, V.C. and Higgs, D.J. (1973) Selenium as a catalyst for the reduction of cytochrome C by glutathione. Biochemistry 12:4591-4595.

18. Wilson, R.L. (1979) In Oxygen Free Radicals and Tissue Damage. Cliba foundation symposium 65 (new series) Excerpta Medica, Amsterdam, Oxford and New York. 19-42.

19. Rotruck, J.T., Pope, A.L., Ganther, H.E. and Hockstra, W.G. (1972) Prevention of oxidative damage to rat erythrocytes by dietary selenium. J. Nutr. 102:689-696.

20. Chow, C.K. and Chen, C.J. (1980) Dietary selenium and age related susceptibility of rat erythrocytes to oxidative damage. J. Nutr. 110:2460-2460.

21. Flohe, L., Gunzler, W.A. and Schock, H.H. (1973) Glutathione peroxidase, a seleno enzyme. FEBS lett. 32:132-134.

22. Sunde, R.A., Ganther, H.E. and Hockstra, W.G. (1978) A comparison of ovine liver and erythrocyte glutathione peroxidase. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37:757.

23. Awasthi, Y.C., Beutler, E., and Srivastava, S.K. (1975) Purification and properties of human erythrocyte glutathione peroxidase. J. Biol. Chem. 250:5144-5149.

24. Forstrom, J.W., Zakowaki, J.J. and Tappel, A.L. (1978) Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry 17:2639-2644.

25. Wendel, A. and Seis, H. (1979) Int. Symp. Selenium and tellurium Chem. 3rd. Melz France (abs.)

26. Huisman, T.H.J., Schillhorn Van Veen, J.M., Dozy, A.M. and Nechtman, C.M. (1964) Studies on animal hemoglobins II. The influence of inorganic phosphate on the physio-chemical and physiological properties of the hemoglobin of the adult chick. Biochem. Biophys. Acta 88:352-366.

27. Moldoreanu, Z., Tenovno, J., Mestecky, J. and Pruitt, K.M. (1982) Human milk peroxidase is derived from milk leukocytes. Biochem. Biophys. Acta 718:103-108.

28. Bell, D.J. and Freeman. B.M. (1971) In Physiology and biochemistry of the domestic fowl. Vol. 2, pp. 858. Academic Press.

29. Jenkins, K.J., Hidiroglou, M. and Ryan, J.F. (1969) Intravascular transport of selenium by chick serum proteins. Can. J. Phys. Pharm. 47:459-467.

30. Motsenbocker, M.A. and Tappel, A.C. (1982) Seleno-cysteine containing protein from rat and monkey plasma. Bioch. Biophys. Acta 704:253-260.

31. Imbach, A. and Sternberg, J. (1967) Metabolic studies with seleniated compounds 1. Kinetic studies with $Se^{75}O_3$ in rats. Int. J. Applied Radiat. Isot. 18:545-556.

32. Sandholm, M. (1973) The initial fate of a trace amount of intravenously administered selenite. Acta Pharmacol. toxicol. 33:1-5.

33. Miller. K.R. (1972) Distribution of Se^{75} in liver, kidney and blood proteins of rats after intravenous injection of sodium selenite. N.Z.J. Agric. Res. 15:547-564.

34. Pedersen, N.D., Whanger, P.D., Weswig, P.H. and Muth, O.H. (1972) Selenium binding proteins in tissues of normal and selenium responsive myopathic lambs. Bioinorg. Chem. 2:37-45.

35. Underwood, E.J. (1977) In Trace elements in human and animal nutrition. 4th Edition. pp 302. Academic Press.

36. Ehligh, C.F., Hogue, D.E., Allaway, W.H. and Hamm, D.J. (1967) Fate of selenium from selenite or selenomethionine with or without vitamin E in lambs. J. Nutr. 92:121.

37. Motsenbocker, M.A. and Tappel, A.L. (1982) Selenium and selenoproteins in the rat kidney. Biochem. Biophys. Acta 709:160-165.

38. Blau, M. and Manske, R.F. (1961) The pancreas specificity of Se^{75}-selenomethionine. J. Nucl. Med. 2:102-105.

39. Bunk, M.J., Cupp, M.S. and Combs, Jr. G.F. (1980) Relationship of selenium-dependent glutathione peroxidase activity to nutritional pancreatic atrophy in the selenium-dependent chick. Fed. Proc. 39:556.

40. Wu, S.H., Oldfield, J.E., Whanger, P.D. and Weswig, P.H. (1973) Effects of selenium, vitamin E, and antioxidants on testicular function in rats. Biol. Reprod. 8:625-629.

41. Prohaska, J.R., Modafy, M. and Ganther, H.E. (1977) Interactions between cadmium, selenium, and glutathione peroxidase in rat testes. Chem. Biol. Interact. 18:253-265.

42. Pollini, V. and Bacci, E. (1979) Rat and bull selenoprotein associated with spermatic mitochondria. J. Submicrosc. Cytol. 11:165-170.

43. Hsieu, M. Steve and Ganther, H.E. (1975) Acid-volatile selenium formation catalyzed by glutathione reductase. Biochemistry 14:1632-1636.

44. Steele, R.D. and Benevenga, N.J. (1979) The metabolism of 3-methyl thiopropionate in rat liver homogenates. J. Biol. Chem. 254:8885-8890.

45. Pierce, S. and Tappel, A.L. (1977) Effects of selenite and selenomethionine on glutathione peroxidase in the rat. J. Nutr. 107:475-479.

46. Forstrom, J.W., Zajowski, J.J. and Tappel, A.L. (1978) Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. 17:2639-2644.

47. Ladenstein, R., Epp, O., Bartels, K., Jones, A., Huber, R. and Albrecht, W. (1979) Structure analysis and molecular model of the selenoenzyme glutathione peroxidase at 2.8 A resolution. J. Mol. Biol. 134:199-218.

36. Ehligh, C.F., Hogue, D.E., Allaway, W.H. and Hamm, D.J. (1967) Fate of selenium from selenite or selenomethionine with or without vitamin E in lambs. J. Nutr. 92:121.

37. Motsenbocker, M.A. and Tappel, A.L. (1982) Selenium and selenoproteins in the rat kidney. Biochem. Biophys. Acta 709:160-165.

38. Blau, M. and Manske, R.F. (1961) The pancreas specificity of Se^{75}-selenomethionine. J. Nucl. Med. 2:102-105.

39. Bunk, M.J., Cupp, M.S. and Combs, Jr. G.F. (1980) Relationship of selenium-dependent glutathione peroxidase activity to nutritional pancreatic atrophy in the selenium-dependent chick. Fed. Proc. 39:556.

40. Wu, S.H., Oldfield, J.E., Whanger, P.D. and Weswig, P.H. (1973) Effects of selenium, vitamin E, and antioxidants on testicular function in rats. Biol. Reprod. 8:625-629.

41. Prohaska, J.R., Modafy, M. and Ganther, H.E. (1977) Interactions between cadmium, selenium, and glutathione peroxidase in rat testes. Chem. Biol. Interact. 18:253-265.

42. Pollini, V. and Bacci, E. (1979) Rat and bull selenoprotein associated with spermatic mitochondria. J. Submicrosc. Cytol. 11:165-170.

43. Hsieu, M. Steve and Ganther, H.E. (1975) Acid-volatile selenium formation catalyzed by glutathione reductase. Biochemistry 14:1632-1636.

44. Steele, R.D. and Benevenga, N.J. (1979) The metabolism of 3-methyl thiopropionate in rat liver homogenates. J. Biol. Chem. 254:8885-8890.

45. Pierce, S. and Tappel, A.L. (1977) Effects of selenite and selenomethionine on glutathione peroxidase in the rat. J. Nutr. 107:475-479.

46. Forstrom, J.W., Zajowski, J.J. and Tappel, A.L. (1978) Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. 17:2639-2644.

47. Ladenstein, R., Epp, O., Bartels, K., Jones, A., Huber, R. and Albrecht, W. (1979) Structure analysis and molecular model of the selenoenzyme glutathione peroxidase at 2.8 A resolution. J. Mol. Biol. 134:199-218.

