

November 22, 1967

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I am submitting herewith a thesis written by Gene Withrow entitled "Metabolism of Selenium-75 in the Erythrocyte of Growing Sheep and Swine." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

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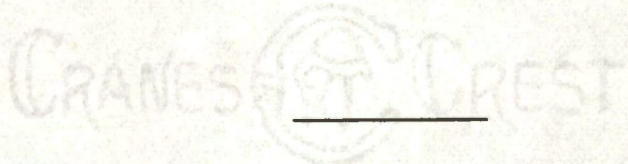
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METABOLISM OF SELENIUM-75 IN THE ERYTHROCYTE
OF GROWING SHEEP AND SWINE



A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Gene Withrow
December 1967

ACKNOWLEDGMENT

I wish to express my deepest gratitude to Dr. M. C. Bell, Professor, UT-AEC Agricultural Research Laboratory, for his stimulating ideas and suggestions in initiating and conducting this investigation, for his willingness to offer help for any problem, and most of all for his constant effort to expose me to the meaning of scientific research.

Sincere appreciation is expressed to Dr. T. P. McDonald, Research Instructor, UT Memorial Research Center, for his special effort of guidance and assistance in establishing proper laboratory procedures for this research problem. Thanks are offered to Dr. W. R. Farkas, Research Assistant Professor, UT Memorial Research Center, and to Dr. R. A. Popp, Biology Division, Oak Ridge National Laboratory, for their contributions to laboratory techniques. Recognition is also given to Dr. C. J. Wust, Biology Division, Oak Ridge National Laboratory, for his suggestions during the initiation of this study. I would also like to thank committee members, Drs. A. P. Levin and C. C. Chamberlain, for their advice and comments pertaining to this thesis.

The author expresses his gratitude to Dr. N. S. Hall, Laboratory Director, for his providing the facilities and materials necessary to conduct this research problem at the UT-AEC Agricultural Research Laboratory. Special thanks are granted to Mr. Nick Sneed for his valuable technical advice; to Mrs. Helen Scott for her patience, devotion, and uplifting humor throughout this investigation; and to Mr. Ernest

Vinsant and his animal caretaking crew for their help and cooperation.

Most of all, I would like to express heartfelt thanks to my wife, Ruth, for her understanding, encouragement, and unselfishness during the preparation of this thesis.



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CHAPTER I

INTRODUCTION

Selenium, recognized as both a toxic and an essential dietary element to the mammalian organism, has been involved in many studies of mammalian metabolism designed to evaluate its role in biological systems. Early research with selenium was very difficult due to the complex analytical techniques necessary for the detection of elemental selenium in submicroamounts. As radioactive tracer techniques were perfected, biological research with selenium became much more simple.

One very important use of a selenium isotope (^{75}Se) tracer technique is the study of the incorporation of selenium into tissue proteins. Selenium-75 has been shown to be incorporated into hemoproteins, cytochrome-c, myoglobin, muscle enzymes (myosin and aldolase), and nucleoproteins (McConnell, 1963). Wright (1965) has shown that ^{75}Se was incorporated into the hemoglobin of the newly-formed sheep erythrocyte where it remains throughout the life of the cell. This incorporation allows for an accurate determination of erythrocyte life span in sheep. The need for an accurate determination of erythrocyte life span in nutritional and physiological studies has been discussed by Judd and Matrone (1962).

Even though much research has been conducted on selenium metabolism in tissue proteins, a very important question still remains as to how selenium is bound to these proteins.

The objectives of these studies were to reinvestigate the value of selenium-75 in estimating the life span of sheep erythrocytes; to establish its value in estimating the life span of swine erythrocytes; to identify the sites of incorporation and the type of binding of ^{75}Se in the erythrocyte of both species; and to compare the differences in selenium utilization in the erythrocyte between these species.

In order to accomplish these goals, it was necessary to determine: (1) selenium-75 uptake of whole blood, plasma, erythrocyte, and erythrocyte wash; (2) incorporation of selenium-75 into erythrocyte hemoglobin and stroma; (3) selenium-75 uptake of liver, spleen, and bone marrow; (4) erythrocyte destruction processes in each species; and (5) presence of ^{75}Se -containing compounds in the globin protein.

CHAPTER II

LITERATURE REVIEW

All of the available information on selenium will not be covered in this chapter due to the vastness of the subject. Only a brief survey of background information and a more complete review of material pertinent to the understanding of this thesis problem will be covered. An expert and comprehensive review of all aspects of selenium has been presented by Rosenfeld and Beath (1964) which was the primary source of information for this review up to 1964.

I. GENERAL INFORMATION

Selenium toxicity to animals. Two livestock diseases known as "alkali disease" and "blind staggers" have plagued livestock producers throughout parts of the western United States for many years. "Alkali disease," once thought to be due to too much alkali salt intake, was later found to be caused by organic selenium compounds which are bound to the proteins of the plant material ingested by the animal. "Blind staggers," named for its symptomology, is actually due to animal intake of plants containing organic selenium compounds that are easily extracted from the plant material by water. These diseases took a heavy toll of livestock throughout fifteen of the western states for many years. Most of this poisoning was attributed to forages of some type (Rosenfeld and Beath, 1964).

No reported connection had been made between the trace element selenium and "alkali disease" and "blind staggers" until 1931 when H. G. Knight suggested the possibility that these toxicity diseases could be caused by ingestion of plants that were absorbing selenium from the soils in affected areas (Ackerson, 1965). In 1933, Robinson published a report showing that non-toxic wheat contained no measurable selenium while toxic wheat yielded as much as 12 p.p.m. Thus one of the first causal connections between livestock poisoning and selenium toxicity to animals was established. Selenium toxicity diseases in livestock are now controlled by several measures such as: (1) recognition and isolation of seleniferous plants; (2) mapping of seleniferous areas; (3) land and crop management on seleniferous soils, i.e., grow plants that are high in selenium uptake on low selenium-containing soil; (4) grazing control (do not overgraze); (5) use of herbicides to destroy undesirable seleniferous plants; and (6) controlled irrigation. In addition, several chemical compounds have shown protective properties against selenium poisoning. Arsenic has exhibited some unexplained protection against selenium poisoning but arsenic administration to animals must be carefully controlled since it can also cause a toxic response. Other compounds which have shown modifying effects on selenosis are: sulfur (sulfides, sulfates), bromobenzene, 2,3-dimercaptopropanol, and dietary proteins (Rosenfeld and Beath, 1964).

Selenium in animal nutrition. Selenium remained classified specifically as an element toxic to animals until 1957 when Schwarz and

Foltz reported that a dietary agent from natural sources was effective in preventing liver necrosis in rats. This dietary agent was coined "Factor 3" since it was the third known compound to prevent rat liver necrosis. Cystine and vitamin E had also been shown to be effective preventives of necrotic liver in rats. Selenium was isolated from "Factor 3" and was shown to be effective in preventing a necrotic liver response in rats on a selenium deficient diet (Schwarz and Foltz, 1957). Cystine's biopotency in preventing liver necrosis in rats may have been partially due to a slight selenium contamination (1-2 $\mu\text{g.}$) of the amino acid used for experimentation (Schwarz, Stesney, and Foltz, 1959).

The studies with liver necrosis in rats, plus many others, have revealed a remarkable synergistic effect between selenium and vitamin E. Animal diseases such as white muscle disease, stiff lamb disease, and nutritional myopathy in calves and lambs were once thought to be due to a dietary deficiency of vitamin E. However, selenium has shown some curative actions on these animal diseases when the animals were on vitamin E deficient diets (Schwarz, 1961). Also, Schwarz et al. (1957) and Patterson, Milstrey, and Stokstad (1957) reported that selenium along with vitamin E protects against the fatal exudative diathesis in chicks. Even though there is a high correlation between the physiological responses to vitamin E and selenium, there are several vitamin E deficiency symptoms which do not respond to selenium administration even in large excess. For example, muscular dystrophy in rabbits (Draper, 1957), resorption of the embryo in pregnant rats (Harris, Ludwig, and Schwarz, 1958), and encephalomalacia in chicks (Dam et al., 1957) gave no

measurable response to selenium administered to the animals. These experimental results strongly suggest that selenium and vitamin E may be two separate nutritional entities; just what their exact chemical and physiological relationship is remains unknown. Recent developments seem to indicate that the interaction between selenium and vitamin E points to a common target effect on the respiratory chain in the mitochondria (Imbach and Sternberg, 1967). This hypothesis is supported by the fact that selenium is incorporated into cytochrome-c in the rat (McConnell and Dallam, 1962) and is possibly involved in the in vivo synthesis of ubiquinone (Green et al., 1961). In vitamin E deficiency, peroxidation due to the reaction of lipid free-radical intermediates with proteins, lipids, and other compounds causes particular damage to the mitochondria and microsomes of the cells (McConnell, 1963). Olcott, Brown, and Van der Veen (1961) suggest that selenomethionine functions as an antioxidant in anhydrous fats and oils by decomposing added peroxides. Selenium has also exhibited antioxidant properties in aqueous lipid emulsions, rubber, and lubricating oils (Olcott, Brown, and Van der Veen, 1961). These findings along with the other tocopherol (vitamin E)-like physiological properties give reason to suspect that selenium and vitamin E may be involved as antioxidants in the mitochondria.

Selenium and man. Selenium research in humans has been very limited. However, it is becoming more evident that selenium may also play a role in human nutrition. Schwarz (1961) reported that a daily supplement of 25 μ g. of selenium as γ,γ' -diselenovaleric acid to

children suffering from a "protein-malnutrition disease" called kwashiorkor, gave an immediate response in weight gain and increase in food intake. No reports have appeared to validate this work, but Burk et al. (1967) have shown an increased in vitro uptake of selenium (selenite) in the erythrocytes of children suffering from kwashiorkor. An increased in vitro uptake of radioselenite by the red cells of "protein-deficient" children suggests that children with kwashiorkor may have reduced stores of selenium (Burk et al., 1967). Perhaps the increased in vitro uptake of selenium in human erythrocytes will prove useful as a clinical diagnosis for kwashiorkor.

The known dietary requirement for this element by a variety of species would imply that it is indeed a required nutrient for man. (Burk et al., 1967.)

Selenium poisoning has also been suggested in man by certain disease conditions of individuals from highly seleniferous regions (Rosenfeld and Beath, 1964). Specific cases of dermatitis were thought to be due to selenium in the diet of the affected individuals (Rosenfeld and Beath, 1964). Small amounts of selenium intake may also aggravate other conditions such as arthritis, degenerative liver, bad teeth, skin discoloration, and gastrointestinal disorders (Anderson et al., 1961). An unusually high number of dental caries have been reported in areas with high amounts of selenium in the soil (Rosenfeld and Beath, 1964). Studies with common foodstuffs in the diets of families in seleniferous areas indicated that eggs, meat, and milk contained considerable amounts of selenium which could be a possible source of selenium poisoning to man (Smith and Westfall, 1937). A selenium concentration of 5 p.p.m. in

common foods and one-tenth this amount in milk or water could be potentially dangerous for human consumption (Rosenfeld and Beath, 1964).

The possibility of a new role for selenium in man has been reported by Siren (1964) who suggests that selenium may be involved in the excitation mechanisms of the photoreceptor of the human eye. It is interesting to note that Siren (1964) found the highest values for selenium concentration in the retina of the tern (aquatic bird) and roedeer (small deer) which are known to have very good visual acuity, and the lowest selenium concentration values in the guinea pig which exhibits very poor sight characteristics. There appears to be a direct correlation between the acuity of sight and the amount of selenium in the retina of the eye.

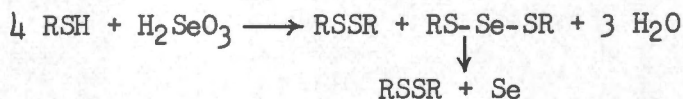
Industrial uses of selenium. Biological research is sometimes stimulated from other areas of interest and vice versa. The possibility that selenium may be involved in the excitation mechanism of the photoreceptor of the eye may have been revealed in part by the use of selenium in manufacturing photoelectric equipment. The electrons of selenium are susceptible to excitation by light; therefore, selenium is used extensively in the manufacture of automatic light switches, which are "turned on and off" by the amount of light available, and in the manufacture of the photographic exposure meter which consists of a selenium cell and a galvanometer which measures the electric current generated from the selenium cell.

The toxic properties of excess selenium have been utilized in the production of various insecticides and pesticides which have proven

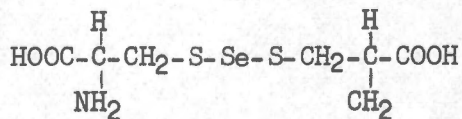
effective because insects have not developed a tolerance to the toxicity of excessive selenium (Rosenfeld and Beath, 1964).

II. CHEMISTRY OF SELENIUM

Reaction of selenites with sulfhydryl compounds. Selenite readily oxidizes sulfhydryl compounds forming disulfide and unstable RS-Se-SR compounds, as shown below:



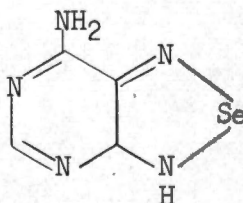
The separation of the selenocompound from disulfide is difficult due to the instability of RS-Se-SR compounds (Painter, 1941). Aqueous solutions of sodium selenite (0.0025 M.) and cysteine hydrochloride (0.01 M.) react to form selenotetracystine $\text{Se}(\text{C}_3\text{H}_6\text{Na}_2\text{S})_4$. Additional studies involving the reaction between selenious acid and cysteine have indicated that a mixture of at least two components was formed. Selenite oxidized two molecules of cysteine to cystine and probably bound two molecules of cysteine to form selenodicysteine (Rosenfeld and Beath, 1964).



The reaction of selenious acid with glutathione (GSH) was reported to be in a mole ratio of 1:4 with the formation of a mixture of oxidized glutathione (GSSG), selenodiglutathione (GS-Se-SG), and water. Selenite appears to be the most active catalyst for the oxidation of glutathione as indicated by the catalytic oxidation of glutathione, cysteine,

dihydrolipoic acid, and coenzyme A by selenite (Rosenfeld and Beath, 1964).

Organoselenium compounds. Dimethyl selenide $[\text{Se}(\text{CH}_3)_2]$, a volatile liquid, is thought to be a metabolic product of inorganic selenium in many biological systems (Rosenfeld and Beath, 1964) which is probably excreted from the animal body via the respiratory system (McConnell and Roth, 1966b). Seleno-amino acids, particularly selenocysteine, selenocystine, and selenomethionine, and their incorporation into various proteins of animal and plant tissue, have been studied extensively. In general, the seleno-amino acids have the same degree of instability as their sulfur analogues. Selenocystine is less stable than selenomethionine, particularly during acid hydrolysis of protein (Rosenfeld, 1961). Several organic carcinostatic and bacteriostatic compounds have been prepared with selenium. Examples are: (1) 6-selenopurine; (2) 2,4-diselenouracil; (3) 2-selenothymine; (4) selenoguanine; (5) selenocytosine; and (6) diselenothymine. These have been produced by attachment of selenium to a cyclic ring structure in some fashion. However, 6-amino-8-selenopurine:



has been produced with selenium placed within the purine nucleus (Rosenfeld and Beath, 1964). These compounds may prove useful as possible purine metabolites for cancer therapy (Rosenfeld and Beath,

1964) and in the study of selenium incorporated into nucleic acids of the biological system.

III. BIOCHEMISTRY OF SELENIUM

Selenium cycle. Shrift (1964) has suggested that a cycle exists in nature for selenium similar to described cycles for sulfur, carbon, and nitrogen. This cycle is shown schematically in Figure 1.

Organoselenium and sulfur analogue antagonism. Analysis of the cells of Chlorella vulgaris by Shrift (1954) indicated that the sulfur metabolite, L-methionine, prevented the absorption of its selenium analogue, selenomethionine. The amount of selenium that entered the cell depended on the external S:Se ratio, not the amount of selenium supplied to the organism. Sulfate did not antagonize the action of selenomethionine. On the other hand, McConnell and Cho (1965) reported that in the transmucosal movement of selenium compounds across a concentration gradient in the intestine of the golden hamster, only L-selenomethionine transport was inhibited by its sulfur analogue. Sulfate administration to rats caused an increased excretion and decreased tissue retention of selenate (Ganther and Baumann, 1962). Also, sulfates have been shown to alleviate 20 to 40 per cent of the growth depression in rats on high selenium containing diets (Halverson and Monty, 1960). The sulfur-selenium antagonism seems to be a competition for a site or carrier at the cell membrane, prior to transport, and the excess of one will inhibit the other (McConnell and Roth, 1965).

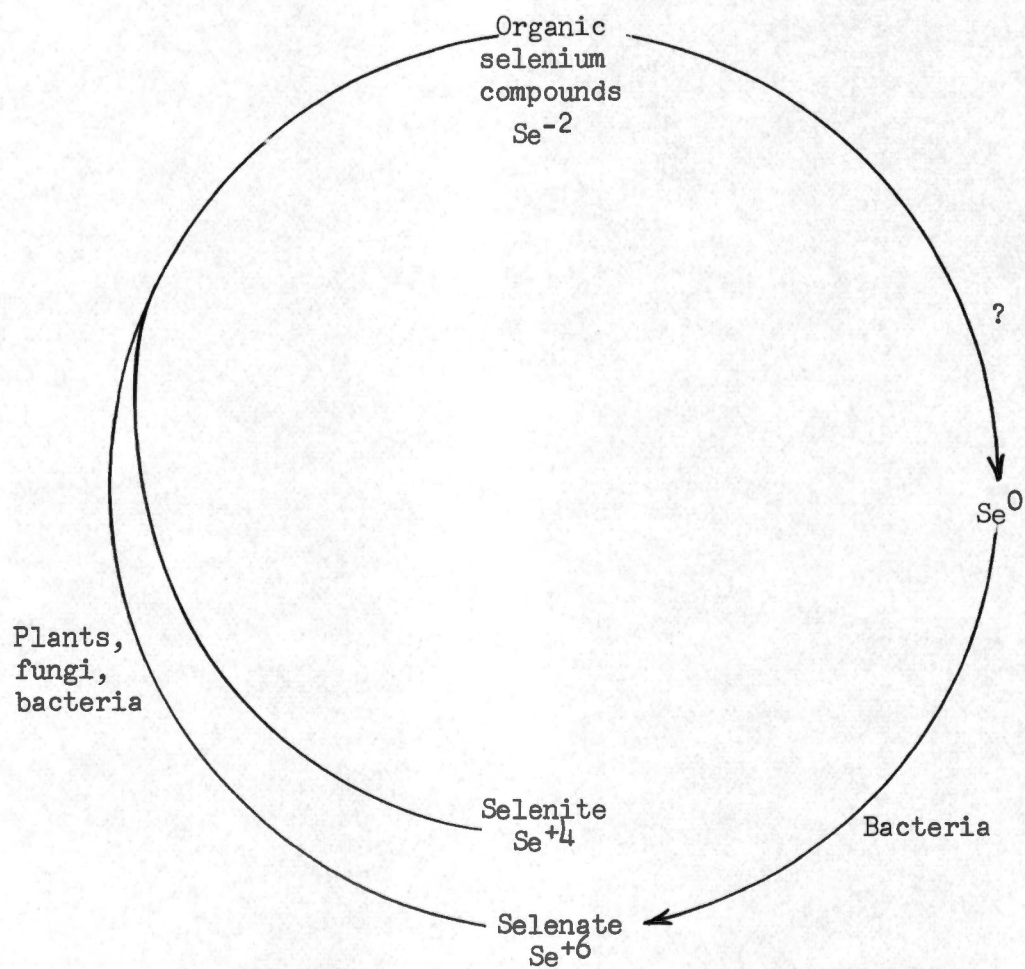


Figure 1. Proposed selenium cycle.

CRANE & CREST

Selenium transmethylation. Investigations have indicated that the ATP-dependent enzymatic activation initiates methyl transfer by an formation of S-adenosylmethionine (Rosenfeld and Beath, 1964). An essential feature of the activation reaction of transmethylation is the conversion of the sulfur atom from a thioether to a sulfonium configuration. The dependence of transmethylation on the sulfur atom and the importance of selenium in many biological systems prompted investigation of selenomethionine's ability to replace methionine in the activation reaction of transmethylation. Selenomethionine proved to be a better substrate than methionine for a methionine-activating yeast enzyme. Se-Adenosylselenomethionine was shown to serve as a methyl donor in a reaction with guanidinoacetic acid to yield Se-adenosylselenohomocystine and creatine (Mudd and Cantoni, 1957). Rosenfeld (1962b) reported a depression of methionine formation in rat liver homogenates by selenate and selenite when the methyl acceptors were homocystine and homocysteine and the methyl donors were choline or betaine. The interference to transmethylation may have been due to: (1) oxidation of sulfhydryl groups; or (2) the methyl capture by "active" seleno-compounds (Rosenfeld, 1962b). Transmethylation of selenite in the liver microsomes has been proposed from the measurement of ^{75}Se -dimethyl selenide from the lungs of rats after radioselenite administration (Imbach and Sternberg, 1967).

