ASSOCIATION OF SELENIUM WITH SERUM PROTEINS IN THE RAT

by

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INTRODUCTION

Recently, attention has been focused on the effect of selenium on organisms. There is abundant evidence that trace quantities of selenium exist in animals without apparent effect and that higher levels of selenium cause toxicity (24) (25) (35, p. 166). Some investigators (13) (14) (40) suggest that selenium may contribute to caries susceptibility in teeth, although others (44) believe that no relationship exists.

In vivo studies with animals have shown that selenium becomes intimately associated with the proteins of several tissues by mechanisms other than adsorption (18) (23) (43). McConnell *et al.* (18) have postulated that since selenium and sulfur have chemical properties which are similar, metabolism of selenium may occur by the same pathways as those of sulfur. Specifically, they propose that selenium may replace sulfur in the sulfur-containing amino acids of the protein molecule. Ion binding of selenium to protein has also been postulated (23) (37).

Selenium is found in relatively high concentrations in the liver, blood, and kidney as compared to other tissues of selenized animals (16) and is transported to all organs, including the teeth, by the blood (5). Therefore, the possibility exists that selenium becomes incorporated in the

protein matrix of teeth, thereby affecting dental caries susceptibility in some manner. Likewise, the toxicity reported in selenium-ingesting animals may be due in part to sulfur replacement in enzymes containing active sulfhydryl groups such as adenosine triphosphatase and other kinases.

This study was undertaken to ascertain whether or not selenium replaces sulfur in the sulfur-containing amino acids of serum proteins after parenteral administration of trace quantities of selenium^{75} to albino rats.

HISTORICAL

When livestock was fed the grains and forages from areas in the western Great Plains of the United States, death occurred due to an unknown toxicosis (24) (25) (35). Soon after the discovery of this toxicosis, agricultural experimental stations began to search for the cause. In 1928, Dr. Kurt W. Franke, at the South Dakota Agricultural Experimental Station, showed the toxicity was due to the feed fed these animals rather than the water they received (10). In 1933, Robinson discovered the presence of selenium in this feed (28). Subsequently, Franke (11) found that this element was confined chiefly to the protein fractions and was present in the soil of all regions where the toxic grain grew. Franke and Potter (12) later showed that selenium was the toxic agent. They concluded this by adding sodium selenate and sodium selenite to the diet of rats and observing that the symptoms produced were exactly the same as those produced by the toxic grains.

Although selenium had been shown to be associated with the proteins in plants, it was not until 1940 that any selenium-containing compounds were isolated from these plants or from any other biological sources. In this year, Horn and Jones (17) separated an amino acid complex containing selenium and sulfur in the ratio of two to one from the Astragalus

pectinatus plant. They tentatively assigned the structural formula which was indicative of a mixture of cystathionine and its selenium analogue.

McConnell and Cooper (18) have shown that the radioactive isotope, selenium^{75}, becomes tightly bound to the serum proteins of dogs after subcutaneous injection of sodium selenate. Recent paper electrophoresis studies in our laboratory (33) show that this binding also occurs in the serum proteins of rats upon administration of sodium selenite containing radioselenium. This phenomenon has been demonstrated in several other animal tissues (21) (22) (23) (43).

Other studies on the distribution of selenium in serum proteins have shown that selenium is bound to all of the proteins examined (18) (23) (43). McConnell et al. (23) have reported that within the first hour after subcutaneous injection of selenium^{75} in dogs, the greatest initial activity is found in the albumin fraction and gradually decreases. As the radioactivity in the albumin fraction decreases, the activity in the globulin fractions, which is low immediately after administration, increases for about 48 hours until reaching a plateau which lasts for several weeks. This apparent shift has been shown to occur in the serum proteins of rats (33). Thus, after long periods of selenium ingestion or administration, the selenium associated with the serum

proteins is found predominantly with the globulins (43).