48. Condell, R.A., and Tappel, A.L. (1982) Amino acid sequence around the active-site selenocysteine of rat liver glutathione peroxidase. Biochem. Biophys. Acta. 709:304-309.

49. Sunde, R.A., and Hockstra, W.G. (1980) Incorporation of selenium from selenite and selenocysteine into glutathione peroxidase in the isolation perfused rat liver. Biochem. Biophys. Res. Commun. 93:1181-1188.

50. Hawkes, W.C., Lyons, D.E., and Tappel, A.L. (1979) Identification and purification of a rat liver selenocysteine-specific transfer RNA. Fed. Proc. 38:820.

51. Tappel, A.L. (1981) In Selenium in Biology and Medicine. Spallholz, J.E., Martin, J.L., and Ganther, H.E., pp. 44. AVI Publishing Company, Inc. Westport, CT.

52. Stryer, L. (1981) In Biochemistry. Second edition. pp. 649. W.H. Freeman and Company.

53. Kerner, B. (1978) The structure of the selenium moiety of bovine red blood cells glutathione peroxidase. Ph.D. thesis, University of Tubingen.

54. Esaki, N., Nakamura, T., Tanaka, H., and Soda, K. (1982) Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. J. Biol. Chem. 257:4386-4391.

CHAPTER V

The Effect of Selenium and Vitamin E Supplementation on Broiler Performance Under Heat Stress

by

M.A. Alayan,[1] P.D. Whanger,[2] and G.H. Arscott[3]

[1]Department of Biochemistry and Biophysics
Oregon State University
Corvallis, Oregon 97331

[2]Department of Agricultural Chemistry
Oregon State University
Corvallis, Oregon 97331

[3]Department of Poultry Science
Oregon State University
Corvallis, Oregon 97331

CONTRIBUTIONS

Mohammad A. Alayan participated in designing the experiment, calibrating the environmental chambers, conducting the animal study, collecting the blood samples, dissecting the chicks to collect the required organs, performing the biochemical analyses, analyzing the data and calculating the results, and writing the paper.

P.D. Whanger participated in the experimental design, directing the study, discussing the results, and editing the written report.

G.H. Arscott helped in the experimental design, setting the animal batteries, optimizing the environmental chambers, and initiating the animal study.

The Effect of Selenium and Vitamin E Supplementation on Broiler Performance Under Heat Stress

ABSTRACT

In this study on the effects of high levels of Se and/or Vitamin E on performance under heat stress, a significant depression in gain and feed consumption was found, regardless of treatment. However, feed efficiency significantly improved in heat stressed chicks fed diets high in selenium and Vitamin E when compared to feed efficiency in animals on other dietary regimes. Significantly lower hematocrit levels were found in heat stressed chicks, as compared to control chicks, which were raised under conventional conditions. Se levels in chick blood, liver, kidney, and pancreas, and plasma Vitamin E levels were directly related to dietary Se or Vitamin E content. Dietary Vitamin E supplementation counteracted the effect of heat stress in depressing blood selenium levels, however, the opposite trend was observed in the other organs studied. The pancreas in heat stressed chicks was larger than chicks raised at conventional temperatures.

THE EFFECT OF SELENIUM AND VITAMIN E SUPPLEMENTATION ON BROILER PERFORMANCE UNDER HEAT STRESS

INTRODUCTION

The effects of elevated temperature upon both chick growth and diet consumption is well recognized. Impaired growth is partly due to diminished feed uptake, but mostly by less efficient feed conversion.[1] A number of dietary manipulations have been conducted in an attempt to ameliorate the effect of heat stress on chick performance.

Fuller and Mora[2] found that reducing the heat increment of the diet by substituting fat for carbohydrate, calorie for calorie, resulted in improving growth in chicks under heat stress. The adjustment of the protein, or even amino acid level, in the diet to compensate for the feed intake reduction due to heat stress proved ineffective in overcoming the reduction in chick performance.[3] Another approach was taken by Krivtsov _et al_.[4] who studied the effect of vitamins, enzymes, zinc bacitracin, and aspirin on the physiological condition and productivity of broilers grown under high temperatures. These authors found that chicks subjected to high temperature had hyperfunctioning adrenals and a decrease in ascorbic acid concentrations. There were signs of lymphopenia, eosinophilia, a decrease in amylolytic and proteolytic activity of gastrointestinal tract enzymes, and an increased NADPH and a fall in the oxidized form of NADPH in blood. Moreover, they found that diet supplementation of

combined enzymes and vitamins resulted in better growth, improved blood pictures, and restored the TCA cycle to normal in chicks under heat stress. However, aspirin was found ineffective against this stress.

In the literature, no evidence exists that the effects of selenium (Se) or vitamin E were studied in ameliorating heat stress, despite the strong indications that these two substances could play certain roles in overcoming the heat stress effects.

It has been found that the erythrocyte count is lower for chicks raised at high environmental temperatures than when raised at recommended heat levels.[5] Meanwhile, oxygen consumption and respiratory rate increases and the hemoglobin level drops in chicks as the environmental temperature rises.[6] These conditions place the red blood cells (RBC) under extremely high stress that can well affect their efficiency as well as their span of life. Se and Vitamin E have been shown to protect erythrocytes against oxidative damage.[7]

The depression in growth resulting from heat stress is mostly the result of less efficient feed conversion from the decrease in amylolytic and proteolytic activity of the digestive enzymes. Since the pancreas is the organ responsible for secreting most of these important digestive enzymes, the integrity of the pancreas would largely be reflected in the activity of digestion and, hence, feed utilization. Selenium's role in protecting the acinar cells against atrophy and fibrosis has been well documented[8] and a high level of

Se in chick diets raised under high temperatures could improve feed conversion.

The purpose of this study was to investigate changes in broiler performance when raised under heat stress upon a diet supplemented with Se and/or Vitamin E.

MATERIALS AND METHODS

A total of 240 one-day-old broiler chicks were divided into two groups to represent each of two environmental conditions, cool (21°C) and hot (33°C). Each chick group was partitioned into four equal treatments, each containing three replicates, which were composed of 5 males and 5 females. The dietary treatments used in this study were:

A) basal diet according to NRC requirements,

B) basal diet supplemented with 2.5 ppm Se in the form of Na_2SeO_3 (25 times the NRC requirement),

C) basal diet supplemented with 1000 I.U./kg Vitamin E as the acetate ester of α-tocophenol (100 times the NRC requirement), and

D) basal diet supplemented with Se (2.5 ppm) and Vitamin E (1000 I.U./kg).

The composition of the basal diet is shown in Table V.1.

The chicks were housed in battery brooders, with raised, wire-floor cages located in thermally controlled rooms. The temperature in the hot (33°C) and cool (21°C) rooms was kept fairly constant using termostat heaters. However, some temporary variation (3°C) occurred due to limitations of the mechanical equipment and climatic fluctuation. Ambient temperature records were kept during the whole testing period using maxima, minima thermometers.

The experimental period lasted for four weeks. Relative humidity was recorded on a daily basis and was 65% and 30% in the cool and hot

Table V.1. Composition of the basal diet.

Ingredients	Composition (%)
Ground yellow corn	60.00
Soybean meal (dehulled)	30.17
Alfalfa (dehydrated, 17%)	2.00
Safflower oil	2.25
Defluoro calcium phosphate	1.90
Limestone (ground)	0.80
Salt (NaCl)	0.30
Vitamin premix and Mineral premix*	0.25
DL-Methionine (98%)	0.05
Premix (of filler)**	2.27
Chemical composition [analyzed (A) or calculated (C)]	
Metabolizable energy (kcal/kg)	3023 (C)
Crude protein (%)	20.00 (A)
Calcium (%)	1.00 (C)
Phosphorous, total (%)	0.71 (C)
Lysine (%)	1.07 (C)
Sulfur amino acids (%)	0.72 (C)
Selenium (ppm)	0.375 (A)
Vitamin E (I.U.)	35.5 (C)

*Vitamin and mineral premixes supplied the following per kg of diet: Vit A, 8872 IU; Vit D, 1100 ICU; Vit E, 25.5 IU; riboflavin, 5.11mg; D-pant. acid, 12.5mg; niacin, 43.5mg; choline, 1375mg; Betain, 70.4mg; Vit B_{12}, 5.5 MCG; Vit K, 0.7mg; pyrodixine, 4.3mg; folacin, 1.52mg; biotin (total) 0.15mg; thiamine, 3.1mg; Mn, 83mg; Fe, 140.8mg; Cu, 414mg; I, 31mg.