Biosynthesis of organic selenium compounds. Selenate or selenite is probably utilized in the same manner as sulfur in the production of essential amino acids by the rumen bacteria in ruminant animals.

Rosenfeld (1962a) isolated selenocystine from the hydrolysate of wool from sheep dosed orally with $\text{H}_2^{75}\text{SeO}_3$. Cowie and Cohen (1957) showed that selenium is incorporated into the cellular proteins of a methionine requiring strain of Escherichia coli (E. coli) possibly as selenocystine. Tuve and Williams (1961) found that selenium partially replaced sulfur in methionine and possibly cysteine in wild type strain B of E. coli. Conversely, Cummins, Martin, and Maag (1966), in a rabbit experiment with radioselenite, stated that "selenocystine and selenomethionine are not synthesized in vivo from selenite."

IV. SELENIUM IN BLOOD STUDIES

Erythrocyte studies. McConnell and Cooper (1950) found that selenium as $\text{Na}_2^{75}\text{SeO}_3$ injected subcutaneously into dogs was taken up in the red cells and was incorporated primarily into the hemin fraction of the erythrocytic hemoglobin. They speculated that the fixation of selenium did not take place in the bone marrow since they could detect no radioactivity in the bone marrow 24 hours after selenium-75 injection. However, McConnell (1963) later stated that selenium remained attached to the red cell of the dog until the death of the cell. Also, selenium in the form of ^{75}Se -selenomethionine has been shown to be concentrated in the bone marrow cells of humans and to be incorporated into the erythrocytic hemoglobin during the early stages of red cell formation (Penner, 1964) which are known to be actively engaged in hemoglobin production. Selenomethionine was shown to be incorporated into the globin fraction of red cell hemoglobin. Selenium-75 labeled

selenomethionine may be very useful as a tracer for studying erythrocyte life spans in humans (Penner, 1966). Substantiating results on the use of ^{75}Se -selenomethionine as a satisfactory marker for human erythrocytes were offered by Awwad et al. (1966a) who also revealed that radioactive selenomethionine was incorporated by reticulocytes in vitro. This in vitro tagging of human erythrocytes should permit the study of erythrocyte survival by the reinjection of in vitro ^{75}Se -labeled red cells. A similar in vitro labelling technique of erythrocytes with inorganic radioactive selenium was used to measure blood volumes in dogs (Gatien et al., 1964). Wright (1965) demonstrated that radioselenite ($\text{H}_2^{75}\text{SeO}_3$) was incorporated into the globin moiety of sheep erythrocytic hemoglobin with no detectable activity in the heme. He postulated that radio-selenium was incorporated into the hemoglobin of the ovine erythrocyte during its formation and remained throughout the cell's life span.

Wright and Bell (1963) showed that the in vitro uptake of radioactive selenium ($\text{H}_2^{75}\text{SeO}_3$) by ovine erythrocytes was inversely proportional to the amount of selenium in the diet of the sheep and that the radioselenite moved into the red blood cells via an oxygen-dependent transport mechanism. These results indicate the possibility for a test of the dietary level of selenium of animals in the field.

Plasma and serum studies. It is in general agreement that radioactive selenium is taken up in the fractions of plasma and serum at a rapid rate but is also cleared rapidly. Data presented by McConnell and Cooper (1950) and Celander (1964) on the dog, Wright (1965) and Jacobsson

(1966a) on sheep, Sternberg and Imbach (1967), Awwad et al. (1966b), and Jacobsson (1966a) on rats and mice, and Awwad et al. (1966a) and Penner (1964b) on humans all showed similarities of ^{75}Se uptake and release from plasma and serum fractions of the blood.

Burk and his associates (1967) and Awwad et al. (1966a) reported a failure of ^{75}Se as $^{75}\text{SeO}_2$ and ^{75}Se -selenomethionine, respectively, to associate with plasma and serum proteins of human blood in vitro. However, Imbach and Sternberg (1967) illustrated a positive uptake of ^{75}Se as $\text{Na}_2^{75}\text{SeO}_3$ into human plasma and serum proteins. These results suggest the possibility of different binding mechanisms for different selenium compounds to proteins.

Leucocyte and platelet studies. An in vitro uptake of radio-selenium as $\text{Na}_2^{75}\text{SeO}_3$ has been demonstrated by Cavalieri et al. (1967) who proposed that the binding mechanism of ^{75}Se to human leucocytes may not be dependent on protein synthesis since puromycin (inhibitor of protein synthesis) had no effect of selenium uptake by the white cells. Cavalieri's results also indicated that two binding processes of ^{75}Se to human leucocytes were probable: an almost immediate binding and a more gradual incorporation. Penner (1966) has reported in vivo uptake of ^{75}Se -selenomethionine by human leucocytes and platelets. Since methionine is concentrated in leucocytes and platelets, these results indicate that further investigations with ^{75}Se -selenomethionine may prove useful for both leucocyte and platelet turnover studies similar to those presented on erythrocytes (Penner, 1966).

The real controversial problem yet to be solved in using selenium-75 in blood studies is the mode of binding. Many possibilities have been set forth but as yet no absolute answer has been found.

V. OTHER TISSUE STUDIES WITH SELENIUM

Liver. Although selenium distribution has been studied in most all the tissues of animals, particular emphasis has been given to those tissues and organs in which a significantly greater amount of selenium is incorporated and which may afford some indication of the metabolic function of selenium in the animal system. Major emphasis has been placed on the liver since it plays a very important role in protein biosynthesis. A significant amount of radioselenium was found in the liver of sheep following administration of ^{75}Se (Rosenfeld, 1964). This concurs with findings of Wright and Bell (1966) in sheep and swine. In addition to a large uptake of selenium-75 in the liver of growing swine following ^{75}Se injection, Buescher, Bell, and Berry (1961) also reported a minor amount of the liver-selenium in the bile. The biliary concentration of selenium was low when compared to tissue concentration. Selenium has also been isolated from the bile of sheep in the form of selenotaurine (Rosenfeld, 1961). Concentration of radioselenium in the liver has been reported for rats and mice (Anghileri and Marqués, 1965; Awwad et al., 1966b; Imbach and Sternberg, 1967).

Pancreas. Analysis of the pancreas after ^{75}Se -selenomethionine administration has proven very valuable both as an elaboration of the

metabolic role of selenium, and as a clinical tool. When ^{75}Se -selenomethionine was injected into dogs, it localized in the pancreas in about 2 hours postinjection. This follows the normal pattern of amino acid concentration found in the pancreas as shown with several ^{14}C and ^{35}S -labeled amino acids which is probably related to the rapid utilization and destruction of pancreatic enzymes in comparison to other body proteins (Blau and Manske, 1961). Blau and Bender (1962) used the pancreas localization characteristic of selenomethionine as a scanning device for studying deep seated lesions of the human pancreas.

Miscellaneous. Selenium has also been found to be incorporated into the proteins of dog hair (McConnell and Kreamer, 1960); the milk of rats (McConnell, 1948), dogs (McConnell and Roth, 1964), and sheep (Jacobsson, Oksanen, and Hansson, 1965); lipoproteins of dogs (McConnell and Levy, 1962); skeletal muscle enzymes, aldolase, and myosin (McConnell and Roth, 1965); ribonucleoproteins of rat liver (McConnell and Roth, 1962); and into rat liver ribosomes (McConnell and Roth, 1966a); plus many others.

The long half-life of ^{75}Se (127 days), together with the fact that this isotope is a gamma-ray emitter, makes radioselenium a unique tool for a variety of experiments, particularly in protein studies both in vitro and in vivo. It is clear, however, that more investigation is needed to clarify the metabolic action of selenium in the animal with major emphasis on the binding mechanisms to proteins.

CHAPTER III

MATERIALS AND METHODS

Separate experiments which make up this study of selenium-75 in the blood of both sheep and pigs include: (1) erythrocyte life span study; (2) red blood cell hemoglobin, hemin, and stroma analysis for radioactivity; (3) chromatographic investigation of erythrocyte globin amino acids; (4) selected tissue analysis for radioselenium; and (5) red cell destruction and preferential binding study on red cells of varying ages.

I. EXPERIMENTAL ANIMALS

Nineteen Pitman-Moore miniature gilts, 10 wether lambs, and 10 ewe lambs of mixed breeding were used for this study. The gilts weighed an average of 34 kg. and the lambs averaged 40 kg. The miniature pigs were selected for these long-term studies because preliminary results showed that their small size and low weight gain made them much more suitable than domestic breeds. Preliminary blood analyses including readings for red blood cells, hemoglobin, white blood cells, platelets, and hematocrit showed all animals to be within normal ranges. Preliminary blood checks were made in an effort to avoid animals with anemia or any other blood abnormalities. The animals were selected for experimentation primarily on hematocrit and red cell numbers. Usually the two animals with the lowest hematocrit and red cell readings were culled

from each group. Animals were also selected on the basis of their weight uniformity.

Before being placed on experiment, pigs and lambs were treated for both internal and external parasites. The miniature gilts were all ovariectomized to prevent interference from the estrous cycle. Females of both species were used in the absence of sufficient numbers of males.

Selected animals were placed in metabolism crates 1 week prior to dosing to allow time for adjustment to environment and experimental diet. After administration of radioactive selenium, the animals were left in the metabolism crates for an additional week to allow the level of radioactivity in the excreta to drop to a "safe" level.

Pigs were fed a standard ground-corn diet (Buescher, Bell, and Berry, 1961) at the rate of 400 gm., three times daily. Sheep were fed the same amount of a standard cottonseed-hull diet.

II. ISOTOPE DOSING PROCEDURE AND SAMPLE COLLECTION

A single dose of 1.5 mCi. of selenium-75 as H_2SeO_3 (33.6 mCi./mg.) in dilute HCl solution¹ was given intravenously into the jugular vein of each animal. Five ml. blood samples were taken from the pigs and sheep on the erythrocyte life span study at 1, 2, 4, 8, 10, 24, 48, 72, and 96 hours; 5, 6, 7, 8, 9, 10, and 14 days; and once weekly thereafter through 182 days after isotope injection. Blood samples of 10 ml. were drawn from the jugular vein of each animal on the red cell destruction and

¹Purchased from New England Nuclear Corp., Boston, Mass.

preferential binding study at 1, 6, 10, 24, 48, 72, and 96 hours; at 7 and 10 days; and once weekly thereafter, for 70 days following administration of ^{75}Se . Heparin² was added as an anticoagulant to each blood sample collected.

Tissue samples of liver, spleen, and bone marrow from sternum, rib, and femur were taken for radioactivity measurements from each of two lambs sacrificed at 2, 4, 49, 56, and 70 days and from each of two pigs sacrificed at 1, 4, 14, 28, and 70 days postinjection with ^{75}Se . These collection times were selected to correspond to the changes in radioactivity found in the red blood cells of animals whose erythrocyte life spans were studied.

III. EXPERIMENTAL PROCEDURE AND LABORATORY ANALYSES

Erythrocyte life span study. Immediately after collection of blood samples, hematocrit readings were taken by standard procedures (Schalm, 1961). The blood aliquots were thoroughly but gently (to avoid hemolysis) mixed and 1 ml. was taken for measurement of radioselenium content. The remaining sample was centrifuged slowly (2,000 r.p.m.)³ for 15 min. in a refrigerated centrifuge. One ml. of plasma was removed for radioactivity measurement and the remainder was aspirated from the packed cells and discarded. The packed cells were washed three times

²"Panheprin," Abbott Laboratories, North Chicago, Ill.

³All centrifugation was done in an International Refrigerated Centrifuge, type PR-2, International Equipment Co., Boston, Mass., unless otherwise cited.

with a total of 30 ml. of 0.9 per cent physiological saline solution to remove unbound ^{75}Se . One ml. each of red cells and red cell wash was collected for analysis of radioselenium content. These separation techniques were similar to those described by Wright (1965). All fractions were assayed in duplicate for selenium-75 activity in a well-type scintillation counter. Radioactive standard solutions of $\text{H}_2^{75}\text{SeO}_3$ were collected from each dosing solution and diluted with distilled water to 10,000, 100,000, and 1 million times volume for radioactive-counting procedures.

Hemoglobin, hemin, and stroma analysis. Erythrocyte samples from the animals involved in the erythrocyte life span study were also used for hemoglobin (Hb) selenium-75 measurements. However, since only a trend of erythrocyte hemoglobin uptake of radioselenium was desired, the hemoglobin measurements were ended at 35 days after isotope administration. One ml. of erythrocytes was lysed by the addition of 7.5 ml. of cold distilled water, thoroughly mixed, and 2.5 ml. of carbon tetrachloride were added. The samples were mixed and centrifuged (4,000 r.p.m.) for 15 min. to separate the stroma from the hemoglobin (McDonald, 1966). One ml. of hemoglobin was drawn for counting of radioactivity, 1 ml. was frozen for later use in preliminary chromatographic analysis, and 2 ml. were cooled in the refrigerator. After sufficient cooling, the 2 ml. of hemoglobin were mixed with 0.3 ml. of cold 0.1 M. HCl. The samples were stored in the cold for 1 hr., mixed with 2 ml. of cold methylethyl ketone (McDonald, 1966), and left in the cold overnight.

A 0.5 ml. aliquot of hemin was drawn for ^{75}Se activity analysis.

Selenium-75 content of globin was derived mathematically by subtracting the counts in the hemin from those in the hemoglobin. Since no activity was detected in the hemin fraction, the globin counts were synonymous with the counts of the hemoglobin. The amount of radio-selenium in the stroma was also calculated mathematically by subtracting the radioactivity in 1 ml. of hemoglobin (1 ml. of diluted Hb times a dilution factor of 8.5) from the activity in 1 ml. of red cells.

Preliminary chromatographic analysis. A limited chromatographic analysis of the globin proteins of both sheep and pigs was run to establish desirable techniques for chromatographic separation of red cell globin amino acids. One-half ml. of hemoglobin (diluted 8.5 times in lysing procedure) was raised to 1 ml. with distilled water to prepare an approximate solution of 2 gm. per cent (2 gm. Hb/100 ml. solution), which seems to be the most desirable concentration of hemoglobin for chromatographic analysis (Popp, 1966). Hemin and globin were separated by adding 10 ml. of chilled (-15°C.) acid-acetone (4 ml. conc. HCl in 100 ml. acetone) to the diluted hemoglobin solution. The acetone-hemoglobin mixture was maintained below 0°C. , continuously stirred, for 10 min., and centrifuged (4,000 r.p.m.) to collect the globin. The globin was washed twice with 10 ml. of cold acetone to remove unbound selenium-75. Globin samples were dried in a vacuum desiccator overnight (Ingram and Stretton, 1962). Reduced temperatures were maintained in a dry ice-acetone bath.

Cystine hydrolysate decomposes when allowed to stand in air (Block, Durrum, and Zweig, 1958). In addition, cysteine, cystine, and methionine have been shown to be very labile to oxidation (Smith, 1960), along with their seleno-analogues which are slightly more unstable than the sulfur-containing amino acids (Peterson and Butler, 1962). To avoid amino acid oxidation, a 1:1 v./v. mixture of 90 per cent formic acid and 6 N. hydrochloric acid was used for protein digestion (Block, Durrum, and Zweig, 1958). Volumes of 5, 10, and 20 ml. of hydrochloric acid-formic acid mixture were tried to determine the minimum amount of acid necessary to produce complete hydrolysis of the erythrocyte globin proteins. The 5 ml. volume gave complete hydrolysis as shown by chromatography and caused the least breakage of sealed digestion tubes. The globin-acid mixture was placed in a combustion tube (20 x 250 mm.)⁴ and frozen in a dry ice-acetone bath. They were carefully thawed under vacuum, heat-sealed, and placed in a 105° C. oven for 48 hr. (Ingram and Stretton, 1962; Farkas, 1966). The tubes were removed from the oven, opened, and the hydrolysate removed. The excess acid was removed from the hydrolysate in vacuo at 35° C. and the resulting film of amino acid hydrochlorides were placed in a vacuum desiccator over Na₂CO₃ for 24 hr. The hydrolysate was then taken up in warm distilled water, filtered, and again evaporated to dryness, and finally taken up in exactly 1 ml. of 10 per cent 2-propanol (Block, Durrum, and Zweig, 1958).

⁴Matheson Scientific Co., Cincinnati, Ohio.

Oxidation of the sulfur-containing amino acids with hydrogen peroxide has been shown to prevent decomposition of these amino acids during chromatography, particularly when using phenolic solvents (Smith, 1960). When oxidation of the sulfur amino acids occurs during chromatography, the spots on the paper appear as streaks or fail to appear at all with a ninhydrin stain (Smith, 1960). Since it is thought that selenium may substitute for sulfur in the sulfur-containing amino acids in animal tissue proteins (McConnell and Wabnitz, 1957; Rosenfeld, 1962a), the chemical state and stability of sulfur-containing amino acids, cysteine, cystine, and methionine, are of special interest to this study. However, in a direct comparison between the one-way chromatographic action of unoxidized and H_2O_2 -oxidized standard ^{75}Se -selenomethionine in a standard amino acid mixture, it was found that H_2O_2 oxidation (0.1 ml. of cold 30 per cent H_2O_2 /ml. of hydrolysate for 2 min.; Smith, 1960) caused considerable spreading of ^{75}Se as indicated by an autoradiogram shown in Figure 2.

The spreading characteristic of hydrogen peroxide oxidation on ^{75}Se radioactivity in paper chromatography of standard ^{75}Se -selenomethionine made it undesirable for the chromatography of sheep and swine globin amino acids in this study.

Several volumes of amino acid hydrolysate were spotted on the chromatographic paper before a compromise could be reached between satisfactory amino acid separation and the minimum amount of radioactivity that must be present for detection by the counting device used and for clear autoradiograms that were developed from X-ray film.

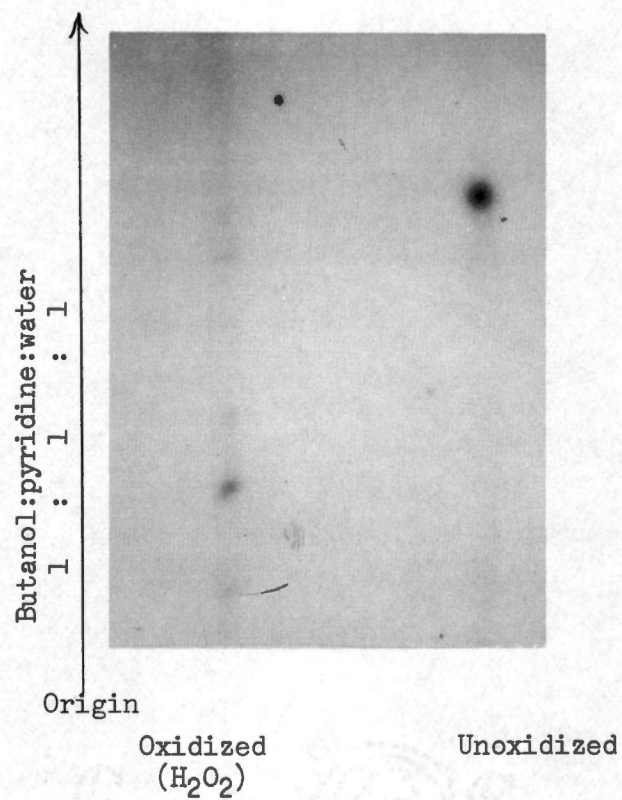


Figure 2. Autoradiogram of one-way chromatogram of oxidized and unoxidized standard amino acid mixtures containing ⁷⁵Se-selenomethionine.

A volume of 50 μ l. was selected as the most desirable amount.

A two-way descending chromatographic system using a butanol-acetic acid-water (4:1:5 v./v./v.) as the first direction solvent (Popp, 1966), and Mallinckrodt Gilt Label liquid phenol (Block, Durrum, and Zweig, 1958) as the second direction solvent proved undesirable for use in the chromatographic analysis. The butanol-acetic acid-water and phenol solvent system gave good separation of amino acids but the spots were lacking in compactness and the amino acid, cystine, was frequently oxidized or decomposed and failed to appear with a ninhydrin stain.

All techniques for two-way descending chromatography were similar to those described by Block, Durrum, and Zweig (1958) except for slight adaptations in equipment to fit the facilities available.