McConnell and Wabnitz (22), using dog liver protein hydrolysates purified by ion-exchange methods, found three areas of greatest activity on overloaded one-dimensional paper chromatograms 48 hours after subcutaneous injection of the dog with selenium^{75} tetrachloride. These areas, they concluded, corresponded to amino acids since precipitation tests and ion-exchange studies showed them to behave as such. These were the areas of cystine, methionine, and leucine. Using two-dimensional paper chromatography and non-purified hydrolysates they obtained essentially the same results. When these hydrolysates were purified by ion-exchange methods, and two-dimensional chromatograms prepared, the amino acid spots contained activity too small for significant assay.

McConnell and Kreamer (19) isolated the cystine-rich protein, keratin, from dog hair and found that it contained radioselenium after subcutaneous injection of selenium^{75} tetrachloride. They further isolated and then purified the cystine fraction by recrystallization and found a small percentage of the activity in this purified fraction.

Westfall and Smith (43) found that removal of the selenium compound(s) from protein-free tryptic liver digests did not parallel that of cystine removal. Liver hydrolysates were prepared from the livers of rabbits which had been fed

selenized oats or selenized wheat for several months. Using several different reagents for the removal of selenium and cystine from the hydrolysates, they found no correlation between the percentages of the two substances removed. They also reported that the selenium analogue of cystine is less stable than cystine itself.

A mixture of S-methylcysteine and its selenium analogue, Se-methylcysteine, was isolated from the plant Astragalus pectinatus by Trelease et al. (36) using ion-exchange and filter paper columns. They found that the elution R_f values for the S-methylcysteine and its selenium analogue were identical.

Several microorganisms are known to synthesize selenomethionine from inorganic selenium. Tuve and Williams (38) reported the identification of selenomethionine in the proteins of Escherichia coli after growth on a sulfur-deficient medium containing radioactive selenium as sodium selenate. The proteins were successively digested enzymatically with pepsin, pancreatin, and erepsin. The selenomethionine was separated from the hydrolysate using ion-exchange columns. To this radioactive selenomethionine was added carrier selenomethionine and the mixture was chromatographed. A radioautograph was made of the chromatogram. The spot size, shape, and location on the radioautograph corresponded "precisely"

with the colored area produced by the ninhydrin test for carrier selenomethionine.

Recently, selenomethionine was isolated by Blau (2) from Saccharomyces cerevisiae grown on a medium containing small amounts of sulfur and high specific activity selenium^{75} as selenious acid. This selenium analogue was separated from the hydrolyzed yeast by ion-exchange columns. Blau showed that the elution R_f values of selenomethionine and selenocystine "closely match" those of methionine and cystine, respectively. This work with microorganisms represents the most conclusive evidence of biosynthesis of the selenium analogues of cystine, cysteine, or methionine, although these analogues have been synthesized by chemical means (26) (27).

Blau and Manske (3), using the selenomethionine synthesized by Saccharomyces cerevisiae in the above experiment, reported in preliminary studies that selenomethionine becomes incorporated into the proteins of the pancreas and liver of dogs after administration by several routes. The highest activities in these tissues were found one to two hours after administration.

High selenium-to-sulfur ratios have been shown to enhance replacement of sulfur by selenium in the proteins of several microorganisms (6) (32) (39). Cowie and Cohen (5), using radioselenite and radiosulfate, have shown selenium and sulfur

to be competitive antagonists in Escherichia coli. This competitive antagonism has been further demonstrated by Schrift (32) in Chlorella vulgaris. Using both selenate and selenomethionine in separate experiments, Schrift showed that the uptake of selenium varies directly with the selenium-to-sulfur ratio and is not dependent upon the absolute amounts of selenium administered.