Used to carry the supplemented selenium (Na_2SeO_3), Vitamin E, or Se and Vitamin E to form diets B, C, and D, respectively. Filler was added to diet A to adjust the density to equal that of the other diets.

rooms, respectively. Chick body weights and feed consumption data were collected every week and feed efficiency was calculated. Mortality records were kept during the length of the experiment.

At the end of the experiment, three male chicks were randomly selected and weighed. Blood samples (6 mls) from the wing vein were collected in heparinized vacutainer evacuated glass tubes. Then the birds were sacrificed by chloroform suffocation and liver, kidney, and pancreas were dissected and weighed to estimate selenium, organ size, and moisture content. Hematocrit (PCV) was determined using the microhematocrit technique. After an aliquot (1 ml) of whole blood was removed for Se analysis, the samples were centrifuged for five minutes at 1000xg. Plasma was aspirated and the cells were washed twice with physiological saline (0.9% NaCl) and then lysed with glass distilled water. RBC lysates were assayed for glutathione peroxidase (GSH-Px) activity by a coupled enzyme assay[9] with the noted modifications[10] using hydrogen peroxide as the substrate. Selenium concentrations in blood and other tissues were determined by a fluorimetric procedure[11] with the noted changes.[12]

Plasma samples were assayed for Vitamin E content (as α-tocophenol) by high performance liquid chromatography (HPLC) according to DeLeeheer _et al_.[13] with some adjustments. The adjustments include the use of Perkin-Elmer 655-105 Fluorescence Spectrophotometer adjusted to 290 nm and 330 nm excitation and emission wave lengths, respectively, and a stainless-steel column,

250 mm x 4.6 mm (ID) packed with 10 μm Lich vos orb RP18. All the data were subjected to statistical analyses using Duncan multiple range test.[14] Standard error of the mean (σ_{n-1}) was also calculated.

RESULTS

Body weight gains were significantly depressed (16-21%, depending on the treatments) in the hot environment (33°C) if compared to chicks raised at a cool (21°C) temperature until 4 weeks of age. Feed consumption was also significantly depressed (11-25%) by exposing chicks to constant heat stress (Table V.2). Results in Figures V.1 and V.2 indicated that the reduction in body gain and feed intake due to heat stress is critical during the last two weeks (3rd and 4th) of the experiment. Within each temperature regimen, supplementing the diets with Se (B and D) or Vitamin E (C and D) did not improve body gain or feed consumption over the control diet (A), as illustrated in Table V.2. In the cool environment (21°C), feed efficiency was insignificantly improved (Table V.2) by the use of high Vitamin E ration (C and D). In the hot environment, insignificant improvement in feed efficiency was observed as a result of Se (B) or Vitamin E (C) fortification. However, supplementing the diet with both Se and Vitamin E (D) improved feed efficiency significantly in heat stressed chicks. The mortality rate was found to be higher, though not significantly different in the hot environment (Table V.2).

Results in Table V.3 indicated that hematocrit levels in chicks are higher in the cool environment than the hot one. Within each temperature regimen, a higher level of hematocrit was observed in the blood of chicks fed a high Se ration (B) if compared to other treatments (A,C and D).

Table V.2. Performance of chicks raised under cool (21°C) and hot (33°C) environmental conditions until 4 weeks old.

Parameters	Diets			
	A	B	C	D
Body weight gain (g/chick)				
Cool	796±21[a*]	817±6[a]	804±38[a]	802±26[a]
Hot	573±30[b]	543±31[b]	546±63[b]	658±58[b]
Hot/Cool (%)	84.5	78.7	80.3	82.0
Feed consumption (g/chick)				
Cool	1445±16[a]	1576±170[a]	1423±183[a]	1416±140[a]
Hot	1284±58[b]	1181±138[b]	1198±85[b]	1112±68[b]
Hot/Cool (%)	88.9	74.9	84.2	78.5
Feed Efficiency (Feed/Grain)				
Cool	1.81±0.16[ab]	193±0.22[b]	1.76±0.15[ab]	1.76±0.13[ab]
Hot	1.91±0.16[ab]	1.84±0.29[ab]	1.78±0.02[ab]	1.69±0.12[a]
Mortality rate				
Cool	6.7±5.8[a]	3.3±5.8[a]	3.3±5.8[a]	0.00[a]
Hot	10.0±10.0[a]	5.7±11.5[a]	23.0±40.0[a]	6.7±5.8[a]

*Mean $\pm\sigma_{n-1}$ within parameters followed by similar superscripts are not different at $P<0.05$.

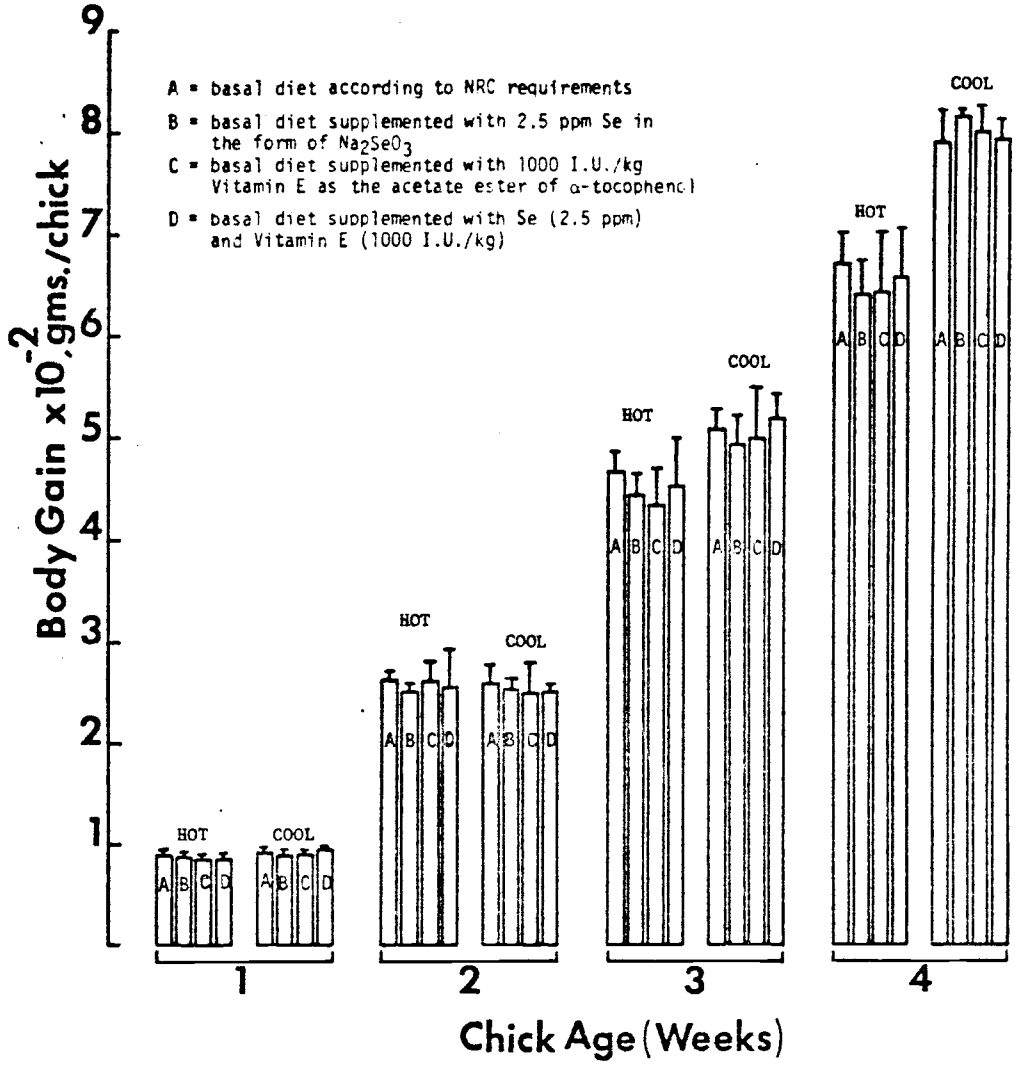


Figure V.1. The effect of dietary treatments (A,B,C, and D) and environmental temperatures (cool versus hot) on body weight gain (gms/chick) at the end of the 1st, 2nd, 3rd, and 4th weeks of age.