Chromatographic and autoradiographic analyses. Preparation of erythrocytic globin samples, amino acid digestion, and chromatographic techniques were identical to those used in the preliminary chromatographic study. Since phenol was shown to be undesirable for use in this study, a milder solvent system that gives little or no oxidation of sulfur or selenium containing amino acids, and gives adequate separation of these amino acids was selected. A two-way descending chromatographic system with butanol-pyridine-water (1:1:1 v./v./v.) as the first direction solvent and butanol-acetic acid-water (4:1:5 v./v./v.) as the second direction solvent (Peterson and Butler, 1962) was used for all the chromatography in this study.

Standard amino acid mixtures of 19 naturally occurring amino acids⁵ were chromatographed with each group of protein hydrolysate from the globin fraction of swine and sheep hemoglobin. Each standard solution was made up in a concentration of 5 mg. of each amino acid/milliliter of 10 per cent 2-propanol. Just enough 6 N. HCl was added to dissolve the compounds not completely soluble in water (Smith, 1960), e.g., cystine, alanine, glutamic acid, leucine, isoleucine, tryptophan, and tyrosine (Weast, Selby, and Hodgeman, 1964). One hundred μ l. of a ⁷⁵Se-selenomethionine solution (100 to 400 mCi./mg.)⁶ were added to each standard mixture for counting and autoradiographic investigations. From this radioactive solution, a 5 μ l. volume was spotted on Whatman No. 3 chromatographic paper which was used in all the partition chromatography. Care was taken to make sure each volume of protein hydrolysate was applied at the same location on each chromatogram. In addition to the standard mixture, each of the 19 standard amino acids plus methionine sulfone and cysteic acid⁷ was chromatographed separately to construct a standard map.

After completion of both directions of each chromatographic run, the papers were air-dried, and selected papers were sprayed with a 0.5 per cent ninhydrin stain in 95 per cent ethyl alcohol solution (Popp, 1966). The color was developed in an oven at 105° C. for 3 min.

⁵Nutritional Biochemical Corp., Cleveland, Ohio.

⁶Nuclear-Chicago Corp., Des Plains, Ill.

⁷Nutritional Biochemical Corp., Cleveland, Ohio.

(Smith, 1960). The remaining undeveloped papers were used for chemical identification of the sulfur-containing amino acids. Several chemical reagents were tried for the detection of sulfur amino acids but the potassium iodoplatinate reagent described by Winegard and Toennies (1948), Block and Bolling (1951), and Toennies and Kolb (1951) provided the most efficient detection of both cystine and methionine. Cystine and methionine (excluding oxidized forms) yielded white spots on red-purple backgrounds when chromatograms were sprayed with the potassium iodoplatinate solution. The exact location of iodoplatinate positive spots was identified by physical measurements and by comparison to the location of white spots on standard chromatograms. The measurements obtained from the iodoplatinate-sprayed papers were compared to those measurements from the blue spots on the ninhydrin-sprayed papers for a positive identification of the sulfur-containing amino acid in reference to other amino acids in both the standard and unknown amino acid mixtures.

The areas containing the radioactive selenium were identified by two methods: (1) sections of each paper were cut out and counted directly in a deep-well scintillation counter; and (2) autoradiograms were made of representative chromatograms of both the sheep and pig erythrocyte globin amino acids. The autoradiograms were prepared by standard procedures (Block, Durrum, and Zweig, 1958). Due to the fact that selenium-75 is a strong gamma emitter (0.265-0.280 mev.) precautions were taken to insure proper exposure of the X-ray film. Preliminary experiments showed that wrapping each paper chromatogram in heavy-duty aluminum foil would stop enough of the lower energy rays to

expose the film. The X-ray film was left with the chromatograms for an exposure period of 10 days.

Chromatograms and autoradiograms were made of pig and sheep red cell globin samples collected at 1, 10, 24, and 72 hours and at 7, 14, 49, and 70 days for the pig; at 1, 10, and 48 hours and at 7, 21, 49, and 70 days for the sheep, after administration of ^{75}Se . Again these times correspond roughly to periods of changing selenium-75 content in the blood.

Tissue analyses. Representative samples of liver and spleen were weighed and counted in triplicate for ^{75}Se activity without further preparation. Fresh smears were made of the bone marrow from the sternum, rib, and femur of each pig and lamb sacrificed in the tissue study. The marrow was suspended in bovine serum⁸ for more uniform smears (Pardue, 1967). Wright's stains and microautoradiograms of the bone marrow smears were prepared by the histology section at The University of Tennessee Agricultural Research Laboratory. After development of the microautoradiograms, the bone marrow slides were microscopically scanned for the identification of sites containing selenium-75.

Erythrocyte destruction and preferential binding study. Five-ml. samples of whole blood were pipetted into adapted disposable 5-ml. syringes and sedimented by centrifugation. Samples were spun at

⁸TC type, Difco Laboratories, Detroit, Mich.

6,200 r.p.m. ($\cong 5,090 \times G.$) for 35 min. in an angled-head centrifuge⁹ (Coopersmith and Ingram, 1966). The plasma was aspirated from the packed cell column, and the remaining cells were frozen. By the use of a proper screw device (Coopersmith and Ingram, 1966), the frozen cells were pushed up and cut off into five separate fractions each being approximately 20 per cent of the height of the frozen red cell column. Care was taken to remove any residue of plasma left on the cell layer after aspiration. Each of the five fractions cut from the frozen red cells contained in 5 ml. of whole blood was weighed and analyzed for ^{75}Se content by previously described counting procedures.

IV. STATISTICAL ANALYSIS OF DATA

Data were statistically analyzed, where applicable, for standard deviations of observations and standard errors of means as outlined by Steele and Torrie (1960) to illustrate data variability.

⁹Servall, type NSE, Ivan Servall, Inc., Norwalk, Conn.

CHAPTER IV

RESULTS AND DISCUSSION

I. ERYTHROCYTE LIFE SPAN DETERMINATIONS

Sheep. Figure 3 shows that the whole blood and plasma content of selenium-75 reached a peak at approximately 4 hr. after radio-selenium administration. The plasma concentration of ^{75}Se dropped rapidly for about 3 weeks postdosing, then decreased slowly for the duration of the study. Uptake of radioselenium in the whole blood was similar to plasma uptake during early sampling periods. However, in about 6 days after the initial injection, the radioactivity level in the whole blood began to increase again due to an increase in ^{75}Se content of the red blood cells. A slight drop in the radioselenium content of whole blood was recorded between 21 and 28 days after dosing, probably as a result of a sharp decline in plasma ^{75}Se . The second peak of ^{75}Se content in sheep whole blood and erythrocytes occurred at 42 and 49 days postdosing, respectively, then decreased slightly, plateaued for a short period, and again decreased. The rapid rate of decrease in ^{75}Se activity of whole blood and erythrocytes at approximately 150 days after dosing was probably due to the death of the red cells originally incorporating the label (Figure 3). These results are similar to data reported in sheep by Wright (1965) and in dogs by McConnell (1963). A slight increase in the radioselenium content of the whole blood and the plasma was measured between 175 and 182 days postdosing,

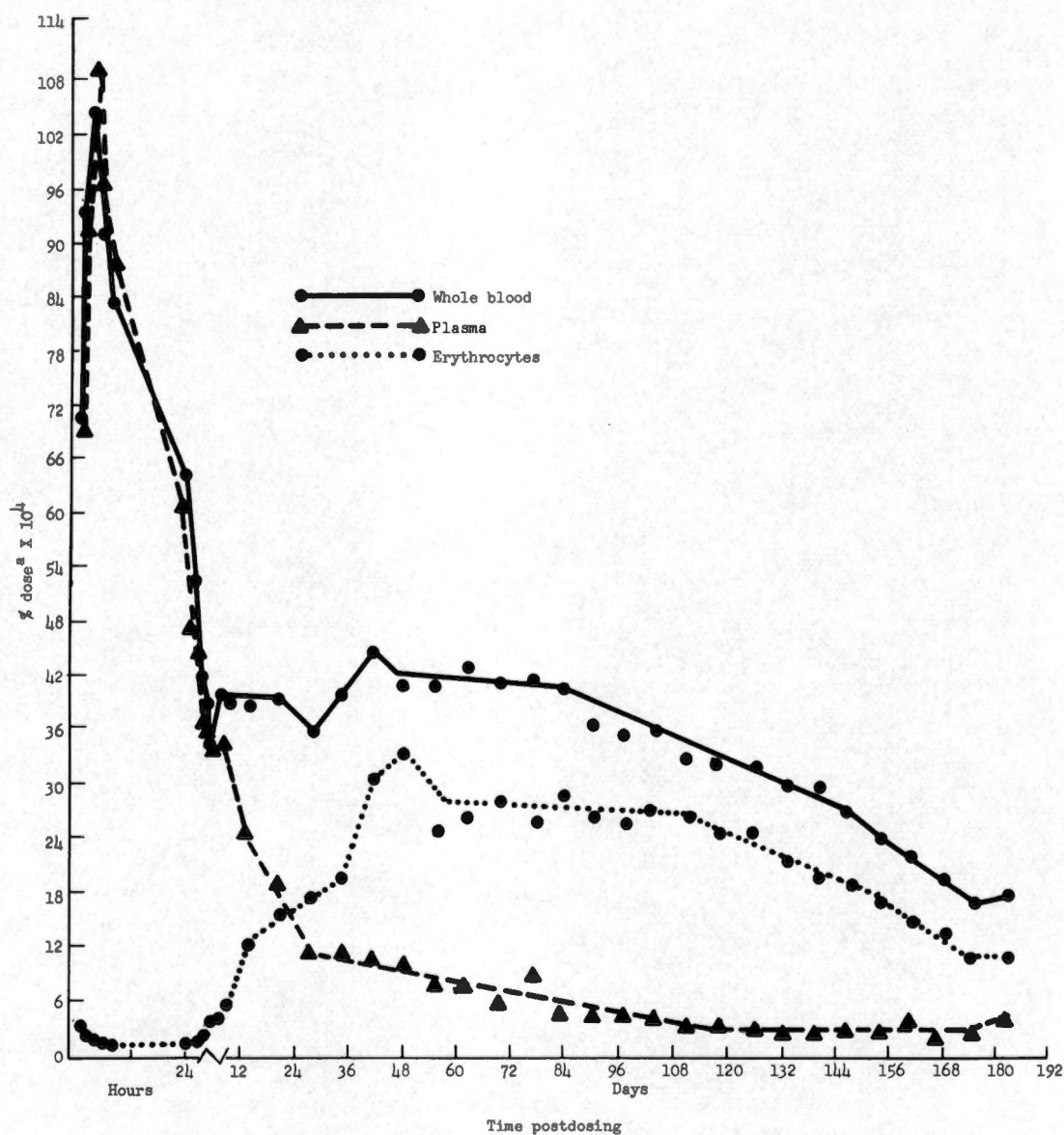


Figure 3. Whole blood, plasma, and erythrocyte content of selenium-75 following a single intravenous dose to growing lambs.

^aPlasma and erythrocyte concentration expressed as $\% \text{ dose/ml.}$ of whole blood by multiplying $\% \text{ dose/ml.}$ of plasma and erythrocytes times mean hematocrit reading at each time period.

presumably accounted for by the release of ^{75}Se from the destroyed erythrocytes. An increase in radioactivity of the red cell saline wash was also noticed at this time (Appendix Table III). The measurements of ^{75}Se in the red cell wash during early blood collection periods (Figure 4) indicated that the majority of the radioselenium in the erythrocyte samples at these times was of an unbound nature.

Life span determinations of ovine erythrocytes were made using the procedure of Neuberger and Niven (1951) which estimates the "mean" life span as the time interval between the point at which the concentration of the red cell label increases to 50 per cent of the maximum amount of label measured in the cells and the point the concentration of label decreases to 50 per cent of the maximum. Estimates of erythrocyte life span of the six sheep used in this study were 81, 112, 112, 140, 154, and 165 days with a mean of 127 days with a standard deviation of 31 days. This mean value is less than estimates reported by Wright (1965) using an in vivo incorporation of ^{75}Se into the globin of mature sheep and by Judd and Matrone (1962) using an in vivo labeling of heme with glycine-2- ^{14}C in sheep. The smaller 127-day estimate calculated in this study was probably due to the large individual animal variation and the expanding blood volume of the growing animals used in the present experiment and not to a difference in erythrocyte life spans of growing versus mature sheep as has been suggested by Judd and Matrone (1962).

Erythrocytes are subjected to two types of destruction processes:

- (1) an age determinate process not subject to environmental hazards; and
- (2) a random destruction by some external force independent of the red

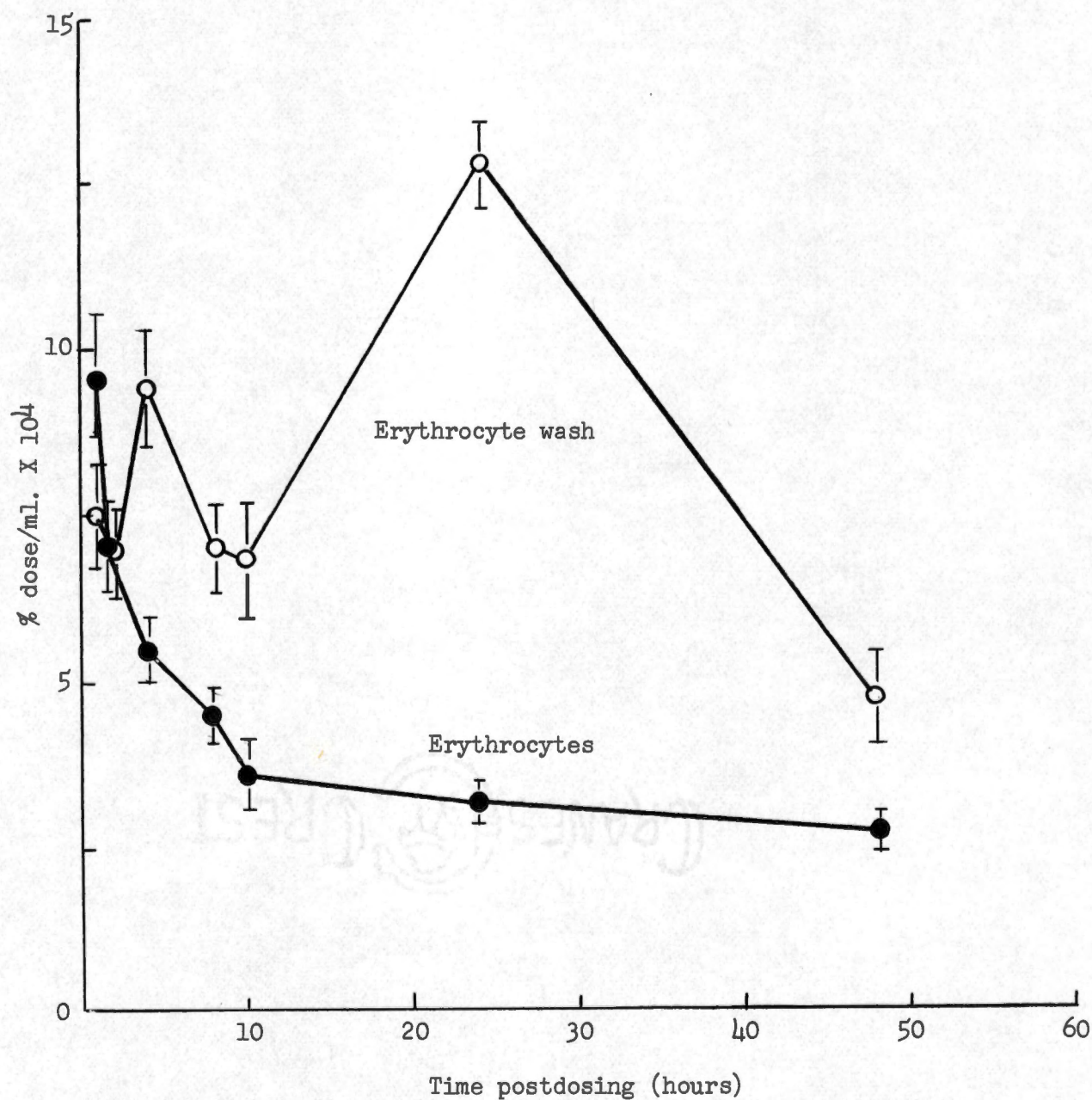


Figure 4. Selenium-75 content of circulating erythrocytes and erythrocyte saline wash following a single intravenous dose to growing lambs.

cell (Dornhorst, 1951). It is thought that these two processes may operate in varying ratios from species to species and between animals within the same species. An interpretation of the results obtained in this study as outlined by Dornhorst (1951) and Lajtha (1961) suggests that both a random and an age-dependent erythrocyte destruction process exist in sheep. However, existing evidence seems to indicate that the age-dependent process is highly predominant. The predominance of the age-dependent process in growing sheep was illustrated in this study by the slow rate of disappearance of ^{75}Se from the circulating erythrocytes after the initial incorporation followed by a drop in radioselenium content between 154 and 161 days after ^{75}Se injection (Figure 3, page 33). Since growing animals were used in this study, the trend in ^{75}Se disappearance from the red cell could have been effected by increasing blood volumes from increasing body sizes of the experimental animals. Similar results were shown by Wright (1965) using ^{75}Se label in older sheep than those used in the present investigation.

Swine. Figure 5 shows that the uptake of ^{75}Se by the porcine whole blood and plasma was similar to that in sheep during the first few hours after isotope administration. There was a rapid uptake of radioselenium in the whole blood and plasma which peaked at 2 and 4 hr. postdosing, respectively. A rapid clearance of selenium from plasma and whole blood then occurred for about 3 weeks followed by a decrease thereafter. The rate of disappearance from the whole blood was slower than that from the plasma due to an increased uptake of ^{75}Se by the erythrocytes. The whole-blood concentration curve failed to illustrate

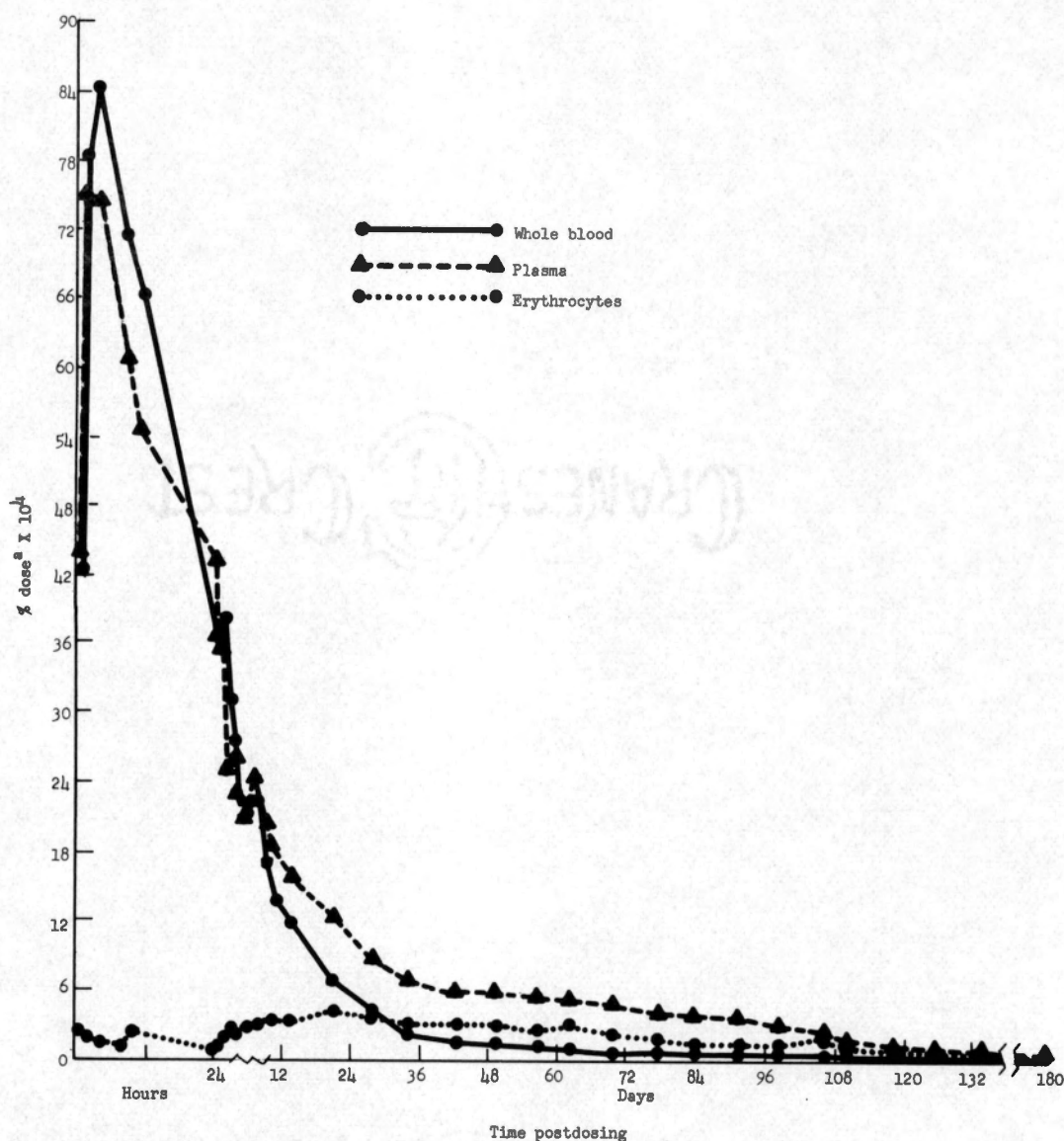


Figure 5. Whole blood, plasma, and erythrocyte content of selenium-75 following a single intravenous dose to growing pigs.