Investigation of the toxic action of selenium has shown that inorganic selenium compounds change chemical form after administration to animals. Rosenfeld and Beath (30) demonstrated that sodium selenate is converted into selenite and a volatile selenium compound by beef liver, spleen, whole blood, and plasma in in vitro experiments; these tissues also converted selenite into both a volatile selenium compound and elemental selenium. McConnell and Portman (20) later showed that this volatile compound was dimethyl selenide. This was found in the respiratory gases of rats after administration of sodium selenate. Therefore, it appears that the forms of selenium existing in an animal are selenate, selenite, selenide, and elemental selenium following the administration of selenate.

Before contemplating the question of whether or not selenium analogues of the amino acids cystine, cysteine, and methionine are synthesized from inorganic selenium, evidence should first be given for the synthesis of these amino acids

from inorganic sulfur. Dziewiatkowski (7) found that small amounts of radioactive sulfur become incorporated in the cystine of hair, liver, skeletal muscle, and skin proteins of rats after subcutaneous injection of sulfur^{35} as sodium sulfide. Before administering the sodium sulfide, cysteine was depleted by feeding bromobenzene. In rats, as well as human beings, bromobenzene is detoxified by conjugation with cysteine. Since cystine is in equilibrium with cysteine, it also was depleted with a resultant weight loss. Immediately after the rats were removed from the bromobenzene diet, sodium sulfide^{35} was injected intraperitoneally for several days. Weight gains occurred, indicating protein synthesis. Subsequent analysis showed small amounts of the sulfur^{35} to be incorporated in the proteins of the previously mentioned tissues.

In another study, Dziewiatkowski and Di Ferrante (9) found that small fractions of the original doses of sulfur^{35} injected intraperitoneally as sodium sulfate could be recovered in cystine and methionine from the serum proteins of rats 24 hours after administration. Relatively large doses were administered (250 uC, 500 uC, 1250 uC carrier-free sulfur^{35} as sodium sulfate). They also found that most of the sulfur^{35} associated with the serum proteins of rats was bound as sulfate and was released upon hydrolysis. Some of

the bound sulfur^{35} could be extracted with trichloroacetic acid from the proteins as part of a non-dialyzable moeity, probably carbohydrate-protein complexes (31) (41). These investigators point out that their work is in accord with other reports which indicate that only an exceedingly small, "sometimes undetectable", fraction of a dose of sulfur^{35} administered as sulfate can be recovered from rat tissues as cystine and methionine sulfur (8) (34).

EXPERIMENTAL

Two male albino rats of the Sprague-Dawley strain, weighing 468 grams and 485 grams, were injected with selenium^{75} (ORNL Se-75-P-2 Processed, Enriched) as sodium selenite. The injection schedule consisted of an initial intracardiac injection of 2 uC. (0.1 ml., 0.4 ug.) of selenium^{75} followed by subcutaneous injections of 0.4 uC. (0.2 ml., 0.08 ug.) every three days. Injections were continued until 45 days had elapsed for the first animal and 60 days for the second. Total selenium^{75} administered was 7.6 uC. (1.5 ug.) and 9.6 uC. (1.9 ug.), respectively.

During the period of selenium^{75} administration, the rats were fed Purina Laboratory Chow and tap water ad libitum. At the end of the respective 45- and 60-day periods, the animals were sacrificed by ether inhalation. The blood was immediately withdrawn by heart puncture and allowed to clot. From this point on, the samples of both animals were treated in an identical manner. The serum was separated from the clot by centrifugation and decantation. The crude serum was assayed for radioactivity in a well-type scintillation detector[1]. Radioactivity was followed by this same assay method during isolation

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[1] Tracerlab Model P20CW with thallium-activated sodium iodide well crystal and Model "1000" scaler.

and purification procedures. To determine the amount of non-dialyzable selenium present, a portion of the crude serum was electrodialyzed and assayed, and then discarded. All dialyses were performed in a Research Specialties Co. Model A-190 electrodialysis unit.