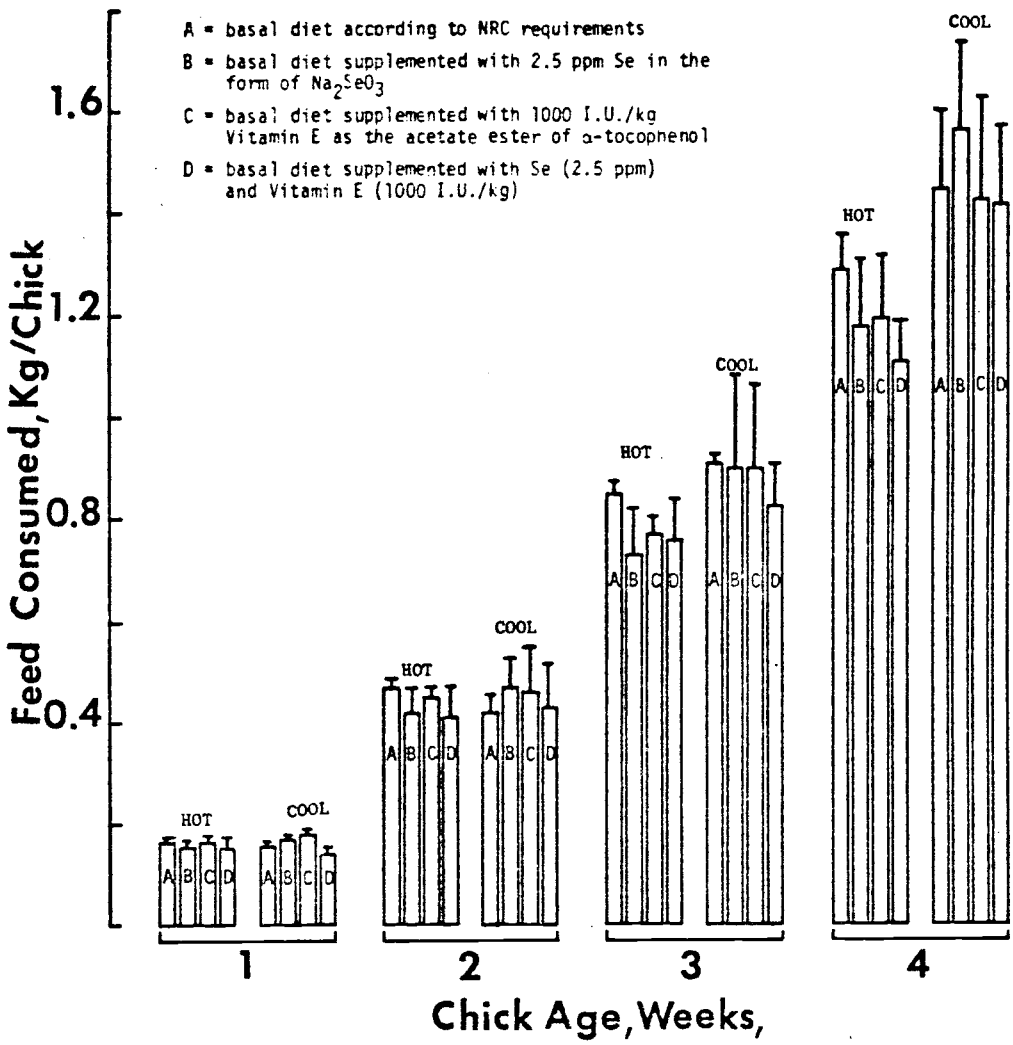


Figure V.2. The effect of dietary treatments (A,B,C and D) and environmental temperature (cool versus hot) on feed consumption (kg/chick) at the end of the 1st, 2nd, 3rd, and 4th weeks of age.

Table V.3. Some parameters in blood of 4-week-old chicks raised under cool (21°C) and hot (33°C) environments and fed diet A, B, C, or D.

Parameter	Diet A	Diet B	Diet C	Diet D
Hematocrit (PCV)				
Cool	33.0±1.7[c*]	36.5±1.5[d]	31.0±1.7[bc]	31.7±1.5[c]
Hot	29.0±1.0[ab]	31.0±2.3[bc]	29.0±2.0[ab]	28.0±1.5[a]
Selenium in whole blood (ppm)				
Cool	0.31±0.03[b]	0.57±0.05[d]	0.34±0.04[b]	0.48±0.01[c]
Hot	0.25±0.03[a]	0.51±0.04[c]	0.33±0.05[b]	0.47±0.08[c]
Erythrocyte GSH-Px activity (EU)**				
Cool	9.38±0.47[a]	13.31±0.08[b]	9.59±1.70[a]	13.89±1.77[b]
Hot	8.87±1.90[a]	15.50±0.65[b]	8.48±1.70[a]	14.03±4.60[b]
Plasma Vitamin E level (μg/ml)				
Cool	16.8±6.2[a]	14.3±3.1[a]	226.9±7.0[b]	226.6±89.1[b]
Hot	16.0±1.0[a]	20.8±7.5[a]	212.0±74[b]	172.0±41.0[b]

* Mean $\pm\sigma_{n-1}$ within parameters followed by different superscripts are significantly different at $P<0.05$.

**Enzyme units (EU) are nmoles NADPH oxidized per min per mg hemoglobin.

The level of Se in chick blood was found directly related to the dietary Se content. Supplementing the basal diet with Na_2SeO_3 (B and C) resulted in a significant increase in blood Se if compared to the unsupplemented diets (A and C) in both environments (Table V.3). The effect of heat stress on blood Se seems to be influenced by dietary Vitamin E supplementation. Heat stress caused a significant depression in blood Se level of chicks fed diets not supplemented with Vitamin E (A and B), but not in chicks consuming high Vitamin E rations (C and D). These results indicate a relationship among heat stress, blood Se content, and dietary Vitamin E. A direct relationship was observed between Se and glutathione peroxidase activity (GSH-Px) in chick blood. Significantly higher levels of GSH-Px activity were noticed in erythrocytes from chicks fed diets fortified with Se (B and D) if compared to unsupplemented diets (A and C) in both environments. Within each treatment, no significant difference was observed in GSH-Px activity of chick RBC due to heat stress.

The level of Vitamin E in plasma is directly affected by the dietary Vitamin E content. Chicks fed diets high in Vitamin E (C and D) accumulated significantly higher levels (approximately 13-fold) of Vitamin E in plasma than chicks raised on unsupplemented diets (A and B). No significant difference appeared in plasma Vitamin E levels of heat-stressed chicks and chicks in the cool environment.

The level of Se in liver, kidney, and pancreas depended on dietary Se and Vitamin E levels and heat stress (Table V.4).

Table V.4. Moisture, selenium and organ size of liver, kidney, and pancreas of chicks raised in hot (33°C) and cool (21°C) environments.