^aPlasma and erythrocyte concentration expressed as % dose/ml. of whole blood by multiplying % dose/ml. of plasma and erythrocytes times mean hematocrit reading at each time period.

the uptake of radioselenium by porcine erythrocytes as clearly as did the sheep whole-blood curves (Figures 3 and 5, pages 33 and 37) due mainly to the lesser amount of ^{75}Se incorporated per red cell in the pig, as compared to the sheep cell (Appendix Tables III and IV). However, Figure 5, page 37, does illustrate that selenium-75 was incorporated into the pig erythrocyte as indicated by the gradual increase in radioactivity in the erythrocytes to a peak at 21 days with a gradual decrease in activity to a sharper decline between 105 and 112 days after ^{75}Se administration. This sudden drop in ^{75}Se content in the pig erythrocytes is important to note since these results can be interpreted to mean that once the selenium is incorporated into the hemoglobin of the red blood cell, it remains until the death of the cell. This fact is supported by similar findings in dogs (McConnell, 1963). As in sheep, the red cell saline wash content of ^{75}Se indicated that the greater percentage of radioactivity was not tightly bound to the erythrocytes during the early hours after dosing (Figure 6). After a peak in activity after 24 hr. postdosing, the radioactivity in the red cell wash decreased sharply without further increases during the duration of this study.

Again by using the methods of Neuberger and Niven (1951), estimates for the erythrocyte life span of the six pigs studied were 67, 68, 68, 68, 74, and 88 days, with a mean of 72 days with a standard deviation of 8 days. This estimate is in good agreement with those presented by Bush et al. (1955) using a glycine-2- ^{14}C labeling technique and Jensen et al. (1956) using a ferrokinetic technique on porcine erythrocytes. These results also agree with those presented by Hansard and Kincaid

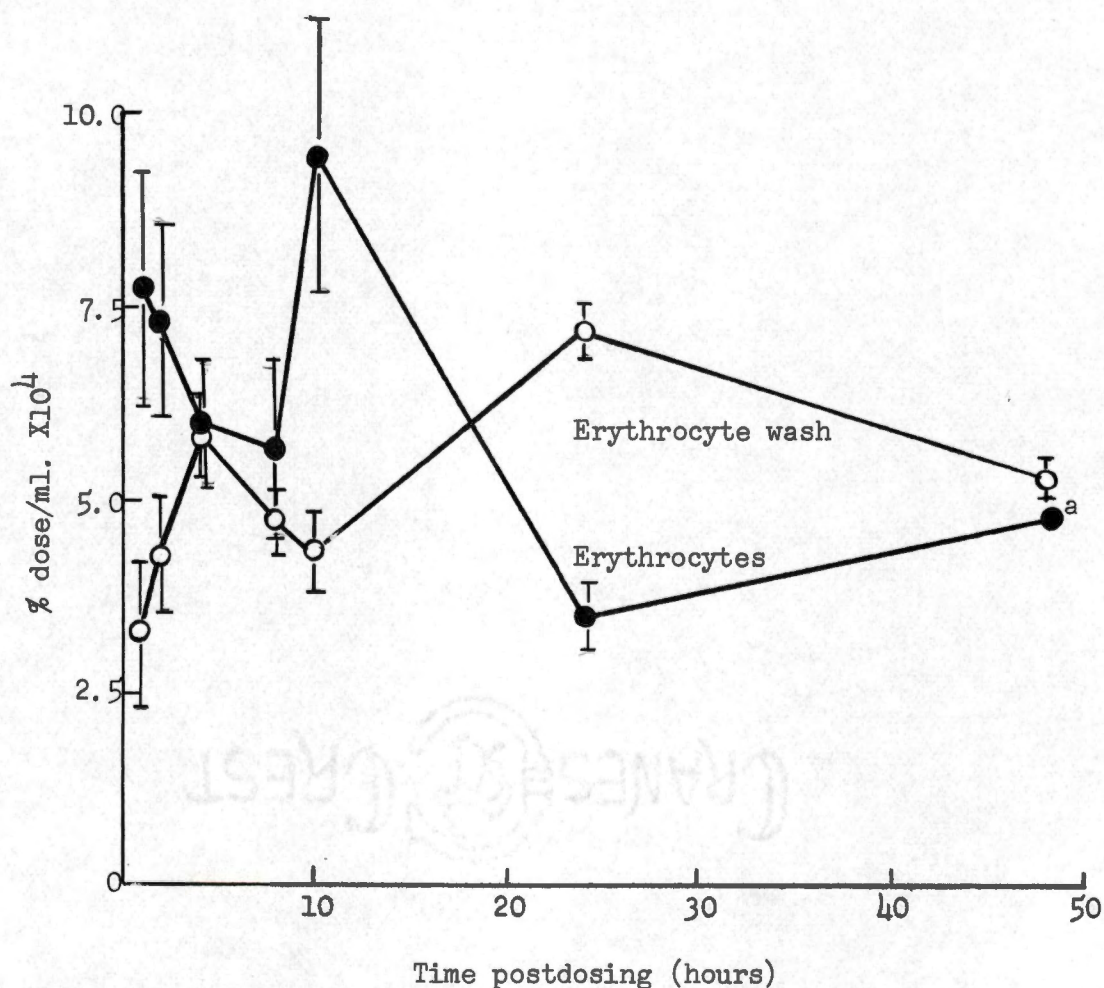


Figure 6. Selenium-75 content of circulating erythrocytes and erythrocyte saline wash following a single intravenous dose to growing pigs.

^aDue to a procedural loss, the value for ^{75}Se content of erythrocytes for 48 hr. postdosing was average of 24 and 72 hr. values; therefore, no standard error of the mean was shown.

(1956) using an erythrocyte reinjection technique with ^{51}C in mature swine.

An interpretation of the data from measurements of selenium-75 content of the pig erythrocytes as illustrated by Dornhorst (1951) and Lajtha (1961) indicated that the radioactive selenium disappears from the porcine erythrocyte at an exponential rate, e.g., there is a predominant random destruction process of red cells in pigs. So, there seems to be a combination of two processes of red blood cell destruction in the growing pig with the random process predominant over the age-dependent process. Supporting evidence is presented by Bush et al. (1955) with a glycine-2- ^{14}C study of erythrocyte destruction in growing swine in which they estimate that approximately 89 per cent of the red blood cells were destroyed randomly. It has been speculated that the high ratio of random erythrocyte destruction in growing swine may be due to a more rapid production of erythrocytes than in mature swine (Bush et al., 1955) which for some unexplained reason subjects the more rapidly produced erythrocytes to external forces in a different manner than the red cells of mature animals. It seems that this theory is not highly probable, however, due to lack of supporting evidence. No direct comparison of erythrocyte life spans and red cell destruction processes between growing and mature swine has been noted.

II. SPECIES COMPARISON OF SELENIUM-75 IN THE ERYTHROCYTE

General. The data collected in this study illustrate a definite species difference in ^{75}Se utilization in the erythrocytes of the sheep

and the pig. First, the sheep erythrocytes incorporated several times the amount of ^{75}Se per cell than that incorporated by the pig red blood cells. Why this happened is not clear at the present time. Perhaps more efficient utilization of the selenium-75 was accomplished in the ruminant animal by resecretion of the mineral element into the rumen where it was incorporated into proteins by the rumen bacteria, then reabsorbed for use in blood metabolism. This occurrence does not seem highly probable, however, since Wright and Bell (1966) showed that the urinary excretion of an intravenous dose of ^{75}Se was severalfold higher than fecal excretion for 120 hr. after injection. They also found very little difference in the total percentage of dose retained by either sheep or swine. Furthermore, if resecretion into the rumen did occur, it is possible that the selenium was not reabsorbed since Peterson and Spedding (1963) have suggested that a reduction of selenite (form used in this study) to an insoluble or unavailable form could occur in the rumen, since they found the selenium in the feces to be in an insoluble form. Wright and Bell (1966) demonstrated that no net absorption of an oral dose of selenium-75 took place in the rumen and only limited absorption occurred in the abomasum. Supporting evidence is offered by Jacobsson (1966b) who reports that a smaller amount of radioselenium was in the tissues of the sheep after an intraruminal tracer dose of ^{75}Se than from a subcutaneous dose. Since it is believed that selenium is closely related to sulfur in protein either by substitution for sulfur during protein synthesis or by some other mechanism, the sulfur-containing amino acid content of the hemoglobin of different species may

offer an explanation to the difference in amounts of selenium incorporated into the erythrocytes of sheep and pigs. For instance, Huisman et al. (1965) in an analysis of the structure of sheep hemoglobin reports that the beta chain of hemoglobins A and B contain two and three residues of methionine, respectively, out of a total of 146 amino acid residues, while the beta chain of pig hemoglobin has been shown to have but one methionine residue out of a total of 146 (Eck and Dayhoff, 1966).

Erythrocyte destruction processes. The difference in rates of radioselenium clearance from the red blood cells of the growing lamb and the growing pig was apparently due to a difference in the ratios of red cell destruction processes. This difference was illustrated by measuring the amount of radioactivity in the red blood cells of varying ages from each species. As the young erythrocyte ages, it becomes smaller, loses water, and its density increases (Spear and Sass, 1964). These aging characteristics of red cells have been utilized to separate a random red cell population into groups of cells with varying ages. Centrifugation of heterogeneous erythrocyte samples results in a sedimentation of older erythrocytes to the bottom of the tube leaving the younger cells at the top (Borun, Figueroa, and Perry, 1957; Coopersmith and Ingram, 1966; O'Connell, Caruso, and Sass, 1965; Régas, 1961) as shown by ^{59}Fe citrate and DF^{32}P -labeling techniques, by enzyme level measurements (glutamic oxaloacetic transaminase and glucose-6-phosphate dehydrogenase) which varies with red cell age, and ^{59}Fe -ultracentrifugation techniques, respectively.

Operating on the hypothesis that ^{75}Se is incorporated into newly formed erythrocytes of the sheep (Wright, 1965) and the pig (Withrow, 1966), it was theorized that in a predominantly age-dependent destruction system of erythrocytes, one should be able to follow the peak of radioactivity from the top of the centrifuged red cell column to the bottom over a period of time as the red cells that originally incorporated the label continue to age and become more dense. On the other hand, if a predominantly random destruction process facilitates red cell death, an initial peak of radioactivity should appear in the younger cells at the top of the sedimented red cell column but gradually disappear as the cells age and move down the column, e.g., after a period of time, there will be very little difference in the amount of radioactivity measured in any of the five fractions of the packed red cell column. Results from averaging the observations of the same fraction (1-5) from six growing lambs at each collection time (Figure 7) showed that the peak of radioactivity appeared in the number one fraction immediately after ^{75}Se administration. Even though the highest concentration of selenium-75 per milligram of red cells remained in the first fraction, it decreased rapidly through 72 hr. after dosing (Appendix Table V). These findings agree with the results shown in Figure 4, page 35, and Appendix Table III that this early concentration of activity was not firmly bound to the erythrocytes. Since these erythrocytes were unwashed, the peak of radioactivity in the upper fractions of the packed cell column during the first few hours after ^{75}Se injection may have been enlarged somewhat by trapped plasma particles even though care was taken to try to cut off

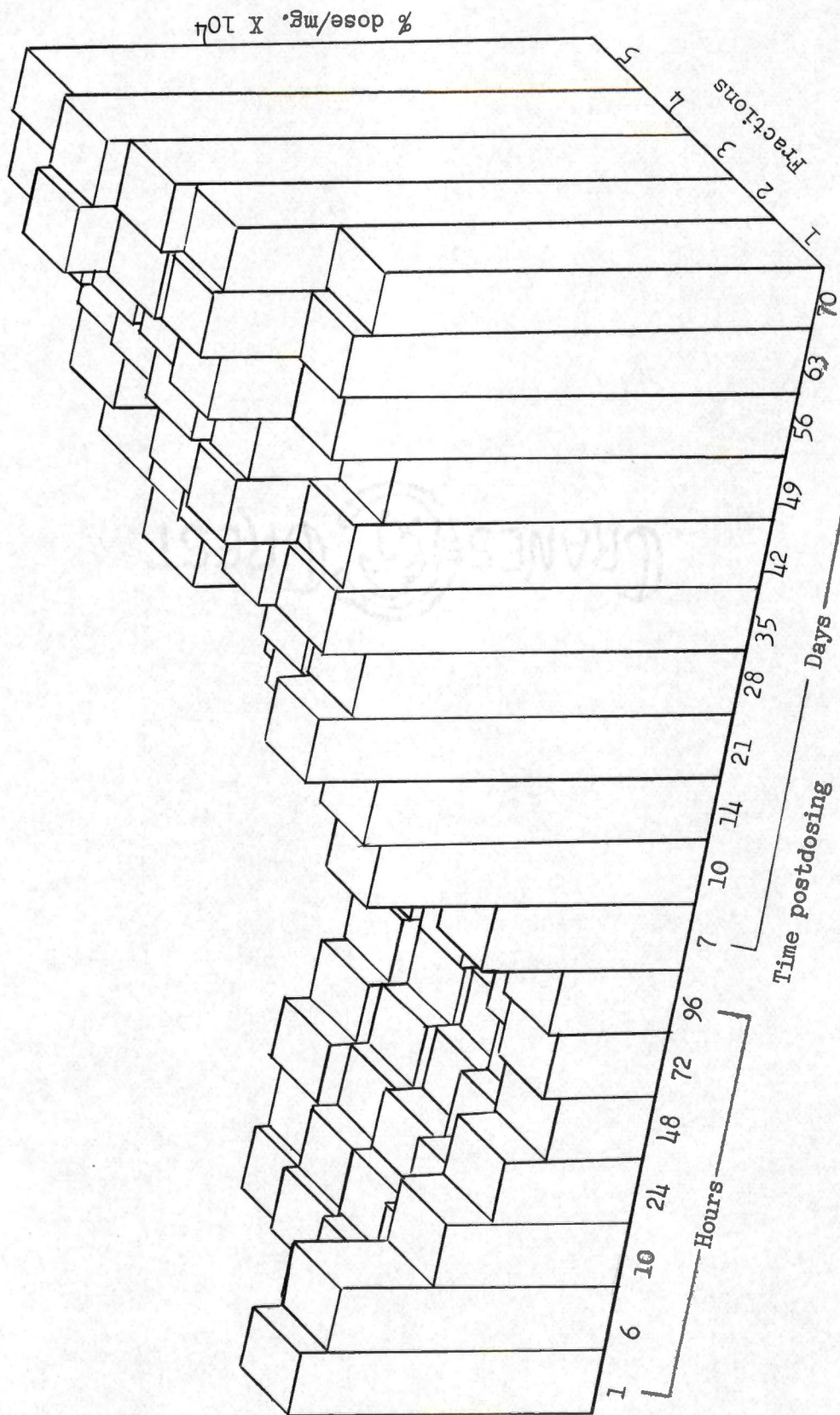


Figure 7. Pattern of ^{75}Se content in sheep erythrocyte age-fractions determined at different times after dosing.

all the remaining plasma and leucocytes.

It is believed that the radioselenium content of the red cell fractions from later sampling periods was not affected by unbound activity or trapped plasma as indicated by the low level of radio-selenium found in the red cell wash and plasma fractions at these times (Appendix Table III). The peak of radioactivity increased after 72 hr. postdosing for the duration of the 70-day period studied. The greatest concentration of radioactivity remained in fraction one of the sheep erythrocytes through the 21-day collection period, and then moved into fraction two. The peak of radioselenium concentration gradually moved down the red cell column and was last measured in fraction number four on the 70th day after the initial ^{75}Se administration. It is unfortunate that this study could not have been continued for a longer period of time, but it seems highly probable that the highest concentration of radioactivity would have been measured in fraction number five, the last, leaviest, and oldest fraction as the cells aged; followed by disappearance of the radioactivity as the cells died. A clearer presentation of the movement of the peak of ^{75}Se activity with a red blood cell population as the cells age was shown by the individual animal measurements of radioactivity in the separate aged fractions over a period of time (Appendix Table V). This variation substantiates the fact that very noticeable differences exist in the red cell metabolism among individual animals.

Similar trends were noted in the uptake of radioselenium in growing pigs in the early periods after ^{75}Se injection. As in the sheep erythrocytes, the measurements of ^{75}Se activity in the centrifugally fractionated porcine red blood cells showed the peak of radioactivity to be in the number one fraction, probably due to unbound ^{75}Se or trapped plasma. Again, this was well illustrated by the large amount of radioactivity in the red cell wash fraction when compared to that in the red cells during the early sampling periods of the red cell life span study (Figure 6, page 39). The ^{75}Se activity decreased through 48 hr. after dosing and began to increase again with the peak of activity in fraction one. As the red cells incorporated the label, the ^{75}Se activity in the number one fraction increased through day 14 and began to decrease again. From this time on, the influence from the age-dependent destruction of the erythrocytes was only slightly noticeable because of an overriding influence from a random destruction process. The peak of activity was measured in fraction one through 21 days; fraction two at 28 days; fraction three at 35, 42, and 49 days; fraction four at 56 and 63 days; and fraction five at 70 days after the initial selenium-75 dose (Figure 8 and Appendix Table VI). Even though a slight peak of radioactivity could be detected throughout the collection period, a gradual decline in the amount of radioselenium was evident with very small differences in selenium-75 content between fractions (Figure 8), particularly during the period between 21 and 63 days postdosing, where ^{75}Se disappearance from the red cell was the most rapid (Figure 5, page 37). Very little re-utilization of the erythrocyte-incorporated selenium

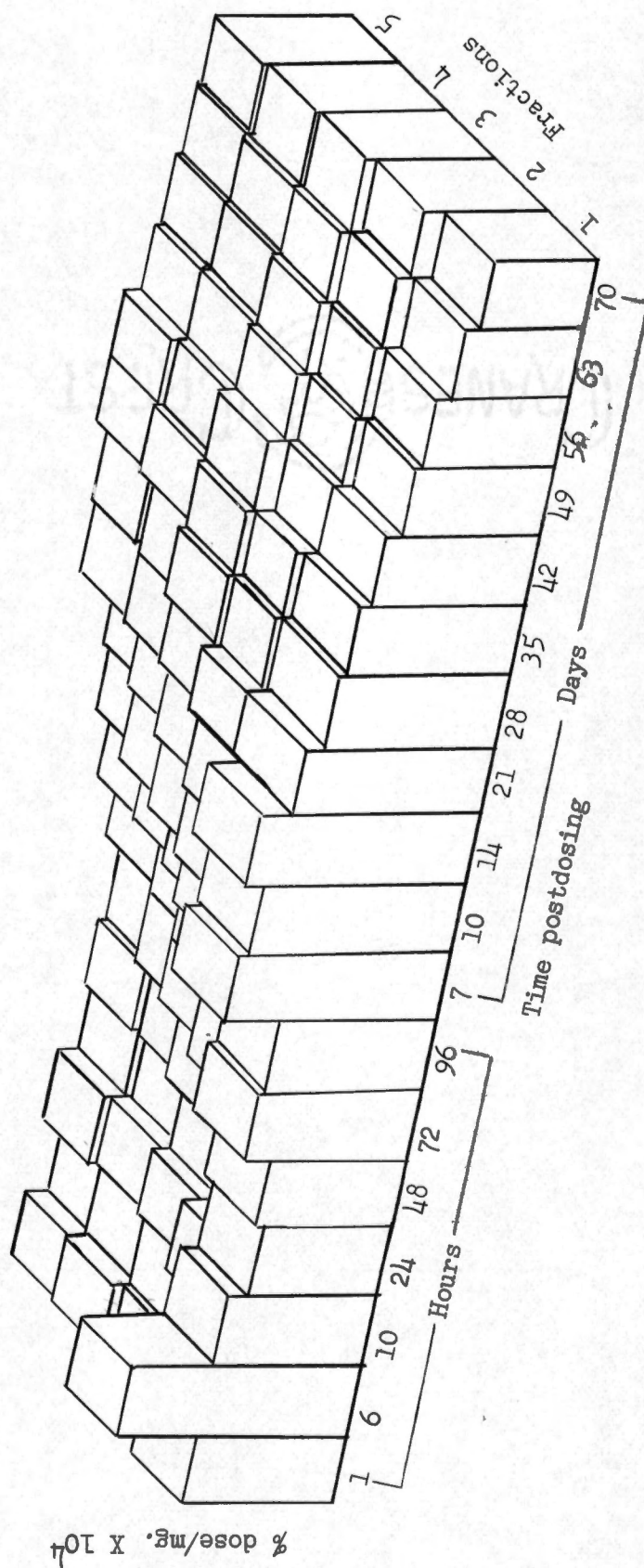


Figure 8. Pattern of ^{75}Se content in swine erythrocyte age-fractions determined at different times after dosing.

was noticed in the young cells at the top of the centrifuged cell column, as indicated by a constant decrease in the radioactivity measured in fraction one. Less animal variation was present in the selenium-75 concentration of the separate red cell age fractions of the pig erythrocytes over the 70-day period studied than in the same measurements in sheep, as illustrated by individual observations (Appendix Tables V and VI). It is obvious that the two species studied have different red cell life spans involving some metabolic factor beyond the scope of this study.