The proteins were precipitated by adding five volumes of acetone to one volume of serum, both at 4°C. After standing overnight at this temperature, the precipitate was collected by centrifugation and divided into two parts for separate hydrolysis. One part was hydrolyzed as precipitated; the other was washed three times with 25 ml. aliquots of cold acetone before hydrolysis. From this point on, both of these isolated protein samples were treated in an identical manner. Each sample was air dried, sealed in a Pyrex glass tube with five times the sample weight of 6 N hydrochloric acid, and hydrolyzed for six hours at 100°C. After cooling, the hydrolysates were removed from the sealed tubes, neutralized with 5 N sodium hydroxide, and purified. Purification consisted of refrigerated centrifugation (16,500 RPM, 30,900 X gravity) for 30 minutes to remove humin, followed by electrodialysis to remove any inorganic selenium^{75} liberated during hydrolysis.

Carbohydrate extraction using trichloroacetic acid was tried as a means of purification. The precipitated serum

proteins were heated at 90°C for 15 minutes with 20 ml. of five per cent trichloroacetic acid. This method of purification was not pursued further, since marked reduction of selenium compounds occurred, as indicated by the red precipitate of elemental selenium.

The purified hydrolysates were separated by two-dimensional descending paper chromatography on 22¼" x 18½" Whatman No. 1 filter paper. Three chromatograms were prepared from each hydrolysate. The solvent used for the first dimension was a propanol-ethanol-water system buffered at pH 8.9 with sodium pyrophosphate (15). A butanol-acetic acid-water solvent was used for the second dimension (4, p. 151, item 10). Control chromatograms were made of the amino acids cystine, cysteine, and methionine for comparison of R_f values with the chromatograms of the purified hydrolysates. The chromatograms were oven dried at 60°C. A solution (0.2 per cent) of ninhydrin in acetone was used to locate the amino acid areas on the chromatograms. These areas were then cut out, placed in a test tube in the scintillation detector, and assayed for radioactivity.

To permit application of relatively large quantities of the sample without overloading, wide one-dimensional descending chromatograms were made on filter paper sheets 9½" x 22¼". Each purified hydrolysate was applied perpendicular to the

flow of the solvent in a long narrow line extending eight inches across the sheet. Each hydrolysate was divided into two portions: one was adjusted to pH 5; the other was adjusted to pH 9. Each portion was then chromatographed to determine whether or not the same distribution of radioactivity occurred in both the acidic and the basic samples. The propanol-ethanol-water solvent previously mentioned was used for development. Again the sulfur-containing amino acids were chromatographed as experimental controls. After color development with ninhydrin, the amino acid areas on all chromatograms were assayed for radioactivity.

The cystine fraction of the purified hydrolysates was isolated to determine the amount of radioselenium present. The procedure consisted of adjusting the purified hydrolysates to pH 5 with hydrochloric acid and cautiously adding cold (4°C), freshly prepared 60 per cent phosphotungstic[1] acid until maximum precipitate was obtained. The precipitates and supernatants were separated by centrifugation and decantation; each was assayed for radioactivity. The precipitates were then dissolved in small amounts of 5 N sodium hydroxide. One-dimensional chromatograms were prepared as described above. The chromatograms were subsequently sectioned and assayed for

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[1] Cystine and the basic amino acids are precipitated by phosphotungstic acid.

radioactivity.

RESULTS

Radioassay indicated that approximately one-sixth of the radioselenium in the crude serum of each animal could be removed by electrodialysis.

Approximately one-half of the original serum activity could be removed from the isolated protein sample by washing the precipitate three times with cold acetone. An additional one-fifth of the original serum sample activity could be removed during purification after hydrolysis. This method of preparation of the serum proteins for chromatography left the purified hydrolysate with approximately 30 per cent of the original serum selenium^{75} activity.

As previously mentioned, the other method of sample preparation for chromatography consisted of isolation of the serum proteins, hydrolysis, and purification of the hydrolysates. This alternate method left the purified hydrolysate with slightly over half of the original crude serum activity.