Organ (Parameter)		Diet A	Diet B	Diet C	Diet D
LIVER					
Moisture (%)	Cool	76.0±2.3[a*]	76.8±1.4[a]	77.0±3.6[a]	78.9±2.0[a]
	Hot	75.6±3.0[a]	79.0±1.4[a]	79.0±2.2[a]	76.0±0.7[a]
Selenium (ppm)	Cool	3.04±0.48[a]	5.88±0.79[b]	3.34±0.27[a]	7.27±1.47[c]
	Hot	3.16±0.81[a]	4.92±1.35[b]	3.21±0.69[a]	4.89±0.17[b]
Organ size (%) of body weight	Cool	2.9±0.3[ab]	3.0±0.3[ab]	3.2±0.3[ab]	3.3±0.7[ab]
	Hot	2.8±0.2[ab]	2.5±0.3[a]	3.0±0.3[ab]	3.1±0.6[ab]
KIDNEY					
Moisture (%)	Cool	80.9±1.4[a]	79.4±2.5[a]	80.4±2.0[a]	79.1±1.3[a]
	Hot	79.9±0.6[a]	79.8±0.5[a]	79.9±0.7[a]	79.4±2.3[a]
Selenium (ppm)	Cool	3.92±0.43[a]	5.80±0.87[c]	4.24±0.24[ab]	6.92±1.02[d]
	Hot	3.59±0.48[a]	5.83±0.23[c]	3.62±0.48[a]	4.78±0.45[b]
Organ size (%) of body weight	Cool	0.9±0.2[a]	0.9±0.2[a]	1.1±0.1[ab]	1.2±0.1[b]
	Hot	0.9±0.2[a]	1.0±0.2[ab]	0.9±0.2[a]	1.1±0.2[ab]
PANCREAS					
Moisture (%)	Cool	73.1±1.5[a]	70.8±2.5[a]	72.4±3.9[a]	73.3±2.6[a]
	Hot	74.9±1.1[a]	73.5±2.3[a]	73.1±1.70[a]	72.3±2.1[a]
Selenium (ppm)	Cool	2.02±0.14[ab]	3.03±0.71[c]	2.05±0.11[ab]	4.77±1.32[d]
	Hot	2.10±0.15[ab]	3.26±0.61[c]	1.80±0.90[a]	2.33±0.29[b]
Organ size (%) of body weight	Cool	0.33±0.05[ab]	0.27±0.04[a]	0.33±0.09[ab]	0.28±0.02[ab]
	Hot	0.34±0.05[bc]	0.37±0.04[c]	0.36±0.01[c]	0.36±0.05[c]

*Mean $\pm\sigma_{n-1}$ within parameters followed by different superscripts are different at $P<0.05$.

Significantly higher levels of Se in liver, kidney, and pancreas were observed in chicks fed diets supplemented with Se (B and D) if compared to unsupplemented diets (A and C) in both environments within treatment D (high in Se and Vitamin E) heat stress caused a significant depression in all organs studied. This effect is not observed in chicks fed treatment B (high in Se only) suggesting a profound effect of Vitamin E on Se utilization by heat-stressed chicks.

DISCUSSION

Results in Table V.2 indicated that heat stress impaired growth and one factor contributing to this is diminished feed uptake. These results are in accordance with findings by Squibb et al.[15]. Supplementing the diets with Se or Vitamin E separately (B and C) did not counteract the depression in gain or feed intake due to heat stress. However, feed efficiency in heat stressed chicks improved significantly when fed a ration high in Se and Vitamin E (D). The reason for this improvement by selenium and Vitamin E is not known. A postulation could be the protection of the acinar cells of the pancreas from the deleterious effect of heat stress, and consequently more efficient digestion of dietary nutrients.

A review of the literature regarding the influence of environmental temperature on the hematological pattern of chick blood[5,16,17,18] indicated that broilers reared at low environmental temperatures have significantly higher hematocrits, erythrocyte counts, and Hb levels when compared to chicks maintained at conventional temperatures. The present study tends to confirm these findings (Table V.3).

Results in Table V.3 demonstrated that blood Se levels and erythrocyte GSH-Px activity is directly related to the Se level in the diet. A similar trend is observed for Vitamin E in plasma. These results are in accordance with Noguchi _et al._[19] and Thompson and Scott.[20]

Heat stress caused a significant depression in blood Se level of chicks fed diets without supplemental Vitamin E (A and B) but not in chicks fed diets supplemented with Vitamin E (C and D). This suggests a relationship among heat stress, blood Se content and dietary Vitamin E. It seems that Vitamin E exerts a sparing effect on the Se level in blood, which agrees with other work. On the contrary, within treatment D (high Se and Vitamin E), heat stress caused a significant depression of Se content in all organs studied. These results indicate a complex relationship exists between Se and Vitamin E in heat-stressed chicks.

Results in Table V.4 confirmed findings in the literature that heat stress did not affect the size of liver and kidney. On the other hand, the size of the pancreas (% of body weight) in heat-stressed chicks was noticed to be bigger than that of chicks raised at cool temperatures, suggesting a significant effect of heat stress on this organ.

From this study we conclude that Se and Vitamin E supplementation has a beneficial effect on feed efficiency of heat-stressed chicks, probably in their effect on the pancreas. A successful way to overcome the reduction in broiler performance due to heat stress, will be to integrate all the beneficial effects of dieatry manipulations in one diet.

REFERENCES

1. Kleiber, M. (1934) Influence of environmental temperature on the utilization of food energy in body chicks. J. Gen. Physiol. 17:701.

2. Fuller, H.L., and Mora, G. (1973) Effect of heat increment of the diet on feed intake and growth of chicks under heat stress. In Proc. Maryland Nutr. Conf. pp. 58-64.

3. Waldroup, P.W. (1982) Influence of environmental temperature on protein and amino acid needs of poultry. Fed. Proc. 41:2821-2823.

4. Krivtsov, I., and Kvitkin, P. Y. (1979) Effect of vitamins, Amilo- and Protosubtilina, Zinc- bacitracin and aspirin on the physiological condition and productivity of broilers under high temperatures. Referativnyi Zhurnal 58:66-71.

5. Huston, T.M. (1960) The effects of high environmental temperature upon blood constituents and thyroid activity of domestic fowl. Poultry Sci. 39:1260.

6. Whittow, G.C. (1976) In Avian Physiology. P.D. Sturkie (editor). Springer-Verlag:New York, NY. pp. 152.

7. Rotruck, J.T., Pope, A.L., Ganther, H.E., and Hockstra, W.G. (1972) Prevention of oxidative damage to rat erythrocytes by dietary selenium. J. Nutr. 102:689-696.

8. Cantor, A.H., Langevin, M.L., Noguchi, T., and Scott, M.L. (1975) Efficacy of selenium in selenium compounds and feed stuffs for prevention of pancreatic fibrosis in chicks. J. Nutr. 105:106-111.

9. Paglia, D.E., and Valentine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.

10. Black, R.S., Tripp, M.J., Whanger, P.D., and Weswig, P.H. (1978) Selenium proteins in ovine tissues: III. Distribution of selenium and glutathione peroxidase in tissue cytosols. Bioinorg. Chem. 8:161-172.

11. Olson, O.E. (1969) Fluorometric analysis of selenium in plants. J. Assoc. Anal. Chem. 52:627-634.

12. Whanger, P.D., Weswig, P.H., Schmitz, J.A., and Oldfield, J.E. (1977) Effects of selenium and Vitamin E deficiencies on reproduction, growth, blood components, and tissue lesions in sheep fed purified diets. J. Nutr. 107:1288-1298.

13. DeLeenheer, A.P., DeBevere, V.O., Cruyl, A.A., and Claeys, A.E. (1978) Determination of Serum α-tocophenol (Vitamin E) by high performance liquid chromatography. Clin. Chem. 2414:585-590.

14. Duncan, D.B. (1955) Multiple range and multiple F tests. Biometrics 11:1-42.

15. Squibb, R.L., Guzman, M.A., and Scrimshaw, N.S. (1959) Growth and blood constituents of immature New Hampshire fowl exposed to a constant temperature of 99°F for 7 days. Poultry Sci. 38:220-221.

16. Barrott, H.G., and Pringle, E.M. (1950) Effect of environment on growth and feed and water consumption on chicksns. III. The effect of temperature of environment during the period from 18-32 days of age. J. Nutr. 41:25.

17. Huston, T.M. (1960) The effects of high environmental temperatures upon blood constituents and thyroid activity of domestic fowl. Poultry Sci. 39:1260.