Tissue analyses. Measurement of radioselenium content of tissue samples from the liver and spleen of the sacrificed lambs indicated a very high concentration in liver and spleen tissue on the second and fourth days following isotope injection. At 49, 56, and 70 days after ^{75}Se administration, the concentration of radioactivity in the tissue samples had decreased to a level that was greatly reduced from the level measured in tissue taken at 2 and 4 days postdosing (Table I). The rate of decrease in tissue ^{75}Se was relatively slow from 49 to 70 days postdosing, possibly as a result of the somewhat stable concentration of radioactivity in the ovine erythrocytes during this collection period (illustrated in Figure 3, page 33, from the erythrocyte life span study).

Measurements of ^{75}Se in the pig liver and spleen tissues showed a similar trend to those in the sheep tissues for the times at which tissue samples were taken (Table I). The high values of ^{75}Se in the liver of both sheep and pigs during the early time periods after

TABLE I

CONCENTRATION OF ^{75}Se IN TISSUE SAMPLES FROM LIVER AND SPLEEN
OF SHEEP AND SWINE (% DOSE/GM. $\times 10^3$ TISSUE)^a

Sheep				Swine			
An. no.	Time postdosing	Liver	Spleen	An. no.	Time postdosing	Liver	Spleen
857	2 days	152	114	831	1 day	92	70
858	2 days	204	116	832	1 day	84	60
	Mean	178 ^b	115 ^b		Mean	88	65
856	4 days	256	101	825	4 days	84	53
843	4 days	163	78	837	4 days	132	94
	Mean	209 ^b	90		Mean	108 ^b	74
858	49 days	50	44	827	14 days	94	69
861	49 days	66	42	828	14 days	50	40
	Mean	58	43		Mean	72	54
849	56 days	66	40	829	28 days	50	37
855	56 days	48	44	830	28 days	42	29
	Mean	57	43		Mean	46	33
844	70 days	44	38	836	70 days	10	7
845	70 days	52	40	838	70 days	10	8
	Mean	48	39		Mean	10	8

^aPer cent dose is average of triplicates of each tissue sample per animal.

^bValues of over 100 per cent of dose were calculated when % dose/gm. was multiplied by 10^3 to obtain whole numbers for all values listed.

injection indicate a possible excretory role for this organ. Similar uptake of selenium-75 in pig liver tissue has been reported by Lindberg and Tanhuanpää (1965) and Orstadius and Åberg (1961), using ^{75}Se -sodium selenite; and in sheep liver tissue by Wright and Bell (1964, 1966) with $\text{H}_2^{75}\text{SeO}_3$. It was hoped that supporting evidence could have been produced to show that a higher ratio of random to age-dependent red cell destruction exists in the pig than exists in the sheep. It seems logical that in the predominantly random destruction system, the radioactivity in these reticuloendothelial cell-containing organs, the liver and especially the spleen, would remain relatively high throughout the life span of the erythrocytes originally incorporating the highest amount of selenium label with a slight increase at the death of these red blood cells. This trend of selenium-75 measurements in the liver and spleen would be in contrast to that in a system where an age-dependent process of erythrocyte destruction predominates. After a rapid drop in ^{75}Se content during early collection periods, the tissue level of ^{75}Se should remain relatively low throughout the life span of the erythrocyte with an increase in tissue ^{75}Se at erythrocyte death. However, an inadequate number of tissue-sampling periods failed to clearly illustrate this species difference.

III. INCORPORATION OF SELENIUM-75 INTO ERYTHROCYTE PROTEINS

Hemoglobin and stroma. Results from hemoglobin analysis of sheep and pig erythrocytes shown in Table II indicated that the injected radio-selenium was incorporated into the globin fraction of the erythrocyte

TABLE II
CONCENTRATION OF ^{75}Se IN SHEEP AND SWINE ERYTHROCYTE
HEMOGLOBIN^a AND STROMA^b (% DOSE/ML. X 10^4)^c

Time postdosing	Sheep		Swine	
	Hemoglobin	Stroma	Hemoglobin	Stroma
Hours				
1	5.95	3.66	4.68	3.10
2	4.76	2.11	4.59	2.80
4	5.27	0.13	7.31	0.00
8	3.23	1.23	3.91	1.75
10	3.57	0.00	3.57	5.83
24	2.55	0.63	3.40	0.04
48	1.96	0.77	2.89	0.85
72	3.40	0.53	4.25	2.85
96	4.93	0.65	6.46	1.58
Days				
5	6.12	1.75	6.80	1.26
6	10.54	2.68	4.76	4.56
7	8.33	6.91	5.95	4.00
8	10.71	5.71	6.04	4.76
9	12.92	7.42	6.20	4.98
10	15.56	9.92	6.88	4.32
14	25.50	12.74	6.97	5.51
21	25.84	21.37	8.76	2.88
28	47.68	9.09	7.90	2.32
35	58.31	3.58	10.03	0.00

^aHemoglobin content of ^{75}Se was also globin content of ^{75}Se since no activity could be measured in the hemin.

^bSelenium-75 in stroma was calculated mathematically by subtracting ^{75}Se content of hemoglobin in 1 ml. of erythrocytes from ^{75}Se content of 1 ml. of erythrocytes.

^cPer cent dose of hemoglobin was calculated per ml. of diluted hemoglobin, e.g., 1 ml. red blood cells plus 7.5 ml. distilled water; count 1 ml. hemoglobin, then multiplied by a dilution factor of 8.5 to derive content of ^{75}Se in hemoglobin of 1 ml. of red blood cells.

hemoglobin with no measurable activity in the hemin fraction which agrees with results presented by Wright (1965). These data also indicated that part of the increase in red cell activity during the first 14 days for the pig and the first 21 days for the sheep after the intravenous injection of ^{75}Se -selenious acid was due to stroma uptake as well as hemoglobin incorporation (Table II, page 51). A sudden drop in stroma activity after 14 and 21 days for the pig and the sheep, respectively, is not understood at present, but it indicates the possibility of more than one binding mechanism for ^{75}Se in the red cell. The radioactivity in the stroma at these early sampling periods may have been due to residual inorganic selenium that was bound to erythrocytes already in circulation at the time of dosing but was not incorporated into the newly formed red cells. Whether this stroma-bound selenium-75 represents a separate binding mechanism from the one responsible for the initial rapid uptake and clearance of radioselenium during the first 48 hours after ^{75}Se administration remains to be established.

Preferential binding by reticulocytes. Hemoglobin incorporation of selenium-75 has been further substantiated by the fact that radio-selenium localizes in the hematopoietically active areas of the bone marrow as shown by autoradiography (Wright, 1965). A microscopic analysis of bone marrow microautoradiograms in this study gave some indication that the ^{75}Se was being incorporated into the reticulocyte stage of the red cell which is known to be actively producing hemoglobin. However, due to a high amount of background activity in the microautoradiograms, radioactivity was not localized enough to give positive

identification of the stages of erythrocyte production that were selectively incorporating the label.

Penner (1964a, 1966) illustrated that ^{75}Se -selenomethionine was incorporated into human hemoglobin by the erythroblasts and reticulocytes. Supporting evidence to the preferential absorption of selenium-75 by the reticulocytes was offered by the centrifugal separation of young and old erythrocytes from both sheep and pigs. Results indicated that the young cells at the top of the centrifuged red cell column definitely took up more label than the older cells at the bottom (Figures 7 and 8, pages 44 and 47). ^{75}Se -selenomethionine has also been shown to be selectively incorporated into the top layers of the centrifugally sedimented red cells from humans (Awwad *et al.*, 1966a). It is interesting to note that Burk *et al.* (1967) failed to show a preferential binding of ^{75}Se -selenite to reticulocytes *in vitro* which may support the idea of more than one binding mechanism for selenium in the erythrocyte.

^{75}Se -selenoamino acids in globin. A two-way descending paper chromatographic analysis of pig and sheep erythrocyte globin amino acids revealed, in both species studied, that the majority of the ^{75}Se activity was located in three areas. Areas 1, 7, and 8, as shown in Figure 9, contained the greatest concentration of selenium-75 when counting data from the representative chromatograms from each collection period were averaged. When compared to a standard map of 19 naturally occurring amino acids (Figure 10), the areas analyzed from representative chromatograms of globin amino acids from sheep and swine (Figure 11)

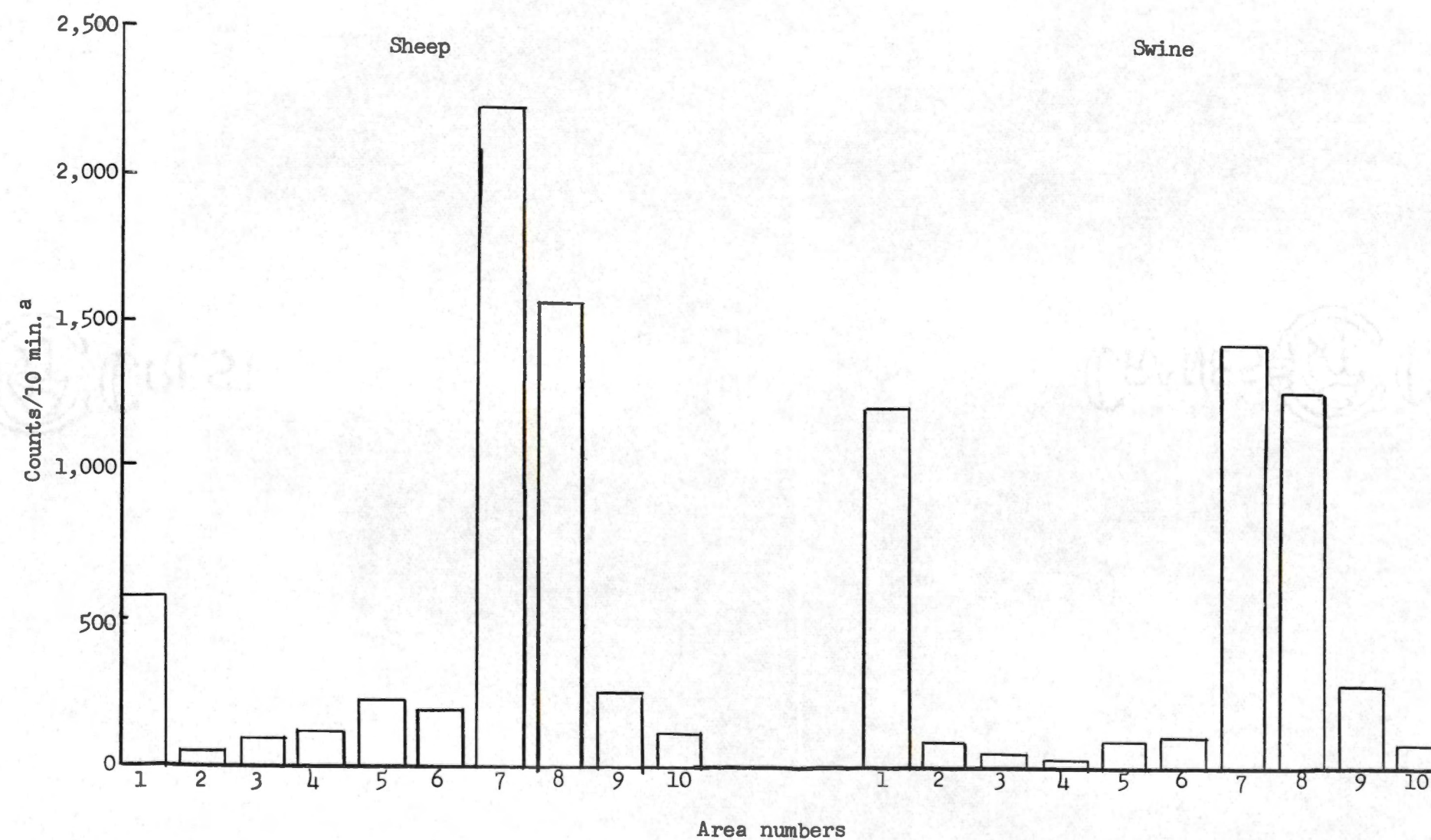
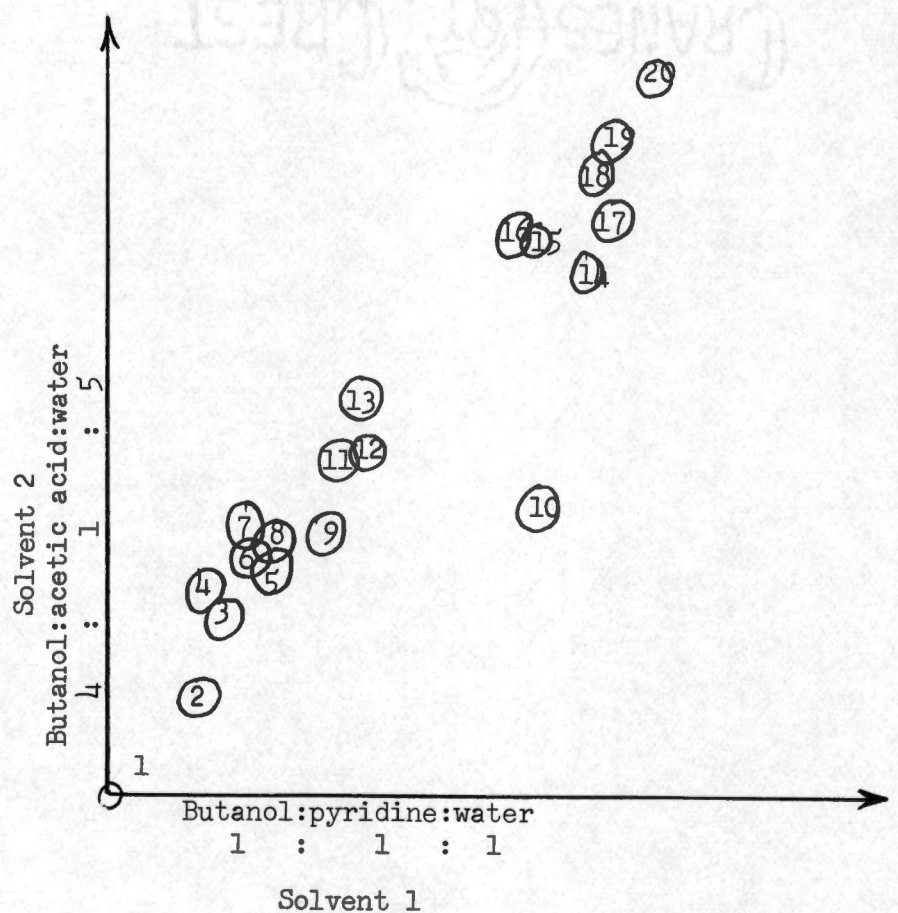


Figure 9. Selenium-75 content of areas of paper chromatograms of globin amino acids of sheep and swine.

^aCounts were adjusted for radioactive decay.



- | | |
|------------------------------|-------------------|
| 1. Origin | 11. Alanine |
| 2. Cystine ^a | 12. Threonine |
| 3. Lysine | 13. Proline |
| 4. Aspartic acid | 14. Tyrosine |
| 5. Histidine | 15. Methionine |
| 6. Arginine | 16. Valine |
| 7. Glutamic acid | 17. Tryptophan |
| 8. Glycine | 18. Isoleucine |
| 9. Serine | 19. Phenylalanine |
| 10. Appeared with methionine | 20. Leucine |

Figure 10. Standard map of 19 naturally occurring amino acids.

^aCysteine was oxidized to cystine in chromatography.

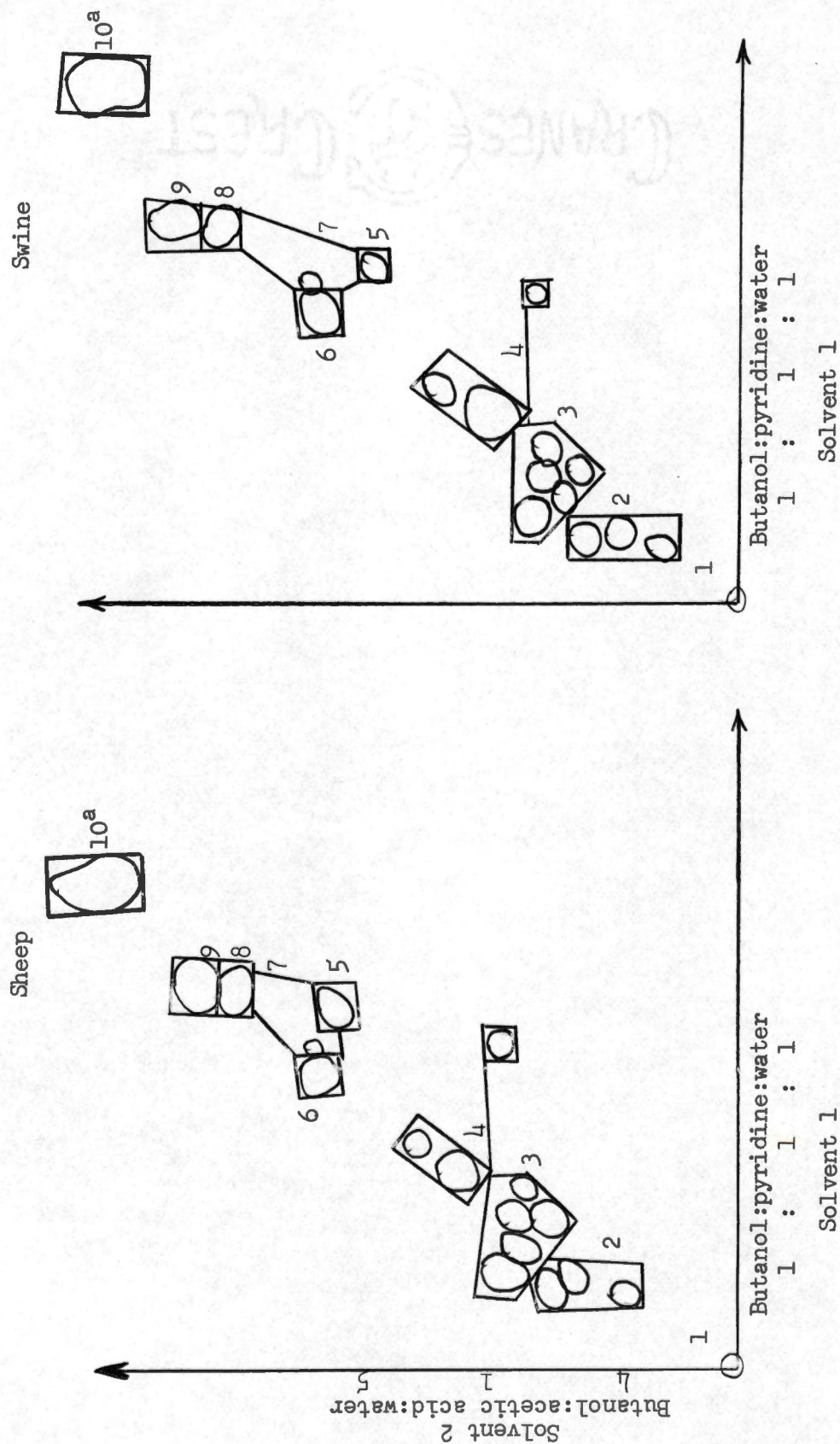


Figure 11. Chromatograms of sheep and swine globin amino acids with areas counted for selenium-75 radioactivity.