The results of the two-dimensional paper chromatographic studies were inconclusive. Application of optimal amounts of the hydrolysate for good separation of the amino acids resulted in areas which, although containing the greatest concentrations of selenium^{75}, were too low for significant assay[1]. Any attempt

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[1] The lower limit for significant assay was taken to be 30 c.p.m. above background since the detector mean value for five one-minute counts of background was observed to vary by that amount.

to apply large enough quantities for significant detection after resolution resulted in streaking and very poor separation. These results provided the rationale for the wide one-dimensional paper chromatograms previously described.

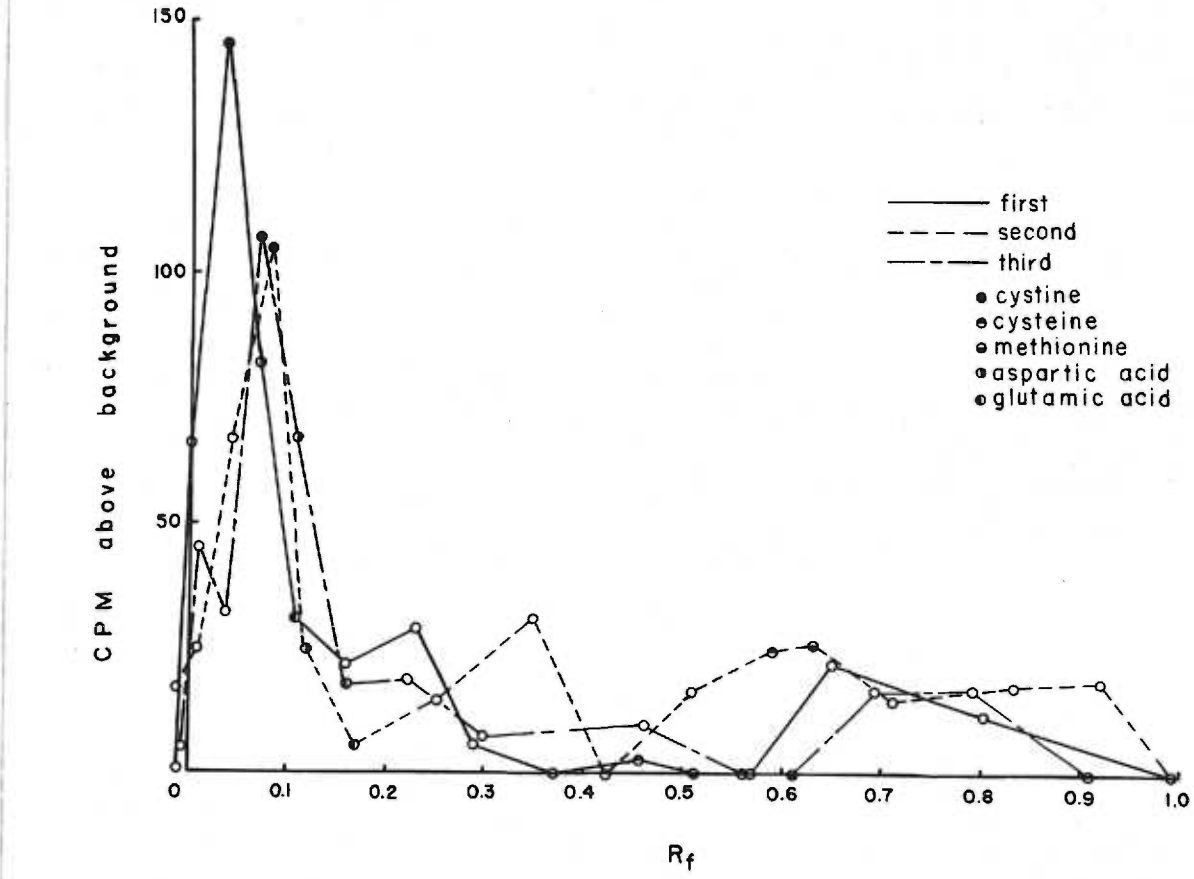

Fig. 1. One-dimensional chromatograms showing the distribution of selenium^{75} in the rat serum protein hydrolysates adjusted to pH 5. Point of application was at R_f=0. The number of the chromatogram and certain amino acid areas are identified. Solvent: propanol-ethanol-water buffered at pH 8.9.