18. Deaton, J.W., Reece, F.N., Nally, E.H., and Tarrer, W.J. (1969) Liver, heart, and adrenal weights of broilers reared under constant temperatures. Poultry Sci. 48:283.

19. Noguchi, T., Cantor, A.H., and Scott, M.L. (1973) Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. J. Nutr. 103:1502-1511.

20. Thompson, J.N. and Scott, M.L. (1970) Impaired lipid and vitamin E absorption related to atrophy of the pancreas in selenium-deficient chicks. J. Nutr. 100:797-809.

BIBLIOGRAPHY

1. Mellor, J.W. (1956) A Comprehensive Treatise on Inorganic and Theoretical Chemistry. John Wiley and Sons, Inc.:New York, NY.

2. Berzelius, J.J. (1817) Annales de chimie et de physique, Paris, Serie 2, Tome 7, 199.

3. Franke, K.W. (1934) A new toxicant occuring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminaty feeding trials. J. Nutr. 8:597-608.

4. Schwarz, K. (1951) A hitherto unrecognized factor against dietary necrotic liver degeneration in American yeast (factor 3). Proc. Soc. Exp. Biol. Med. 78:852-856.

5. Scott, M.L. (1951) Studies on the enlarged hock disorder in turkeys. 3. Evidence of the detrimental effect of fish liver oil and the beneficial effect of dried brewers' yeast and other materials. Poul. Sci. 30:846-855.

6. Schwarz, K. and Foltz, C.M. (1957) Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79:3292-3283.

7. Schwarz, K., Bierl, J.G., Briggs, G.M. and Scott, M.L. (1957) Prevention of exudative diathesis in chicks by factor 3 and selenium. Proc. Soc. Exp. Biol. Med. 45:621-625.

8. Muth, O.H., Oldfield, J.E., Remmert, L.F., and Schubert, J.R. (1958) Effects of selenium and Vitamin E on white muscle disease. Science 128:1090.

9. Proctor, J.E., Hague, D.E., and Warner, R.G. (1958) Selenium, Vitamin E, and linseed oil meal as preventives of muscular dystrophy in lambs. J. Am. Sci. 17:1183-1187.

10. Andrews, E.D., Hartley, W.J., and Grant, A.B. (1968) Selenium-responsive diseases of animals in New Zealand. N.Z. Vel. J. 16:3-17.

11. Nelson, A.A., Fitzhugh, O.G., and Calvery, H.O. (1943) Liver tumors following cirrhosis caused by selenium in rats. Cancer Res. 3:230-236.

12. Tinsley, I.J., Harr, J.R., Weswig, P.H. and Yamamoto, R.S. (1967) Selenium toxicity in rats. I. Growth and longevity. In Symposium: Selenium in Biomedicine. O.H. Muth (editor). AVI Publishing Co.:Westport, CT.

13. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.C., and Hoekstra, W.G. (1973) Selenium, biochemical role as a component of glutathionine peroxidase. Science 179:588-590.

14. Noguchi, T., Langevin, M.L., Combs, G.F.Jr., and Scott, M.L. (1973) Biochemical and histochemical studies of the selenium deficient pancreas in chicks. J. Nutr. 103:44.

15. Noguchi, T. Contor, A.H., and Scott, M.L. (1973) Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. J. Nutr. 103:1502.

16. Frost, D.V. (1972) The two faces of selenium--can selenophobia be cured? CRC Crit. Rev. Toxicol. 1:467-514.

17. Shapiro, J.R. (1972) Selenium and carcinogenesis: a review. In Organic selenium and tellurium chemistry. Ann. N.Y. Acad. Sci. 192:215-219.

18. Greeder, G.A., Poiner, K.A., and Milner, J.A. (1980) Selenium and Cancer. Illinois Research (Winter issue) 8-9.

19. Chen, X., Chen, X., Yang, G., Wen, Z., Chen, J., and Ge, K. (1979) Observations on the effect of sodium selenite in prevention of Keshan disease. Chin. Med. J. 92:471-478.

20. Money, D.F.L. (1970) Vitamin E and selenium deficiencies and their possible actiological role in the sudden death in infants syndrome. N.Z. Med. J. 71:32-34.

21. Noguchi, T., Langerin, M.L., Combs, G.F.Jr., and Scott, M.L. (1973) Biochemical and histochemical studies of the selenium deficient pancreas in chicks. J. Nutr. 103:444-453.

22. Burk, R.F., Lawrence, R.A., and Correia, M.A. (1980) Sex differences in biochemical manifestations of selenium deficiency in rat liver with special reference to heme metabolism. Biochem. Pharmacol. 29:39-42.

23. Burch, R.E., Sullivan, J.F., Jetton, M.M., and Hahn, H.K.J. (1979) The effect of aging on trace element content of various rat tissues. I. Early stages of aging. Age 2:103-107.

24. Spallholz, J.E. (1981) Anti-inflammatory, immunologic, and carcinostatic attributes of selenium in experimental animals. In Diet and Resistance to Disease. American Chemical Society Symposium. Plenum Press. pp. 43-62.

25. Wu, S.H., Oldfield, J.E., Whanger, P.D., Weswig, P.H. (1973) Effects of selenium, Vitamin E, and antioxidants on testicular function in rats. Biol. Reprod. 8:625-629.

26. Whanger, P.D. (1981) Selenium and heavy metal toxicity. In Selenium in Biology and Medicine. Spallhols, J.E., Martin, J.L., and Ganther, H.E. (editors). AVI Publishing Company, Inc.:Westport, CT.

27. Curzon, M.E.J. and Crocker, D.C. (1978) Relationships of trace elements in human tooth enamel to dental cavies. Arch. Oral Biol. 23:647-653.

28. Aaseth, J., Munthe, E., Forre, O, and Steinnes, E. (1978) Trace elements in serum and urine of patients with rheumatoid arthritis. Scand. J. Rheumatol. 7:237-240.

29. Ganther, H.E. (1965) The fate of selenium in animals. World Review of Nutrition and Dietetics. 5:38.

30. Jenkins, K.J. and Hidiroglou (1969) Intravascular transport of selenium by chick serum proteins. Can J. Phys. Pharmacol. 47:459-467.

31. Lee, M., Dong, A., and Yano, J. (1969) Metabolism of Se^{75}-selenite by human whole blood *in vitro*. Can. J. Biochem. 47:791-797.

32. Sandholm, M. (1973) The metabolism of selenite in cow blood *in vitro*. Acta Pharmacol. et Toxicol. 33:6-16.

33. Sandholm, M. (1975) Function of erythrocytes in attaching selenite-Se onto specific plasma proteins. Acta Pharmacol. Toxicol. 36:321-327.

34. McMurray, C.H., and Davidson, W.B. (1979) *In vitro* metabolism of selenite in sheep blood, factors controlling the distribution of selenium and the labelling of plasma proteins. Biochem. Biophys. Acta. 583:332-343.

35. Proter, E.K., Karle, J.A., and Shrift, A. (1979) Uptake of selenium-75 by human lymphocytes *in vitro*. J. Nutr. 109:1901-1908.

36. Plenner, J.A. (1964) Selenomethionine incorporation into plasma proteins. Clin. Res. 12:277.

37. Awwad, H.K., Potchen, E.J., Adelstein, S.J., and Dealy, J.B. Jr. (1966) Se^{75}-selenomethionine incorporation into human plasma proteins and erythrocytes. Metabolism. 15:626-640.

38. Sternberg, J., and Inbach, A. (1967) Metabolic studies with seleniated compounds, II. Turnover studies with Se^{75}-methionine in rats. Int. J. Appl. Radiat. Isot. 18:557-568.

39. Schwarz, K. and Foltz, C.M. (1967) Selenium as an integral part of factor 3 against necrotic liver degeneration. J. Am. Chem. Soc. 79:3293-3300.

40. Eggert, R.G., Patterson, E., Akers, W.J., and Stokstad, E.L.R. (1957) The role of Vitamin E and selenium in the nutrition of the pig. J. An. Sci. 16:1037.