^aArea 10 was phenol red marker used to control length of solvent flow.

were shown to contain the following: (1) origin; (2) cystine, lysine, and aspartic acid; (3) histidine, arginine, glycine, serine, and glutamic acid; (4) alanine, threonine, proline, and an unidentified spot appearing in the same location as a spot appearing with the methionine standard; (5) tyrosine; (6) methionine and valine; (7) isoleucine and tryptophan; (8) phenylalanine; (9) leucine; and (10) phenol red marker. Isoleucine did not appear on pig globin chromatograms with a ninhydrin stain and has been reported not to be present in sheep hemoglobin (Huisman et al., 1965). Tryptophan is destroyed by acid hydrolysis (Block, Durrum, and Zweig, 1958) and only appeared on the standard chromatograms.

A comparison of the measurements of the potassium iodoplatinate positive amino acid spots, cystine and methionine (Block and Bolling, 1951; Toennies and Kolb, 1951; Winegard and Toennies, 1948) in a standard amino acid mixture with the measurements of the migration of ninhydrin-stained spots of individual standard cystine and methionine was made for positive identification of these sulfur amino acids. Cystine and methionine have been reported to have identical paper chromatographic movement to their selenium analogues.

Measuring from the origin (Rf's were not used since the solvent front traveled too far for accurate determinations), values for standard cystine when spotted singly were 5.0 cm. down and 4.6 cm. across; for methionine measured in the same fashion, values were 26.2 cm. down and 21.5 cm. across. Average values for the white "iodoplatinate" spots in the globin protein were: (1) pig--5.4 cm. down, 4.4 cm. across for

cystine; 24.6 cm. down, 20.8 cm. across for methionine; (2) sheep--5.3 cm. down, 4.4 cm. across for cystine; 24.3 cm. down, 21.4 cm. across for methionine. These data illustrated that cystine and methionine occupied the same positions on the chromatograms of pig and sheep globin amino acids as they did on the standard chromatograms. It was also found that standard ^{75}Se -selenomethionine (see Materials and Methods, Chapter II) migrated almost identically to methionine when each was spotted separately (Appendix Table VII). This finding concurs with other reports that the chromatographic behavior of selenocystine and selenomethionine was indistinguishable from their corresponding sulfur compounds (Imbach and Sternberg, 1967; McConnell and Wabnitz, 1957; Peterson and Butler, 1962; Rosenfeld, 1962a; Schoental, 1967).

Due to the unavailability of commercial ^{75}Se -selenocystine, no comparison of chromatographic migration was made between cystine and selenocystine, but there was no reason to suspect any difference due to the evidence just presented. However, autoradiograms of the chromatograms of the globin amino acids from sheep and pigs used in this study and of a standard amino acid mixture containing ^{75}Se -selenomethionine, shown in Figure 12, revealed that the spot of radioactivity was below the selenomethionine-methionine spot and more in the vicinity of isoleucine and phenylalanine (area 7, Figure 11, page 56; spot 18, Figure 10, page 55) (area 8, Figure 11, page 56; spot 19, Figure 10, page 55). These results were confirmed by a radioactivity counting analysis of the amino acids in a standard amino-acid solution containing ^{75}Se -selenomethionine as illustrated in Figure 13. The highest amount of

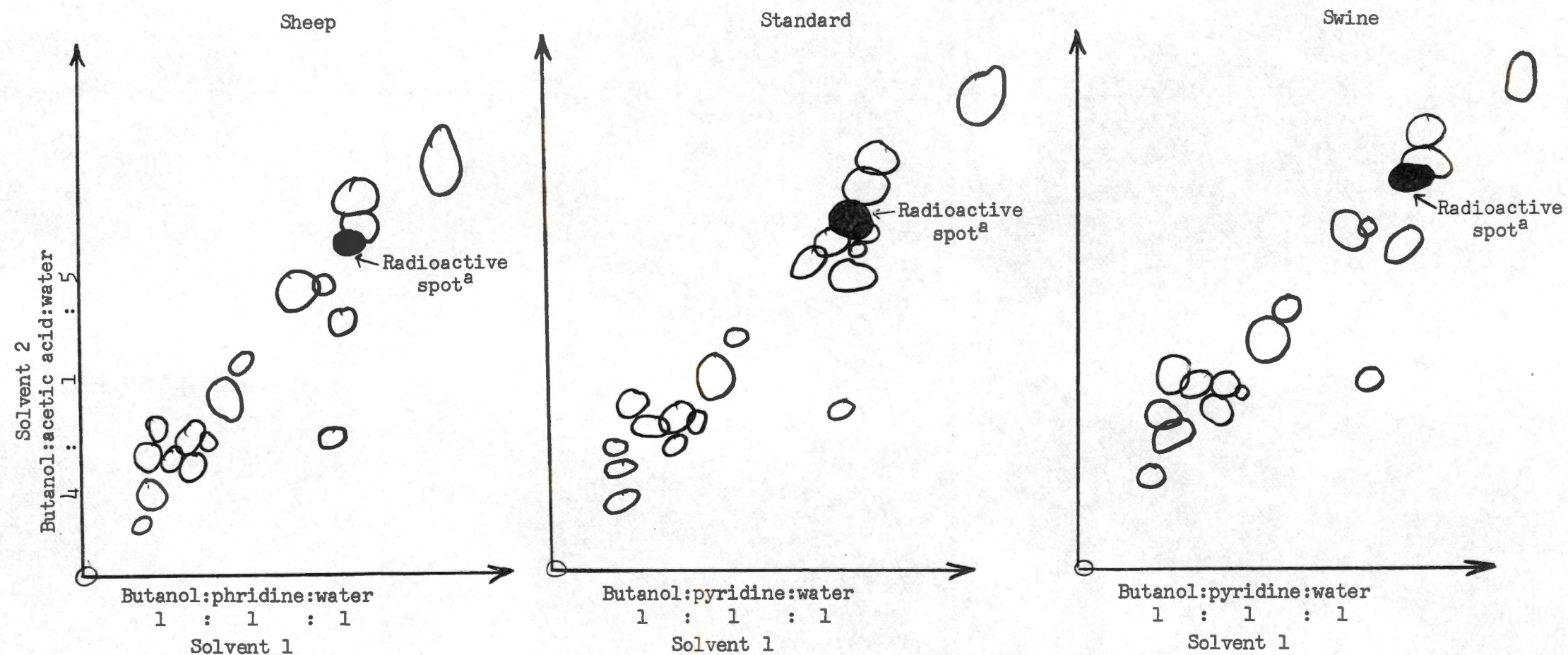


Figure 12. Representative chromatograms of sheep and swine globin amino acids compared to chromatogram of standard amino acid mixture.

^aRadioactive spot was shown by autoradiographic overlay.

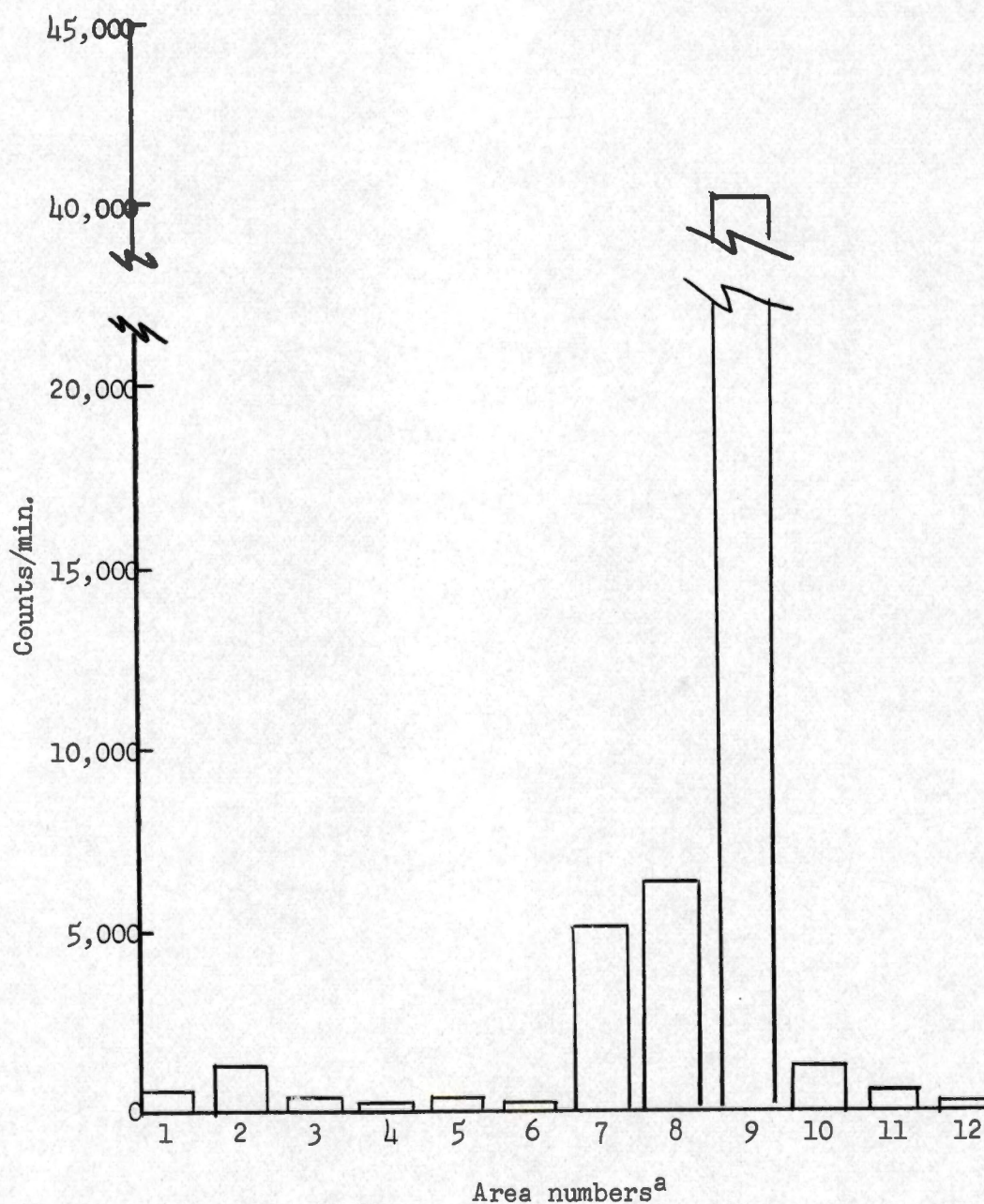


Figure 13. Selenium-75 content of areas of chromatogram of standard amino acid mixture.

^aAreas 1-6 are the same as previously shown (Figure 11, page 56); area 7 contains methionine; area 8 contains tryptophan; area 9 contains isoleucine; area 10 contains phenylalanine; area 11 contains leucine; and area 12 contains phenol red marker.

radioactivity was found in area 9 (isoleucine). Considerable amounts of activity were also found in area 8 (tryptophan) and area 7 (methionine) in that order of decreasing magnitude. The location of the majority of the applied radioactivity in three areas on the standard chromatograms may be explained by the large amount of ^{75}Se -selenomethionine used for chromatography (Materials and Methods, Chapter II) in order to count the radioactivity. The discrepancy between the measurement of the ninhydrin-stained spot of the standard selenomethionine and the location of the radioactivity on the autoradiograms is not entirely explainable by present results.

There is a possibility that the selenomethionine may run differently in an amino acid mixture than when spotted alone in this system due to bonding of selenomethionine to other amino acids by the anionic attachment of selenium to the basic endings of other amino acids (Imbach and Sternberg, 1967). This single spot of radioactivity shown on the autoradiograms of the chromatograms of the sheep and pig globin amino acids strongly suggest that the ^{75}Se in areas 7 and 8 (Figure 11, page 56) may belong to one compound. A comparison of spots obtained from the globin amino acids of both species with the spots obtained from spotting cysteic acid and methionine sulfone indicated that no recognizable oxidation of the sulfur-containing amino acids occurred during chromatography.

IV. BINDING MECHANISMS OF ^{75}Se IN THE ERYTHROCYTE

It is obvious from the results of this study that at least two binding mechanisms exist for selenium-75 in both sheep and swine erythrocytes. One is a rapid and almost immediate binding of selenium to the erythrocyte possibly by an exchange type of reaction of SeO_3^{--} for $-\text{SH}$, Se for S or an addition of SeO_3^{--} to RSH to give RS-SeH (Cavalieri *et al.*, 1967). The rapid uptake of selenium-75 by this initial binding mechanism indicates that enzymes or protein synthesis was not involved. The fact that cyanide inhibits the *in vitro* uptake of radioactive selenite by sheep red blood cells (Wright and Bell, 1963) also suggests a reaction with sulfhydryl groups of erythrocyte proteins since cyanide reacts with cysteine residues of proteins (Cavalieri *et al.*, 1967). Perhaps the initial binding of ^{75}Se to the erythrocyte is merely an exchange for non-radioactive selenium already present in the proteins which might be necessary for maintenance of the essential thiol groups in their reactive state (Schoental, 1967).

The second and much slower binding process as illustrated by the gradual incorporation of selenium-75 into sheep and pig hemoglobin over a period of several days is involved in globin protein synthesis. The preferential binding of selenium-75 to the reticulocyte layer of the centrifuged red cell column of both the sheep and the pig (Figures 7 and 8, pages 44 and 47) strongly supports this fact. It appears that this second mechanism is similar in both sheep and swine since the radioactivity was found in the same location on the chromatograms from both

species (Figures 9 and 12, pages 54 and 59). This suggests a mechanism of incorporation other than substitution of Se for S, in the sulfur-containing amino acids, by the rumen bacteria of sheep, particularly after an intravenous injection for reasons previously discussed, and since no evidence is available to indicate that the nonruminant animal can synthesize sulfur amino acids from inorganic sulfur (Rosenfeld, 1964). In all probability, the compound in the globin of both species that contained the ^{75}Se radioactivity was seleniated methionine, since it had similar chromatographic migration to the selenomethionine in the standard amino acid mixture.

The fact that selenium radicals may also be bound to the basic endings of other amino acids such as leucine (Imbach and Sternberg, 1967; McConnell and Wabnitz, 1957) could account for some of the activity in the phenylalanine and isoleucine areas (areas 7 and 8, Figure 11, page 56), but the majority of the activity found on the chromatograms of the sheep and swine globin was between these two areas as shown by autoradiography. This anionic bonding of Se to other amino acids also leads to the possibility that the chromatographic position of this seleno-compound may be due to a S:Se or Se:Se bond in methionine which may bind two amino acids. A seleno-sulfur type bonding is also suggested by the fact that no radioactivity was found in the cystine area.

This high amount of ^{75}Se found at the origin of the amino acid hydrolysate spotting on the chromatograms would indicate that the selenium was bound to cystine in the $\text{HOOC}-\underset{\text{NH}_2}{\text{CH}}-\text{CH}_2-\text{S}-\text{Se}-\text{SCH}_2-\underset{\text{NH}_2}{\text{CH}}\text{COOH}$ form.

Painter (1941) has shown that RS-Se-SR compounds are unstable and may separate to give RSSR + Se. In addition, selenocystine has been shown to decompose in a water solution on electrodialysis to give elemental selenium (Rosenfeld and Beath, 1964). No other spots containing radioactivity were detected to indicate that selenocystine migrates differently from cystine in this chromatographic system. There is a possibility that binding of Se in cystine and methionine in globin proteins is not identical.

It is evident that selenium metabolism varies from animal to animal and from species to species. It is also evident that selenium may be utilized differently in different tissues of the animal and that much more research is needed to clarify these differences. An exploration of the field of selenium metabolism has proven very rewarding to present and offers many more challenges and opportunities for the future.

CHAPTER V

SUMMARY AND CONCLUSIONS

Selenium-75 was selectively bound by the reticulocytes and was incorporated into the globin moiety of both sheep and swine hemoglobin following a single intravenous dose of $\text{H}_2^{75}\text{SeO}_3$. The radioselenium remained incorporated in the ovine and porcine erythrocyte throughout its life span. Estimates of erythrocyte life span calculated from ^{75}Se turnover averaged 127 days for the growing lambs and 72 days for the growing pigs.

Results indicated that both an age-dependent and a random-destruction process of erythrocytes operated in both sheep and swine. The age-dependent process was predominant in sheep erythrocyte destruction, but random-erythrocyte destruction was higher in the pigs.

Data obtained in the erythrocyte life span study indicated at least two different binding mechanisms of ^{75}Se in sheep and swine erythrocytes: (1) an immediate and very rapid binding and release process possibly an exchange-type reaction of Se for S or SeO_3 --for SH, or an addition of Se to S in the erythrocyte proteins; (2) a slower binding involving protein synthesis of the erythrocyte hemoglobin. The remote possibility of a third type of binding mechanism was implicated by the measurement of a considerable amount of selenium-75 in the erythrocyte stroma during the first 21 days after ^{75}Se administration to the animal.

A two-way paper chromatographic analysis of the erythrocyte globin amino acids of sheep and swine indicated that ^{75}Se was incorporated into an amino acid compound exhibiting similar chromatographic behavior to standard ^{75}Se -selenomethionine. Neither the standard ^{75}Se -selenomethionine nor the ^{75}Se -containing compound in the sheep and pig globin migrated to the same chromatographic location as methionine. Whether this indicates a difference in chromatographic behavior of selenomethionine and methionine in the solvent system used in this study or a binding of selenium and/or selenomethionine to the basic endings of other amino acids is not understood. A binding mechanism other than a mere substitution of selenium-75 for sulfur in the sulfur-containing amino acids was suggested by the fact that the chromatographic location of the selenium-containing compound in the globin protein was identical for both the ruminant (sheep) and the nonruminant (pig). The fact that no radioactivity was measured in the cystine area suggests an unstable Se to S type bonding for cystine since considerable amounts of ^{75}Se were found at the origin of amino acid hydrolysate spotting on the paper chromatograms.

There is a definite need for further investigation of selenium metabolism in the erythrocyte not only to elucidate the possible clinical and diagnostic uses of selenium in blood research, but to clarify the nutritional and physiological role of selenium in the biological system and to add to the knowledge of erythrocyte metabolic processes and their variations in different species.

LITERATURE CITED



LITERATURE CITED

- Ackerson, C. W. 1965. Selenium--the bad and the good. *Feed Age* 15:32.
- Anderson, M. S., H. W. Lakin, K. C. Beeson, F. F. Smith, and E. Thacker (eds.). 1961. *Selenium in Agriculture*. Agriculture Handbook No. 20, U. S. Department of Agriculture, Washington, D. C.
- Anghileri, L. J., and R. Marqués. 1965. Fate of injected Se^{75} -methionine and Se^{75} -cystine in mice. *Arch. Biochem. Biophys.* 111:580.
- Awwad, H. K., E. J. Potchen, S. J. Adelstein, and J. B. Dealy, Jr. 1966a. Se^{75} -selenomethionine incorporation into human plasma proteins and erythrocytes. *Metabolism* 15:626.
- Awwad, H. K., E. J. Potchen, S. J. Adelstein, and J. B. Dealy, Jr. 1966b. The regional distribution of Se^{75} -selenomethionine in the rat. *Metabolism* 15:370.
- Baker, N. F., and J. R. Douglas. 1957. The pathogenesis of tri-chostrongyloid parasites. II. Ferrokinetic studies in ruminants. *Am. J. Vet. Res.* 18:295.
- Blau, M., and M. A. Bender. 1962. Se^{75} -selenomethionine for visualization of the pancreas by isotope scanning. *Radiology* 78:974.
- Blau, M., and R. F. Manske. 1961. The pancreas specificity of Se^{75} -selenomethionine. *J. Nuc. Med.* 2:102.
- Block, R. J., and D. Bolling (eds.). 1951. *The Amino Acid Composition of Proteins and Foods* (2nd ed.). Charles C. Thomas, Springfield, Ill.
- Block, R. J., E. L. Durrum, and G. Zweig (eds.). 1958. *A Manual of Paper Chromatography and Paper Electrophoresis* (2nd ed.). Academic Press, Inc., New York.
- Borun, E. R., W. G. Figueroa, S. M. Perry. 1957. The distribution of Fe^{59} tagged human red cells in centrifuged specimens as a function of age. *J. Clin. Invest.* 36:676.
- Buescher, R. G., M. C. Bell, and R. K. Berry. 1961. The effect of excessive calcium on selenium-75 in swine. *J. Animal Sci.* 20:368.