One-dimensional chromatography gave good separation into several areas; however, in an area where two or more amino

acids had relatively close R_f values, overlapping occurred. The chromatograms were therefore cut and assayed by isolated sections rather than individual amino acid areas. The chromatograms of the purified hydrolysates adjusted to pH 5 showed the area of cystine to contain the greatest activity (Fig. 1). The areas adjacent to cystine, the application area and the aspartic acid area, contained the next greatest amounts of radioselenium. The radioactivity of each subsequent section was much lower than that of the cystine area. The areas containing cysteine and methionine contained little or no activity. On the second chromatogram in Fig. 1, the area between cystine and the application area was the second greatest in activity, but gave no color reaction with ninhydrin.

A different pattern of radioactivity was obtained with the purified samples adjusted to pH 9. In these chromatograms, the greatest areas of activity were those containing aspartic acid and glutamic acid. The aspartic acid area also contained histidine; the glutamic acid area also contained serine. The cystine area of activity was markedly changed from the experiment utilizing the hydrolysates adjusted to pH 5, being relatively much lower in radioselenium content. The areas containing cysteine and methionine again contained little or no activity.

The graphs of the one-dimensional chromatograms show

areas of radioactivity to the left of the application area which did not give a color reaction with ninhydrin. This suggests diffusion of radioselenium, probably in the inorganic form.