41. Scott, M.L., Bieri, J.G., Briggs, G.M., and Schwarz, K. (1957) Prevention of exudative diathesis by factor 3 in chick on Vitamin E-deficient formula yeast diets. Poultry Sci. 36:1155.

42. Combs, G.F.Jr., and Bunk, M.J. (1981) In Selenium in Biology and Medicine. Spallholz, J.E., Martin, J.L., and Ganther, H.E. (editors). AVI Publishing Company, Inc.:Westport,CT. pp. 70-85.

43. Baumgartner, W.A. (1979) In Trace metals in health and disease. Kharasch, N. (editor). Raven Press:New York. pp. 287-305.

44. Bhuyan, K.C., Bhuyan, D.K., and Podos, S.M. (1981) In Selenium in Biology and Medicine. Spallholz, J.E., Martin, J.L., and Ganther, H.E. (editors). AVI Publishing Company, Inc.:Westport:CT. pp. 403-412.

45. Levander, O.A., Morris, V.C., and Ferretti, R.J. (1977) Comparative effects of selenium and Vitamin E in lead-poisoned rats. J. Nutr. 107:378-382.

46. Thomson, C.D. and Robinson, M.F. (1980) Selenium in human health and disease with emphasis on those aspects peculiar to New Zealand. Am. J. Clin. Nutr. 33:303-323.

47. Adams, R.L., Andrews, F.N., Gardiner, E.E., Fontaine, W.E., and Carrick, C.W. (1962) The effect of environmental temperature on the growth and nutritional requirements of the chick. Poultry Sci. 41:588.

48. McCorrick, Garligh, J.D., and Eders, F.W. (1979) The effect of P and Ca nitrition on the tolerance of chicks to heat stress. Fed. Proc. 38:766.

49. Dale, N.M. and Fuller, H.L. (1979) Effects of diet composition on feed intake and growth of chicks under head stress. I. Dietary fat levels. Poultry Sci. 58:1529-1534.

50. Dale, N.M. and Fuller, H.L. (1980) Effect of diet composition on feed intake and growth of chicks under heat stress. II. Constant vs. cycling temperatures. Poultry Sci. 59:1434-1441.

51. Waldroup, P.W. (1982) Influence of environmental temperature on protein and amino acid needs by poultry. Fed. Proc. 41:2821-2823.

52. Deaton, J.W., Reece, F.N., McNally, E.H., and Torrer, W.J. (1969) Liver, heart, and acrenal weights of broilers reared under constant temperatures. Poultry Sci. 48:283.

53. Bunk, M.J., and Combs, G.F.Jr. (1980) Effect of selenium on appetite in the selenium-deficient chick. J. Nutr. 110:743-749.

54. Imbach, A. and Sternberg, J. (1967) Metabolic studies with seleniated compounds. I. Kinetic studies with $Se^{75}O_3$ in rats. Int. J. Appl. Radiat. Isot. 18:545-556.

55. Burk, R.F. (1973) Effect of dietary selenium level on ^{75}Se binding to rat plasma proteins. Proc. Soc. Exp. Biol. Med. 143:719-722.

56. Sandholm, M. (1973) The initial fate of a trace amount of intravenously administered selenite. Acta Pharmacol. Toxicol. 33:1-5.

57. Sandholm, M. (1974) Selenium carrier proteins in mouse plasma. Acta Pharmacol. Toxicol. 35:424-428.

58. Jenkins, K.J., and Hidiroglou, M. (1972) Comparative metabolism of Se^{75}-selenite, Se^{75}-selenate, and Se^{75}-selenomethionine in bovine erythrocytes. Can. J. Physiol. Pharmacol. 50:927-935.

59. Wright, P.L., and Bell, M.C. (1963) Selenium and vitamin E influence upon the in vitro uptake of Se^{75} by ovine blood cells. Proc. Soc. Exp. Biol. Med. 14:379-382.

60. Paglia, D.E., and Valentine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.

61. Gunzler, W.A., Kremers, H., and Flohe, L. (1974) An improved coupled test procedure for glutathione peroxidase (E.C. 1.11.1.9) in blood. 2 Klin. Chem. Klin Biochem. 12:444-448.

62. Cooper, T.G. (1977) In The Tools of Biochemistry. John Wiley and Sons, Inc.:New York, NY. pp. 169-193.

63. Moore, S., and Stein, W.H. (1963) Chromatographic determination of amino acids by the use of automatic recording equipment. Method. Enzymol. 6:819-820.

64. Diplock, A.T., Caygin, C.P.T., Jeffery, E.H., and Thomas, C. (1973) The nature of the acid volatile selenium in the liver of the male rat. Biochem. J. 134:283-293.

65. Beilstein, M.A., Tripp, M.J., and Whanger, P.D. (1981) Evidence for selenocysteine in ovine tissue organelles. J. Inorg. Biochem. 15:339-347.

66. Duncan, D.B. (1955) Multiple range and multiple F tests. Biometrics. 11:1-42.

67. Edwardly, J.S. (1981) In Effect of Supplemental Selenium on the Performance and Se^{75} Metabolism in Chicks. TEMA-4, Howell, J. McC., Gawthorne, M.J., and White, C.L. (editors). Australian Academy of Science, Canberra. pp. 226-229.

68. White, C.L., and Hockstra, W.G. (1979) The metabolism of selenite and selenomethionine in mouse fibroblasts grown in tissue culture. Biol. Trace. Elem. Res. 1:243-257.

69. Bell, D.J., and Freeman, B.M. (1971) In Physiology and Biochemistry of the Domestic Fowl, Vol. 2. Academic Press:New York, NY. pp. 858.

70. Butler, J.A., Whanger, P.D., and Tripp, M.J. (1982) Blood selenium and glutathione peroxidase activity in pregnant women: comparative assays in primates and other animals. Am. J. Clin. Nutr. 36:15-23.

71. Ganther, H.E. (1968) Selenotrisulfides formation by the reaction of thiols with selenious acid. Biochemistry 8:2898-2905.

72. Ganther, H.E. (1971) Reduction of the selenotrisulfide derivative of glutathione to a persulfide analog by glutathione reductase. Biochemistry 10:4089-4098.

73. Ganther, H.E., and Corcoran, C. (1969) Selenotrisulfides. II. Cross-linking of reduced pancreatic ribonuclease with selenium. Biochemistry 8:2557.

74. Gasiewicz, T.A., and Smith, J.C. (1977) Similar properties of cadmium and selenium complex formed in rat plasma _in vivo_ and _in vitro_. Fed. Proc. 36:1152.

75. Steele, R.D., and Benevenga, N.J. (1979) The metabolism of 3-methyl thio propionate in rat liver homogenates. J. Biol. Chem. 254:8885-8890.

76. Esaki, N., Nakamura, T., Tanaka, H., Suzuki, T., Morino, Y., and Soda, K. (1981) Enzymatic synthesis of selenocysteine in rat liver. Biochemistry 20:4492-4496.

77. Esaki, N., Nakamura, T., Tanaka, H., and Soda, K. (1982) Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. J. Biol. Chem. 257:4386-4391.

78. Hutcheson, L.M., Hill, D.C. and Jenkins, K.J. (1963) Influence of dietary fat on the effecacy of agents protecting against muscular dystrophy in the chick. Poultry Sci. 42:846-855.

79. Thompson, J.N. and Scott, M.L. (1970) Impaired lipid and vitamin E absorption related to atrophy of the pancreas in selenium-deficient chicks. J. Nutr. 100:797.

80. Cantor, A.H., Scott, M.L. and Noguchi, T. (1975) Biological availability of selenium in feed stuffs and selenium compounds for prevention of exudative diathesis in chicks. J. Nutr. 105:96.

81. Cantor, A.H., Langerin, M.L., Noguchi, T. and Scott, M.L. (1975) Efficiency of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. J. Nutr. 105:106.

82. Black, R.S., Tripp, M.J., Whanger, P.D. and Weswig, P.H. (1978) Selenium proteins in ovine tissues: III. Distribution of selenium and glutathione peroxidase in tissue cytosols. Bioinorg. Chem. 8:161-172.