- Burk, R. F., Jr., W. N. Pearson, R. P. Wood, II, and F. Viteri. 1967. Blood-selenium levels and in vitro red blood cell uptake of ^{75}Se in kwashiorkor. *Am. J. Clin. Nutr.* 20:723.
- Bush, J. A., N. I. Berlin, W. N. Jensen, A. B. Brill, G. E. Cartwright, and M. M. Wintrobe. 1955. Erythrocyte life span in growing swine as determined by glycine- ^{14}C . *J. Exp. Med.* 101:451.
- Cavalieri, R. R., R. Dickard, G. L. Searle, and K. G. Scott. 1967. Uptake of selenite labelled with selenium-75 by human leucocytes in vitro. *Nature* 215:1092.
- Celander, D. R. 1964. Biosynthesis of radioactive plasma proteins through the use of Se^{75} . *Am. Osteopathic Assn. J.* 63:871. (Abstr.).
- Coopersmith, A., and M. Ingram. 1966. Personal communication. University of Rochester Atomic Energy Project, Rochester, New York.
- Cowie, D. B., and G. N. Cohen. 1957. Biosynthesis by Escherichia coli of active altered proteins containing selenium instead of sulfur. *Biochim. et Biophys. Acta* 26:252.
- Cummins, L. M., J. L. Martin, and D. D. Maag. 1966. Is selenocystine and selenomethionine synthesized in vivo from sodium selenite? *Am. Chem. Soc. 152nd Meeting.* (Abstr.).
- Dam, H., G. K. Nielsen, I. Prange, and E. Søndergaard. 1957. The exudative diathesis produced by *Torula* yeast. *Experientia* 13:493.
- Dornhorst, A. C. 1951. The interpretation of red cell survival curves. *Blood* 6:1284.
- Draper, H. H. 1957. Ineffectiveness of selenium in the treatment of nutritional muscular dystrophy in the rabbit. *Nature* 180:1419.
- Eck, R. V., and M. O. Dayhoff. 1966. Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, Md.
- Farkas, W. R. 1966. Personal communication. University of Tennessee Memorial Research Center, Knoxville, Tenn.
- Ganther, H. E., and C. A. Baumann. 1962. Selenium metabolism. II. Modifying effects of sulfate. *J. Nutr.* 77:408.
- Gatien, L., E. Celander, G. Gordon, M. Jacquo, Jr., and D. R. Celander. 1964. In vivo erythrocyte labeling with inorganic radioselenium. *Am. Osteopathic Assn. J.* 63:871. (Abstr.).

- Green, J., T. Deplick, J. Bunyan, E. E. Edwin, and D. McHale. 1961. Ubiquinone (coenzyme Q) and the function of vitamin E. *Nature* 190:318.
- Halverson, A. W., and K. J. Monty. 1960. An effect of dietary sulfate on selenium poisoning in rats. *J. Nutr.* 70:100.
- Hansard, S. L., and E. Kincaid. 1956. Red cell life span of farm animals. *J. Animal Sci.* 15:1300. (Abstr.).
- Harris, P. L., M. I. Ludwig, and K. Schwarz. 1958. Ineffectiveness of Factor 3-active selenium compounds in resorption-gestation bioassay for vitamin E. *Proc. Soc. Exp. Biol. Med.* 97:686.
- Huisman, T. H. J., C. A. Reynolds, A. M. Dozy, and J. B. Wilson. 1965. The structure of sheep hemoglobins. *J. Biol. Chem.* 240:2455.
- Imbach, A., and J. Sternberg. 1967. Metabolic studies with seleniated compounds. I. Kinetic studies with Se^{75}O_3 in rats. *Intern't'l. J. App. Rad. and Iso.* 18:545.
- Ingram, V. M., and O. W. Stretton. 1962. Human hemoglobin A₂. I. Comparison of hemoglobin A₂ and A. *Biochim. et Biophys. Acta* 62:456.
- Jacobsson, S. O. 1966a. Metabolism of selenium in sheep and mice studied with Se^{75} -sodium selenite, Se^{75} -selenomethionine, and Se^{75} -selenocystine. Royal Veterinary College, Stockholm, Sweden.
- Jacobsson, S. O. 1966b. Uptake of Se^{75} in tissues of sheep after administration of a single dose of Se^{75} -sodium selenite, Se^{75} -selenomethionine, or Se^{75} -selenocystine. *Acta Vet. Scand.* 7:303.
- Jacobsson, S. O., H. E. Oksanen, and E. Hansson. 1965. Excretion of selenium in the milk of sheep. *Acta Vet. Scand.* 6:299.
- Jensen, W. N., J. A. Bush, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1956. The kinetics of iron metabolism in normal growing swine. *J. Exp. Med.* 103:145.
- Judd, J. T., and G. Matrone. 1962. Sheep erythrocyte life span in estimation of hemoglobin turnover in iron metabolism studies. *J. Nutr.* 77:264.
- Lajtha, L. G. 1961. *The Use of Isotopes in Haematology.* Blackwell Scientific Publications, Oxford.
- Lindberg, P., and E. Tanhuanpää. 1965. Retention of selenium in tissues of swine after a single intramuscular administration of sodium selenite. *Acta Vet. Scand.* 6:268.

- McConnell, K. P. 1948. Passage of selenium through the mammary glands of the white rat and the distribution of selenium in the milk proteins after subcutaneous injections of sodium selenate. *J. Biol. Chem.* 173:653.
- McConnell, K. P. 1963. Metabolism of selenium in the mammalian organism. *Agr. Food Chem.* 11:385.
- McConnell, K. P., and G. J. Cho. 1965. Transmucosal movement of selenium. *Am. J. Physiol.* 208:1191.
- McConnell, K. P., and B. J. Cooper. 1950. Distribution of selenium in serum proteins and red blood cells after subcutaneous injection of sodium selenate containing radioselenium. *J. Biol. Chem.* 183:459.
- McConnell, K. P., and R. D. Dallam. 1962. Time-distribution examination of the in vivo incorporation of selenium into cytochrome-c of the rat and its turnover. *Nature* 193:746.
- McConnell, K. P., and A. E. Kreamer. 1960. Incorporation of selenium-75 into dog hair. *Proc. Soc. Exp. Biol. Med.* 105:170.
- McConnell, K. P., and R. S. Levy. 1962. Presence of selenium-75 in lipoproteins. *Nature* 195:774.
- McConnell, K. P., and D. M. Roth. 1962. ⁷⁵Se in rat intracellular liver fractions. *Biochim. et Biophys. Acta* 62:503.
- McConnell, K. P., and D. M. Roth. 1964. Passage of selenium across the placenta and also into the milk of the dog. *J. Nutr.* 84:340.
- McConnell, K. P., and D. M. Roth. 1965. Selenium and rabbit skeletal muscle aldolase and myosin. *Proc. Soc. Exp. Biol. Med.* 120:88.
- McConnell, K. P., and D. M. Roth. 1966a. Incorporation of selenium into rat liver ribosomes. *Arch. Biochem. Biophys.* 117:366.
- McConnell, K. P., and D. M. Roth. 1966b. Respiratory excretion of selenium. *Proc. Soc. Exp. Biol. and Med.* 123:919.
- McConnell, K. P., and C. H. Wabnitz. 1957. Studies on the fixation of radioselenium in proteins. *J. Biol. Chem.* 226:765.
- McDonald, T. P. 1966. Personal communication. University of Tennessee Memorial Research Center, Knoxville, Tenn.
- Mudd, S. H., and G. L. Cantoni. 1957. Selenomethionine in enzymatic transmethylation. *Nature* 180:1052.

- Neuberger, A., and J. S. F. Niven. 1951. Hemoglobin formation in rabbits. *J. Physiol.* 112:292.
- O'Connell, D. J., C. J. Caruso, and M. D. Sass. 1965. Separation of erythrocytes of different ages. *Clin. Chem.* 11:771.
- Olcott, H. S., W. D. Brown, and J. Van der Veen. 1961. Selenomethionine as an antioxidant. *Nature* 191:1201. (Abstr.).
- Orstadius, K., and B. Åberg. 1961. Distribution of Se^{75} -tagged sodium selenite in pigs. *Acta Vet. Scand.* 2:60.
- Painter, E. P. 1941. The chemistry and toxicity of selenium compounds, with special reference to the selenium problems. *Chem. Rev.* 28:179.
- Pardue, J. W. 1967. Personal communication. University of Tennessee Agricultural Research Laboratory, Oak Ridge, Tenn.
- Patterson, E. L., R. Milstrey, and E. L. R. Stokstad. 1957. Effect of selenium in preventing exudative diathesis in chicks. *Proc. Soc. Exp. Biol. Med.* 95:617.
- Penner, J. A. 1964a. Selenomethionine incorporation into hemoglobin. *Clin. Res.* 12:228.
- Penner, J. A. 1964b. Selenomethionine incorporation into plasma proteins. *Clin. Res.* 12:277. (Abstr.).
- Penner, J. A. 1966. Investigation of erythrocyte turnover with selenium-75-labelled methionine. *J. Lab. Clin. Med.* 67:427.
- Peterson, P. J., and G. W. Butler. 1962. Paper chromatographic and electrophoretic systems for the identification of sulphur and selenium amino acids. *J. Chromatog.* 8:70.
- Peterson, P. J., and D. J. Spedding. 1963. The excretion by sheep of selenium-75 incorporated into red clover (Trifolium paratense L): the chemical nature of the excreted selenium and its uptake by three plant species. *New Zealand J. Agr. Res.* 6:13.
- Popp, R. A. 1966. Personal communication. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.
- Rigas, D. A., and R. D. Koler. 1961. Ultracentrifugal fractionation of human erythrocytes on the basis of cell age. *J. Lab. Clin. Med.* 58:242.
- Robinson, W. O. 1933. Determination of selenium in wheat and soils. *J. Assn. Official Agr. Chem.* 16:423.

- Rosenfeld, I. 1961. Biosynthesis of seleno-compounds from inorganic selenium by sheep. Fed. Proc. 20:10. (Abstr.).
- Rosenfeld, I. 1962a. Biosynthesis of seleno-compounds from inorganic selenium by sheep. Proc. Soc. Exp. Biol. Med. 111:670.
- Rosenfeld, I. 1962b. Effect of selenium on methionine formation in vivo and in vitro. Proc. Soc. Exp. Biol. Med. 109:624.
- Rosenfeld, I. 1964. Metabolic effects and metabolism of Se in animals. Wyoming Agr. Exp. Sta. Bul. 414.
- Rosenfeld, I., and O. A. Beath (eds.). 1964. Selenium, Geobotany, Biochemistry, Toxicity, and Nutrition. Academic Press, New York.
- Schalm, O. W. 1961. Veterinary Hematology. Lea and Febiger, Philadelphia, Pa.
- Schoental, R. 1967. The use of radioactive selenium isotope (^{75}Se) as an indicator for sulfur compounds. Biochem. J. 103:27p. (Abstr.).
- Schwarz, K. 1961. Development and status of experimental work on Factor-3 selenium. Fed. Proc. 20:666.
- Schwarz, K., J. G. Bieri, G. M. Briggs, and M. L. Scott. 1957. Prevention of exudative diathesis in chicks by Factor 3 and selenium. Proc. Soc. Exp. Biol. Med. 95:621.
- Schwarz, K., and C. M. Foltz. 1957. Selenium as an integral part of Factor-3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79:3293.
- Schwarz, K., J. A. Stesney, and C. M. Foltz. 1959. Relation between selenium traces in L-cystine and protection against dietary liver necrosis. Metabolism 8:88.
- Shrift, A. 1954. Sulfur-selenium antagonism. II. Antimetabolite action of selenomethionine on the growth of Chlorella vulgaris. Am. J. Botany 41:345.
- Shrift, A. 1964. A selenium cycle in nature? Nature 201:1304.
- Siren, M. J. 1964. Is selenium involved in the excitation mechanism of photoreceptors? Science Tools 11:37.
- Smith, I. (ed.). 1960. Chromatographic and Electrophoretic Techniques (2nd ed.). Interscience Publishers, Inc., New York.

- Smith, M. I., and B. B. Westfall. 1937. Further field studies on the selenium problem in relation to public health. Public Health Rpts. (U. S.) 52:1375.
- Spear, P. W., and M. D. Sass. 1964. Some current concepts of red cell metabolism. II. Metabolism in the aging red cell and in certain diseases of the red cell. Metabolism 13:911.
- Steele, R. G. D., and J. H. Torrie (eds.). 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.
- Sternberg, J., and A. Imbach. 1967. Metabolic studies with seleniated compounds. II. Turnover studies with Se^{75} -methionine in rats. J. App. Rad. Iso. 18:557.
- Toennies, G., and J. J. Kolb. 1951. Techniques and reagents for paper chromatography. Anal. Chem. 23:823.
- Tuve, T., and H. H. Williams. 1961. Metabolism of selenium by Escherichia coli: biosynthesis of selenomethionine. J. Biol. Chem. 236:597.
- Weast, R. C., S. M. Selby, and C. D. Hodgeman (eds.). 1964. Handbook of Chemistry and Physics (45th ed.). The Chemical Rubber Co., Cleveland, Ohio.
- Winegard, H. M., and G. Toennies. 1948. Detection of sulfur-containing amino acids on paper chromatograms. Science 108:506.
- Withrow, G. 1966. Unpublished preliminary results. University of Tennessee Agricultural Research Laboratory, Oak Ridge, Tenn.
- Wright, P. L. 1965. Life span of ovine erythrocytes as estimated from selenium-75 kinetics. J. Animal Sci. 24:546.
- Wright, P. L., and M. C. Bell. 1963. Selenium and vitamin E influence upon in vitro uptake of Se^{75} by ovine blood cells. Proc. Soc. Exp. Biol. Med. 114:379.
- Wright, P. L., and M. C. Bell. 1964. Selenium-75 metabolism in the gestating ewe and fetal lamb: effects of dietary α -tocopherol and selenium. J. Nutr. 84:49.
- Wright, P. L., and M. C. Bell. 1966. Comparative metabolism of selenium and tellurium in sheep and swine. Am. J. Physiol. 211:6.

APPENDIX



TABLE III
CONCENTRATION OF ^{75}Se IN WHOLE BLOOD, PLASMA,
ERYTHROCYTES, AND ERYTHROCYTE WASH OF
GROWING LAMBS (% DOSE/ML. $\times 10^4$)

Time postdosing ^b	Means $\pm$ standard errors ^a			
	Whole blood	Plasma	Erythrocytes	Erythrocyte wash
Hours				
1	70.37 $\pm$ 1.53	103.75 $\pm$ 2.40	9.61 $\pm$ 1.14	7.52 $\pm$ 1.17
2	93.23 $\pm$ 2.60	132.37 $\pm$ 4.94	6.87 $\pm$ 1.03	6.83 $\pm$ 0.81
4	104.56 $\pm$ 5.70	162.48 $\pm$ 7.77	5.39 $\pm$ 0.73	9.19 $\pm$ 1.19
8	91.00 $\pm$ 2.36	139.45 $\pm$ 3.69	4.46 $\pm$ 0.59	6.99 $\pm$ 0.61
10	83.24 $\pm$ 2.64	127.80 $\pm$ 4.37	3.54 $\pm$ 0.65	6.78 $\pm$ 1.03
24	64.06 $\pm$ 0.89	89.31 $\pm$ 4.00	3.17 $\pm$ 0.34	12.80 $\pm$ 0.54
48	47.03 $\pm$ 0.91	71.97 $\pm$ 2.65	2.73 $\pm$ 0.27	4.72 $\pm$ 0.65
72	44.01 $\pm$ 0.79	64.41 $\pm$ 2.89	3.93 $\pm$ 0.78	3.17 $\pm$ 0.34
96	41.80 $\pm$ 1.34	57.62 $\pm$ 3.33	5.58 $\pm$ 1.54	3.64 $\pm$ 0.49
Days				
5	36.45 $\pm$ 3.43	53.97 $\pm$ 2.54	7.87 $\pm$ 2.42	4.15 $\pm$ 0.46
6	33.88 $\pm$ 3.13	49.35 $\pm$ 2.15	13.21 $\pm$ 4.68	1.86 $\pm$ 0.30
7	39.19 $\pm$ 2.11	48.05 $\pm$ 1.88	15.24 $\pm$ 5.71	2.38 $\pm$ 0.20
8	39.42 $\pm$ 2.65	46.44 $\pm$ 1.87	14.75 $\pm$ 5.65	2.33 $\pm$ 0.25
9	38.81 $\pm$ 2.86	46.14 $\pm$ 2.29	20.33 $\pm$ 6.15	3.05 $\pm$ 0.47
10	38.63 $\pm$ 2.93	43.33 $\pm$ 2.31	25.47 $\pm$ 7.75	2.31 $\pm$ 0.34
14	38.22 $\pm$ 2.37	35.22 $\pm$ 1.14	38.24 $\pm$ 6.95	2.00 $\pm$ 0.12
21	39.14 $\pm$ 3.57	28.15 $\pm$ 1.49	47.20 $\pm$ 8.43	1.99 $\pm$ 0.16
28	35.45 $\pm$ 3.97	15.86 $\pm$ 2.32	56.77 $\pm$ 9.99	1.78 $\pm$ 0.28
35	39.46 $\pm$ 4.10	16.22 $\pm$ 1.09	61.89 $\pm$ 8.24	1.48 $\pm$ 0.15
42	44.46 $\pm$ 4.99	14.65 $\pm$ 1.48	97.94 $\pm$ 10.70	1.94 $\pm$ 0.38
49	40.94 $\pm$ 5.60	14.67 $\pm$ 1.27	100.34 $\pm$ 11.59	2.24 $\pm$ 0.25
56	40.93 $\pm$ 3.99	11.45 $\pm$ 0.59	75.07 $\pm$ 7.20	0.87 $\pm$ 0.15
63	42.65 $\pm$ 4.05	11.99 $\pm$ 0.70	76.18 $\pm$ 5.84	0.89 $\pm$ 0.11
70	40.91 $\pm$ 4.06	9.40 $\pm$ 0.55	75.78 $\pm$ 8.57	1.36 $\pm$ 0.10
78	41.64 $\pm$ 3.69	12.80 $\pm$ 2.95	76.57 $\pm$ 7.51	2.53 $\pm$ 0.58
84	40.12 $\pm$ 3.48	6.81 $\pm$ 0.38	80.77 $\pm$ 7.96	0.35 $\pm$ 0.09
91	36.68 $\pm$ 3.52	6.13 $\pm$ 0.47	78.32 $\pm$ 7.95	0.62 $\pm$ 0.11
98	35.34 $\pm$ 3.72	6.17 $\pm$ 0.47	74.54 $\pm$ 7.91	0.77 $\pm$ 0.10
105	35.45 $\pm$ 4.06	6.15 $\pm$ 0.92	77.98 $\pm$ 9.21	0.70 $\pm$ 0.16
112	32.97 $\pm$ 3.79	4.75 $\pm$ 0.45	76.34 $\pm$ 7.86	0.71 $\pm$ 0.19
119	32.02 $\pm$ 3.40	4.99 $\pm$ 0.60	69.25 $\pm$ 7.80	0.69 $\pm$ 0.20

TABLE III (continued)

Time postdosing ^b	Means $\pm$ standard errors ^a			
	Whole blood	Plasma	Erythrocytes	Erythrocyte wash
126	31.69 $\pm$ 3.71	4.28 $\pm$ 0.46	66.48 $\pm$ 7.15	0.91 $\pm$ 0.12
133	29.64 $\pm$ 3.37	3.42 $\pm$ 0.22	62.35 $\pm$ 7.29	0.31 $\pm$ 0.09
140	29.21 $\pm$ 3.13	3.76 $\pm$ 0.44	55.30 $\pm$ 4.91	0.26 $\pm$ 0.04
147	26.41 $\pm$ 2.79	3.58 $\pm$ 0.42	52.04 $\pm$ 5.05	0.29 $\pm$ 0.10
154	23.88 $\pm$ 2.51	3.54 $\pm$ 0.37	50.84 $\pm$ 5.06	0.27 $\pm$ 0.04
161	21.82 $\pm$ 2.24	5.11 $\pm$ 1.19	44.93 $\pm$ 4.48	0.47 $\pm$ 0.10
168	19.39 $\pm$ 2.03	3.45 $\pm$ 0.45	39.50 $\pm$ 4.09	0.16 $\pm$ 0.00
175	16.57 $\pm$ 1.97	3.79 $\pm$ 1.00	32.64 $\pm$ 3.50	0.64 $\pm$ 0.38
182	17.57 $\pm$ 1.64	5.32 $\pm$ 1.81	32.14 $\pm$ 4.05	1.22 $\pm$ 0.36

^aMeans were average of % dose of six animals.