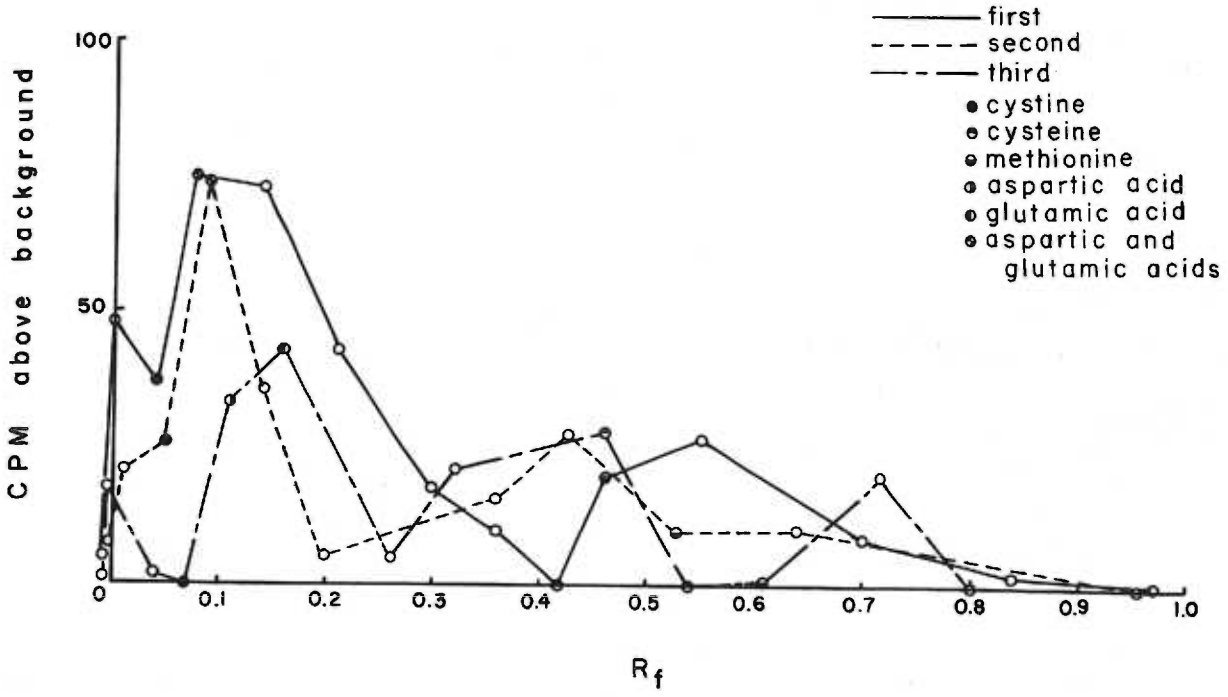

Fig. 2. One-dimensional chromatograms showing the distribution of selenium^{75} in the rat serum protein hydrolysates adjusted to pH 9. Point of application was at R_f=0. The number of the chromatogram and certain amino acid areas are identified. Solvent: propanol-ethanol-water buffered at pH 8.9.

Chromatograms of the samples that were washed three times with cold acetone before hydrolysis contained areas

with concentrations of activity too low for significant assay. Attempts to purify the samples after hydrolysis with means other than centrifugation and electrodialysis resulted in chromatograms which again contained areas with activity concentrations too low for significant assay.

Using phosphotungstic acid, 64 per cent of the activity could be precipitated from the unwashed, purified hydrolysates. One-dimensional chromatograms of the dissolved precipitates were cut and assayed by sections. Significant activity was found in the cystine area, but activity of similar magnitude was also found in the adjacent non-colored areas. The activity appeared to vary inversely with the R_f value, decreasing as the R_f value increased.

DISCUSSION

The experimental results show that selenium administered as selenite becomes intimately associated with the serum proteins of rats. Some of the possible mechanisms which have been postulated are as follows.

1. Replacement of sulfur by selenium. If sulfur replacement does occur, it would probably do so in both cystine and cysteine, since the two sulfur-containing amino acids are reversibly convertible to one another in vivo. The sulfur replacement in cystine could be single or double to form -S-Se- or -Se-Se- bonds.

2. Ion binding. The two types of ion binding which have been suggested are anion-protein combination (23) and sulfhydryl group binding (37). Neither type of ion-binding mechanism has been clearly elucidated as yet; however, experimental results (37) show that selenium administered to microorganisms as selenous acid will bind cysteine in the ratio of one to four, forming a selenium tetracysteine.

3. Adsorption. This might be a combination of both the weak physical van der Waal's adsorption and the stronger chemical adsorption. This mechanism for association of selenium with serum proteins may be of minor importance (18) (23).

Should selenium become associated with the serum proteins by more than one of the above mechanisms, various quantities

would be liberated during the processes of isolation, purification, and hydrolysis, depending upon the energy supplied in each process. This tendency was noted in the results of this study.

Due to the large quantities of radioselenium found to be unassociated in the crude serum and the protein hydrolysates, it was deemed necessary to purify the hydrolysates before chromatography. Although the crude serum appeared to contain ample radioactivity for subsequent analysis, the purification procedures used in this study left the samples with barely sufficient activity for significant assay after chromatographic resolution. The purification method utilizing the acetone washes resulted in samples which did not contain enough activity to continue the study. Carbohydrate extraction with trichloroacetic acid was found to be undesirable as a purification procedure because of the reduction of selenium compounds that occurred. Other workers (22) have found that sample purification using ion-exchange resins also resulted in samples with activity concentrations too low for chromatographic analysis.

The activity remaining in the sample after purification with the methods used in this study may have been due to several factors. One factor might be the de novo synthesis of selenocystine. The chromatograms of the hydrolysates adjusted to

pH 5 suggest that this could be the case. However, chromatograms of the hydrolysates adjusted to pH 9 show that the greatest activity is not associated with the cystine area. This suggests that some moeity was present in the hydrolysates which has chromatographic characteristics similar to selenocystine but which is probably not selenocystine itself. It should be noted here that no pure organic selenium compounds were available for R_f comparison; however, several investigators (2) (36) have shown that sulfur-containing amino acids and their selenium analogues have identical or nearly identical R_f values.