83. Jenkins, K.J. and Hidiroglou, M. (1972) Comparative metabolism of Se^{75}-selenate and Se^{75}-selenomethionine in bovine erythrocytes. Can J. Phys. Pharm. 50:927-935.

84. McConnel, K.P. (1963) Metabolism of selenium in the mammalian organism. J. Agr. Food Chem. 11:385.

85. Halliwell, B. (1978) Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. Is it a mechanism for hydroxyl radical production in biochemical systems? FEBS lett. 92:321-326.

86. Levander, O.A., Morris, V.C. and Higgs, D.J. (1973) Selenium as a catalyst for the reduction of cytochrome C by glutathione. Biochemistry 12:4591-4595.

87. Wilson, R.L. (1979) In Oxygen Free Radicals and Tissue Damage. Cliba foundation symposium 65 (new series) Excerpta Medica, Amsterdam, Oxford and New York. 19-42.

88. Rotruck, J.T., Pope, A.L., Ganther, H.E. and Hockstra, W.G. (1972) Prevention of oxidative damage to rat erythrocytes by dietary selenium. J. Nutr. 102:689-696.

89. Chow, C.K. and Chen, C.J. (1980) Dietary selenium and age related susceptibility of rat erythrocytes to oxidative damage. J. Nutr. 110:2460-2460.

90. Flohe, L., Gunzler, W.A. and Schock, H.H. (1973) Glutathione peroxidase, a seleno enzyme. FEBS lett. 32:132-134.

91. Sunde, R.A., Ganther, H.E. and Hockstra, W.G. (1978) A comparison of ovine liver and erythrocyte glutathione peroxidase. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37:757.

92. Awasthi, Y.C., Beutler, E., and Srivastava, S.K. (1975) Purification and properties of human erythrocyte glutathione peroxidase. J. Biol. Chem. 250:5144-5149.

93. Forstrom, J.W., Zakowaki, J.J. and Tappel, A.L. (1978) Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry 17:2639-2644.

94. Wendel, A. and Seis, H. (1979) Int. Symp. Selenium and tellurium Chem. 3rd. Melz France (abs.)

95. Huisman, T.H.J., Schillhorn Van Veen, J.M., Dozy, A.M. and Nechtman, C.M. (1964) Studies on animal hemoglobins II. The influence of inorganic phosphate on the physio-chemical and physiological properties of the hemoglobin of the adult chick. Biochem. Biophys. Acta 88:352-366.

96. Moldoreanu, Z., Tenovno, J., Mestecky, J. and Pruitt, K.M. (1982) Human milk peroxidase is derived from milk leukocytes. Biochem. Biophys. Acta 718:103-108.

97. Motsenbocker, M.A. and Tappel, A.C. (1982) Seleno-cysteine containing protein from rat and monkey plasma. Bioch. Biophys. Acta 704:253-260.

98. Miller. K.R. (1972) Distribution of Se^{75} in liver, kidney and blood proteins of rats after intravenous injection of sodium selenite. N.Z.J. Agric. Res. 15:547-564.

99. Pedersen, N.D., Whanger, P.D., Weswig, P.H. and Muth, O.H. (1972) Selenium binding proteins in tissues of normal and selenium responsive myopathic lambs. Bioinorg. Chem. 2:37-45.

100. Underwood, E.J. (1977) In Trace elements in human and animal nutrition. 4th Edition. pp 302. Academic Press.

101. Ehligh, C.F., Hogue, D.E., Allaway, W.H. and Hamm, D.J. (1967) Fate of selenium from selenite or selenomethionine with or without vitamin E in lambs. J. Nutr. 92:121.

102. Motsenbocker, M.A. and Tappel, A.L. (1982) Selenium and selenoproteins in the rat kidney. Biochem. Biophys. Acta 709:160-165.

103. Blau, M. and Manske, R.F. (1961) The pancreas specificity of Se^{75}-selenomethionine. J. Nucl. Med. 2:102-105.

104. Bunk, M.J., Cupp, M.S. and Combs, Jr. G.F. (1980) Relationship of selenium-dependent glutathione peroxidase activity to nutritional pancreatic atrophy in the selenium-dependent chick. Fed. Proc. 39:556.

105. Prohaska, J.R., Modafy, M. and Ganther, H.E. (1977) Interactions between cadmium, selenium, and glutathione peroxidase in rat testes. Chem. Biol. Interact. 18:253-265.

106. Pollini, V. and Bacci, E. (1979) Rat and bull selenoprotein associated with spermatic mitochondria. J. Submicrosc. Cytol. 11:165-170.

107. Hsieu, M. Steve and Ganther, H.E. (1975) Acid-volatile selenium formation catalyzed by glutathione reductase. Biochemistry 14:1632-1636.

108. Steele, R.D. and Benevenga, N.J. (1979) The metabolism of 3-methyl thiopropionate in rat liver homogenates. J. Biol. Chem. 254:8885-8890.

109. Pierce, S. and Tappel, A.L. (1977) Effects of selenite and selenomethionine on glutathione peroxidase in the rat. J. Nutr. 107:475-479.

110. Forstrom, J.W., Zajowski, J.J. and Tappel, A.L. (1978) Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. 17:2639-2644.

111. Ladenstein, R., Epp, O., Bartels, K., Jones, A., Huber, R. and Albrecht, W. (1979) Structure analysis and molecular model of the selenoenzyme glutathione peroxidase at 2.8 A resolution. J. Mol. Biol. 134:199-218.

112. Kleiber, M. (1934) Influence of environmental temperature on the utilization of food energy in body chicks. J. Gen. Physiol. 17:701.

113. Fuller, H.L., and Mora, G. (1973) Effect of heat increment of the diet on feed intake and growth of chicks under heat stress. In Proc. Maryland Nutr. Conf. pp. 58-64.

114. Waldroup, P.W. (1982) Influence of environmental temperature on protein and amino acid needs of poultry. Fed. Proc. 41:2821-2823.

115. Krivtsov, I., and Kvitkin, P.Y. (1979) Effect of vitamins, Amilo- and Protosubtilina, Zinc- bacitracin and aspirin on the physiological condition and productivity of broilers under high temperatures. Referativnyi Zhurnal 58:66-71.

116. Huston, T.M. (1960) The effects of high environmental temperature upon blood constituents and thyroid activity of domestic fowl. Poultry Sci. 39:1260.

117. Whittow, G.C. (1976) In Avian Physiology. P.D. Sturkie (editor). Springer-Verlag:New York, NY. pp. 152.

118. Olson, O.E. (1969) Fluorometric analysis of selenium in plants. J. Assoc. Anal. Chem. 52:627-634.

119. Whanger, P.D., Weswig, P.H., Schmitz, J.A., and Oldfield, J.E. (1977) Effects of selenium and Vitamin E deficiencies on reproduction, growth, blood components, and tissue lesions in sheep fed purified diets. J. Nutr. 107:1288-1298.

120. DeLeenheer, A.P., DeBevere, V.O., Cruyl, A.A., and Claeys, A.E. (1978) Determination of Serum α-tocophenol (Vitamin E) by high performance liquid chromatography. Clin. Chem. 2414:585-590.

121. Squibb, R.L., Guzman, M.A., and Scrimshaw, N.S. (1959) Growth and blood constituents of immature New Hampshire fowl exposed to a constant temperature of 99°F for 7 days. Poultry Sci. 38:220-221.

122. Barrott, H.G., and Pringle, E.M. (1950) Effect of environment on growth and feed and water consumption on chicksns. III. The effect of temperature of environment during the period from 18-32 days of age. J. Nutr. 41:25.

123. Deaton, J.W., Reece, F.N., Nally, E.H., and Tarrer, W.J. (1969) Liver, heart, and adrenal weights of broilers reared under constant temperatures. Poultry Sci. 48:283.

124. Thompson, J.N. and Scott, M.L. (1970) Impaired lipid and vitamin E absorption related to atrophy of the pancreas in selenium-deficient chicks. J. Nutr. 100:797-809.