^bTime postdosing = time following single intravenous injection of ⁷⁵Se.

TABLE IV
CONCENTRATION OF ^{75}Se IN WHOLE BLOOD, PLASMA,
ERYTHROCYTES, AND ERYTHROCYTE WASH OF
GROWING PIGS (% DOSE/ML. $\times 10^4$)

Time postdosing ^b	Means $\pm$ standard errors ^a			
	Whole blood	Plasma	Erythrocytes	Erythrocyte wash
Hours				
1	44.09 $\pm$ 4.95	68.96 $\pm$ 8.25	7.78 $\pm$ 1.50	3.30 $\pm$ 0.35
2	75.14 $\pm$ 7.38	120.89 $\pm$ 11.12	7.39 $\pm$ 1.29	4.31 $\pm$ 0.61
4	74.07 $\pm$ 7.03	132.96 $\pm$ 9.13	5.98 $\pm$ 0.92	5.88 $\pm$ 0.56
8	62.66 $\pm$ 4.52	107.47 $\pm$ 5.31	5.66 $\pm$ 1.28	4.65 $\pm$ 0.36
10	55.49 $\pm$ 7.18	96.70 $\pm$ 4.45	9.40 $\pm$ 1.54	4.28 $\pm$ 0.63
24	42.12 $\pm$ 3.35	73.56 $\pm$ 3.34	3.43 $\pm$ 0.53	7.54 $\pm$ 0.56
48	35.24 $\pm$ 1.09	57.20 $\pm$ 1.17	Procedural loss	Procedural loss
72	26.74 $\pm$ 0.93	44.94 $\pm$ 1.11	7.10 $\pm$ 1.09	1.31 $\pm$ 0.13
96	27.66 $\pm$ 1.06	38.18 $\pm$ 0.97	8.04 $\pm$ 1.10	2.72 $\pm$ 0.22
Days				
5	25.24 $\pm$ 1.02	33.56 $\pm$ 1.32	8.06 $\pm$ 0.91	1.59 $\pm$ 0.11
6	21.87 $\pm$ 0.77	31.10 $\pm$ 1.17	9.31 $\pm$ 0.88	1.00 $\pm$ 0.05
7	24.12 $\pm$ 1.12	31.90 $\pm$ 1.32	9.95 $\pm$ 1.04	1.77 $\pm$ 0.21
8	22.39 $\pm$ 1.01	31.26 $\pm$ 2.06	10.80 $\pm$ 0.64	1.30 $\pm$ 0.13
9	20.42 $\pm$ 1.29	25.74 $\pm$ 1.59	11.17 $\pm$ 1.01	0.97 $\pm$ 0.08
10	18.73 $\pm$ 0.99	23.04 $\pm$ 1.65	11.19 $\pm$ 0.85	0.60 $\pm$ 0.05
14	16.50 $\pm$ 0.78	16.97 $\pm$ 0.95	12.47 $\pm$ 1.00	0.55 $\pm$ 0.05
21	12.31 $\pm$ 0.73	11.03 $\pm$ 0.97	11.63 $\pm$ 1.04	0.61 $\pm$ 0.10
28	9.27 $\pm$ 0.81	6.51 $\pm$ 0.86	10.22 $\pm$ 0.75	0.74 $\pm$ 0.33
35	7.54 $\pm$ 0.56	4.17 $\pm$ 0.46	9.78 $\pm$ 0.81	0.25 $\pm$ 0.00
42	5.57 $\pm$ 0.38	2.94 $\pm$ 0.26	9.26 $\pm$ 0.70	0.22 $\pm$ 0.00
49	6.33 $\pm$ 0.40	3.99 $\pm$ 0.30	7.80 $\pm$ 0.58	0.21 $\pm$ 0.00
56	4.89 $\pm$ 0.26	2.11 $\pm$ 0.13	7.86 $\pm$ 0.50	0.03 $\pm$ 0.00
63	4.84 $\pm$ 0.36	1.80 $\pm$ 0.11	7.56 $\pm$ 0.57	0.04 $\pm$ 0.00
70	4.40 $\pm$ 0.36	1.42 $\pm$ 0.09	6.35 $\pm$ 0.60	0.04 $\pm$ 0.00
78	3.72 $\pm$ 0.18	0.97 $\pm$ 0.04	5.49 $\pm$ 0.49	0.02 $\pm$ 0.00
84	3.61 $\pm$ 0.33	0.77 $\pm$ 0.00	5.11 $\pm$ 0.61	0.03 $\pm$ 0.00
91	3.14 $\pm$ 0.35	0.97 $\pm$ 0.05	4.91 $\pm$ 0.84	0.02 $\pm$ 0.00
98	3.41 $\pm$ 0.51	0.83 $\pm$ 0.04	4.03 $\pm$ 0.77	0.03 $\pm$ 0.00
105	2.72 $\pm$ 0.53	0.75 $\pm$ 0.04	4.47 $\pm$ 0.97	0.03 $\pm$ 0.00
112	1.78 $\pm$ 0.29	0.64 $\pm$ 0.04	1.97 $\pm$ 0.39	0.02 $\pm$ 0.00
119	1.27 $\pm$ 0.17	0.61 $\pm$ 0.04	1.99 $\pm$ 0.34	0.02 $\pm$ 0.00
126	0.88 $\pm$ 0.12	0.46 $\pm$ 0.00	1.37 $\pm$ 0.18	0.02 $\pm$ 0.00
133	0.81 $\pm$ 0.09	0.42 $\pm$ 0.00	1.16 $\pm$ 0.19	0.02 $\pm$ 0.00

TABLE IV (continued)

Time postdosing ^b	Means $\pm$ standard errors ^a			
	Whole blood	Plasma	Erythrocytes	Erythrocyte wash
140	0.68 $\pm$ 0.10	0.41 $\pm$ 0.09	0.96 $\pm$ 0.15	0.02 $\pm$ 0.00
147	0.45 $\pm$ 0.05	0.24 $\pm$ 0.00	0.87 $\pm$ 0.18	0.02 $\pm$ 0.00
154	0.38 $\pm$ 0.04	0.20 $\pm$ 0.00	0.52 $\pm$ 0.08	0.02 $\pm$ 0.00
161	0.33 $\pm$ 0.00	0.18 $\pm$ 0.00	0.45 $\pm$ 0.09	0.02 $\pm$ 0.00
168	0.26 $\pm$ 0.00	0.16 $\pm$ 0.00	0.38 $\pm$ 0.06	0.03 $\pm$ 0.00
175	0.20 $\pm$ 0.00	0.13 $\pm$ 0.00	0.25 $\pm$ 0.04	0.03 $\pm$ 0.00
182	0.24 $\pm$ 0.00	0.13 $\pm$ 0.00	0.31 $\pm$ 0.04	0.03 $\pm$ 0.00

^aMeans were average of % dose for six animals.

^bTime postdosing = time following single intravenous injection of ⁷⁵Se.

TABLE V

SELENIUM-75 CONTENT OF SHEEP ERYTHROCYTE FRACTIONS OF
VARYING AGES^a (% DOSE/MG. X 10⁴)

Animal number	Time postdosing	Fraction number ^b				
		1	2	3	4	5
844	Hours					
	1	71.44	31.31	30.15	29.45	27.95
	6	59.01	26.49	26.60	27.60	26.77
	10	37.06	24.05	23.57	26.32	24.75
	24	32.85	18.21	18.68	18.61	18.68
	48	20.82	13.20	13.27	12.65	12.56
	72	28.69	16.62	13.22	12.58	11.56
	96	31.11	21.09	15.43	13.35	13.15
	Days					
	7	52.48	43.43	36.42	19.68	17.82
	10	95.75	63.02	42.47	32.64	30.48
	14	106.48	75.54	60.64	44.42	37.72
	21	103.67	92.95	80.45	69.45	64.05
	28	105.89	100.95	93.12	89.13	85.53
	35	111.20	110.13	106.10	99.40	94.09
	42	105.14	135.14	109.99	105.39	101.75
	49	99.43	124.59	112.32	105.68	102.96
	56	109.63	128.95	123.54	116.92	113.20
	63	125.60	127.57	127.95	122.13	120.77
	70	83.83	123.87	126.61	125.45	121.96
860	Hours					
	1	85.00	24.50	24.40	24.43	23.58
	6	55.01	26.31	24.28	23.12	21.53
	10	43.77	22.59	21.39	20.99	19.73
	24	29.10	16.47	15.64	15.17	14.35
	48	19.21	13.20	12.34	11.98	11.68
	72	17.34	12.29	11.26	10.46	10.53
	96	17.69	14.11	11.52	11.03	9.80
	Days					
	7	34.48	25.04	15.65	12.36	11.13
	10	52.47	40.84	30.26	21.62	16.98
	14	67.95	41.96	29.82	23.60	20.27
	21	71.76	52.67	42.66	37.26	34.91
	28	Procedural loss -----				
	35	88.36	81.98	70.52	66.58	63.79
	42	84.15	82.82	79.53	74.76	71.64

TABLE V (continued)

Animal number	Time postdosing	Fraction number ^b				
		1	2	3	4	5
851	49	72.59	85.23	83.43	82.41	80.80
	56	88.15	92.15	92.15	93.36	91.68
	63	72.06	99.09	97.27	102.28	102.30
	70	85.75	99.56	104.20	106.52	105.66
	Hours					
	1	44.87	21.69	18.95	15.50	14.63
	6	35.52	24.91	17.94	16.28	12.99
	10	31.05	19.76	16.76	13.58	12.02
	24	22.41	10.71	8.99	8.58	7.48
	48	15.43	9.71	6.96	5.83	5.45
	72	21.33	18.62	11.84	7.28	6.51
	96	21.75	18.41	15.59	11.19	8.74
	Days					
	7	23.25	31.43	32.82	24.98	19.58
	10	25.21	36.44	43.91	44.90	40.77
	14	30.62	49.92	55.88	48.68	42.38
	21	55.78	65.14	67.23	65.13	62.67
	28	37.62	42.68	29.20	72.25	42.44
	35	39.20	63.59	73.95	83.82	86.26
	42	45.09	67.18	79.58	87.83	92.62
	49	40.09	67.11	80.14	86.95	88.67
	56	53.09	90.77	81.28	89.93	93.59
	63	52.09	72.38	85.36	92.27	95.69
	70	62.76	76.52	83.17	90.17	92.04
845	Hours					
	1	30.09	22.93	23.26	18.29	18.17
	6	29.77	18.31	16.65	15.61	14.52
	10	18.24	14.76	14.01	13.01	12.64
	24	13.69	11.25	10.68	9.05	9.87
	48	10.29	7.50	7.15	6.84	6.45
	72	12.11	7.48	6.44	6.31	5.47
	96	9.53	7.27	6.54	6.33	3.34
	Days					
	7	19.63	9.01	7.58	6.68	5.73
	10	63.13	27.65	18.87	18.41	13.28
	14	36.56	25.47	20.25	17.38	14.63
	21	54.78	43.53	35.80	29.70	26.97
	28	36.24	56.61	66.43	73.57	72.59

TABLE V (continued)

Animal number	Time postdosing	Fraction number ^b				
		1	2	3	4	5
852	35	66.74	57.39	48.11	40.34	36.79
	42	69.21	62.44	54.93	45.65	41.94
	49	71.97	59.66	51.36	45.40	42.71
	56	80.37	69.23	58.06	44.90	52.67
	63	82.25	74.80	68.30	61.03	58.76
	70	81.34	80.06	73.45	66.46	60.33
	Hours					
	1	24.13	22.14	19.70	18.16	16.65
	6	43.51	18.81	16.91	15.85	14.17
	10	27.33	17.92	17.05	14.24	14.42
	24	17.47	11.57	10.58	9.98	9.08
	48	11.84	8.53	7.82	7.50	7.05
	72	10.12	7.99	7.10	6.13	5.59
	96	9.73	7.81	7.01	6.99	6.01
	Days					
	7	17.12	11.70	7.53	6.29	5.65
	10	33.41	22.69	16.85	12.87	11.82
	14	32.35	23.11	16.33	14.62	11.18
	21	41.31	37.53	33.80	33.74	32.66
	28	60.52	50.27	41.87	34.04	32.84
	35	42.02	48.14	50.38	53.08	54.15
	42	38.55	50.71	57.36	60.91	63.28
	49	35.21	46.88	59.03	64.80	69.16
	56	36.71	51.07	64.79	69.99	73.08
	63	42.97	66.20	68.38	75.81	78.30
	70	42.27	57.21	69.71	79.36	84.10
865	Hours					
	1	32.65	20.95	20.50	19.67	18.00
	6	42.19	21.57	20.58	20.72	18.77
	10	22.13	18.07	17.82	16.22	16.64
	24	21.07	13.66	12.50	10.15	10.88
	48	14.02	10.22	8.90	8.36	8.41
	72	11.25	9.68	8.53	7.67	7.20
	96	12.93	10.42	8.71	7.88	7.48
	Days					
	7	35.00	21.08	12.90	9.52	8.11
	10	36.98	20.33	16.96	14.94	11.42
	14	48.13	26.37	16.11	9.51	10.86

TABLE V (continued)

Animal number	Time postdosing	Fraction number ^b				
		1	2	3	4	5
	21	63.14	49.07	37.19	27.98	23.45
	28	61.46	52.87	44.38	39.50	35.62
	35	53.29	51.37	49.38	46.74	46.67
	42	52.90	56.84	57.29	53.77	57.19
	49	52.04	57.75	59.53	60.69	59.82
	56	57.29	61.50	63.92	64.58	61.78
	63	50.36	64.52	68.70	92.10	72.43
	70	57.96	66.29	70.16	72.49	69.49

^aErythrocytes were separated into age groups by centrifugation with the younger cells at the top of the tube and older cells at the bottom.

^bEach fraction $\approx$ 20 per cent of the height of the red cell column.

TABLE VI

SELENIUM-75 CONTENT OF SWINE ERYTHROCYTE FRACTIONS OF
VARYING AGES^a (% DOSE/MG. X 10⁴)

Animal number	Time postdosing	Fraction number ^b				
		1	2	3	4	5
833	Hours					
	1	37.65	14.36	13.25	13.28	13.42
	6	45.87	14.76	14.42	14.26	13.75
	10	29.92	17.72	15.84	14.93	13.97
	24	20.09	9.62	9.25	9.58	8.98
	48	13.68	9.76	7.79	6.32	5.99
	72	24.70	11.99	10.37	8.51	7.87
	96	17.72	14.49	10.94	8.47	8.03
	Days					
	7	35.12	23.68	18.08	14.66	12.39
	10	35.08	28.89	24.37	21.21	18.84
	14	38.03	31.76	27.25	22.78	19.00
	21	25.46	26.78	24.92	23.85	21.77
	28	29.18	29.45	27.73	26.32	24.12
	35	26.83	28.16	27.95	26.81	25.06
	42	23.74	25.86	25.64	24.82	24.14
	49	20.72	23.68	23.62	23.25	23.71
	56	21.40	22.77	23.00	23.88	22.80
	63	17.56	19.29	21.44	22.31	22.33
	70	14.73	17.30	20.18	20.85	21.57
834	Hours					
	1	24.52	15.16	14.86	14.65	15.70
	6	Procedural loss				
	10	22.06	13.26	10.85	10.26	9.76
	24	21.81	9.07	6.96	6.30	6.11
	48	20.05	9.08	5.89	4.90	4.72
	72	23.51	15.82	10.54	8.43	7.39
	96	28.23	17.88	12.73	9.92	9.19
	Days					
	7	28.88	21.32	15.38	13.55	12.40
	10	26.45	22.88	20.08	19.07	18.02
	14	29.63	26.67	23.66	21.04	18.36
	21	22.06	20.85	19.26	17.68	16.05
	28	26.20	26.89	26.16	24.60	23.24
	35	25.15	25.56	25.59	24.94	23.74
	42	21.90	24.17	25.09	24.54	23.66
	49	21.45	23.11	22.99	23.23	22.87

TABLE VI (continued)

Animal number	Time postdosing	Fraction number ^b				
		1	2	3	4	5
835	56	19.54	21.78	21.82	22.44	22.26
	63	18.58	20.29	21.34	23.00	20.75
	70	16.92	18.50	19.59	20.29	21.13
	Hours					
	1	8.71	4.25	4.37	4.33	4.36
	6	18.24	6.37	5.53	4.98	4.56
	10	19.13	10.27	9.13	8.79	7.65
	24	25.93	10.26	9.04	7.56	7.18
	48	15.75	8.61	7.72	6.53	6.46
	72	20.46	14.81	10.60	8.54	8.49
	92	29.03	15.76	11.98	10.78	10.31
	Days					
	7	25.76	19.59	16.68	14.53	13.16
	10	30.36	25.66	23.10	21.45	17.95
	14	33.49	28.01	24.21	22.10	19.45
	21	23.81	23.25	22.48	21.66	21.46
	28	26.98	26.42	24.91	23.14	21.87
	35	24.67	24.68	24.72	24.20	23.39
	42	20.05	22.79	23.48	23.20	22.21
	49	19.03	20.87	21.28	21.00	21.13
	56	19.69	20.30	20.89	20.29	19.78
	63	18.40	19.85	19.89	19.76	19.65
	70	16.42	17.71	18.15	18.70	18.86
836	Hours					
	1	7.61	2.50	2.03	2.16	2.47
	6	38.89	9.16	8.01	7.33	6.61
	10	15.32	12.26	11.28	9.91	9.42
	24	19.80	10.53	9.83	9.72	9.18
	48	36.11	18.62	19.54	19.02	22.41
	72	22.65	16.60	14.69	13.19	13.64
	96	19.75	16.40	13.81	13.51	13.93
	Days					
	7	30.64	22.19	18.11	13.93	15.12
	10	26.77	23.70	21.59	20.67	19.16
	14	29.28	25.95	23.71	21.67	19.92
	21	33.75	31.72	29.61	27.57	24.94
	28	20.55	21.39	21.60	21.78	21.30
	35	19.22	20.87	21.42	21.22	21.47
	42	18.22	19.43	19.91	19.34	20.00

TABLE VI (continued)

Animal number	Time postdosing	Fraction number ^b				
		1	2	3	4	5
	49	15.20	16.88	12.48	17.51	17.44
	56	15.68	16.47	16.70	16.70	16.79
	63	14.15	15.46	15.85	15.78	15.84
	70	11.85	13.64	13.97	14.57	14.92
	Hours					
838	1	31.75	23.20	22.00	21.62	19.95
	6	31.57	13.39	12.32	10.34	10.19
	10	26.74	11.53	10.37	9.91	9.67
	24	13.27	7.96	6.75	6.28	5.97
	48	16.28	7.29	5.69	4.37	4.42
	72	25.99	17.29	9.79	7.59	5.39
	96	21.65	12.84	8.92	7.16	6.36
	Days					
	7	26.28	16.72	12.61	9.48	8.22
	10	27.36	21.04	18.08	14.33	12.95
	14	26.13	21.72	18.30	15.42	12.75
	21	27.80	25.52	24.07	22.70	20.98
	28	21.21	20.53	19.89	18.14	16.76
	35	19.30	19.76	19.45	18.80	17.98
	42	18.20	18.58	19.37	18.38	17.59
	49	16.48	18.07	17.83	17.50	16.31
	56	15.28	16.55	16.61	17.35	17.16
	63	14.71	15.86	16.50	16.84	16.99
	70	12.41	14.37	14.95	15.97	16.78

^aErythrocytes were separated into age groups by centrifugation with the younger cells at the top of the tube and older cells at the bottom.

^bEach fraction $\approx$ 20 per cent of height of red cell column.

TABLE VII
MEASUREMENT OF MIGRATION OF INDIVIDUAL STANDARD AMINO
ACIDS ON A TWO-WAY PAPER CHROMATOGRAM
(CM. FROM ORIGIN)

Amino acid	Distance ^a	
	Down	Across
Cystine	5.0	4.6
Lysine	8.6	6.0
Aspartic acid	9.7	5.2
Histidine	10.7	8.4
Arginine	11.6	7.5
Glutamic acid	13.0	7.1
Glycine	12.0	8.3
Serine	12.4	11.1
Alanine	15.6	12.2
Threonine	16.2	13.1
Proline	18.9	13.2
Tyrosine	24.8	23.9
Methionine	26.2	21.5
Selenomethionine	26.6	22.0
Spot appearing with methionine	13.1	21.3
Valine	26.8	20.6
Tryptophan	28.0	25.2
Isoleucine	29.7	24.4
Phenylalanine	31.1	25.4
Leucine	34.0	26.8

^aThe distance each amino acid migrated was measured vertically up from the origin and horizontally across from that point to the amino acid spot.