Near the termination of this study, Tuve and Williams (39) reported the presence of a selenocystine-like compound while isolating selenomethionine from the proteins of Escherichia coli grown on a medium containing radioselenium as selenous acid. These workers indicate that although this compound had R_f values similar to selenocystine on paper chromatograms, it was not the selenium analogue of cystine.

At this time no explanation can be made for the existence of the entity possessing similar chromatographic and ion-exchange properties (39). It is possible, however, to postulate mechanisms for its existence. One explanation might be that this moeity is a selenium-amino acid complex. Since removal of all trace quantities of inorganic selenium from the

hydrolysates is impossible, the compound described might be a complex formed from inorganic selenium and different amino acids, depending upon the pH of the purified hydrolysate. Upon interaction with the buffered solvent during development, the complex would either remain associated or it would dissociate, giving a different pattern of radioactivity on the chromatograms. Dziewiatkowski and Di Ferrante (9) reported a nondialyzable radioactive component in digests of rat serum proteins after administration of methionine containing radiosulfur. They further reported that this nondialyzable moeity accounted for 30 per cent of the sulfur^{35} originally associated with the serum proteins.

The previously mentioned selenocystine-like entity could be the oxidation product of a selenium analogue. Since no pure organic selenium compounds were commercially available, it could not be determined in this study whether or not destruction of biosynthetic analogues was occurring during hydrolysis. Several workers (2) (43) have suggested that the stability of selenomethionine is less than that of methionine. An analysis of bond energies indicates that this is probably the case. If the stability of any or all of the selenium analogues of amino acids were such that extensive destruction did occur during hydrolysis, then this moeity referred to above might well be the oxidation product of some selenium compound(s).

The possibility exists that the entity under consideration was merely inorganic selenium traveling freely in the solvent during chromatographic resolution. This is feasible since again it is not possible to remove all trace amounts of radioselenium by electrodialysis.

Although selenomethionine has been isolated from the protein of microorganisms after growth on inorganic selenium, it is known that little or no methionine is synthesized by the rat. No mechanism is known for its synthesis in the rat. Methionine has been shown to be essential for all animals investigated (42, p. 1123). In rats, the sulfur for protein synthesis is derived almost entirely from cystine and methionine, little being supplied by inorganic sulfur compounds. Several workers (8) (34) have reported that only very small, if any, quantities of sulfate sulfur can be recovered as cystine and methionine from the tissues of rats. Inorganic selenite, if metabolized by the same route as inorganic sulfate, would then be expected to give the same result upon administration and be barely detectable, if detectable at all.

Selenium and sulfur have been shown to be competitive antagonists in several microorganisms (6) (32) (39). If this competitive antagonism occurs in rats, then the administration of higher selenium-to-sulfur ratios might have resulted in detectable sulfur replacement. It should be noted that the

selenium-to-sulfur ratios in this study were quite low. To assume competitive antagonism of selenium and sulfur in higher organisms, however, is to assume identical metabolic pathways of these elements. This would result in the formation of selenium analogues, a process which has not been supported by experimental evidence to date.

Although no selenium analogues of amino acids have been isolated in pure crystalline form from either animals or microorganisms, the studies of several investigators (2) (38) (39) strongly support their biosynthesis in several microorganisms. If this is the case, possibly selenium analogues are formed in animals by the intestinal microflora and cannot be demonstrated due to insufficient levels for detection. The work with animals is incomplete at this time and cannot be interpreted as being conclusive. The question of whether or not animals can metabolize selenium in the same manner as microorganisms still remains to be answered.

SUMMARY AND CONCLUSIONS

Selenium^{75} was administered as sodium selenite to albino rats by both intracardiac and subcutaneous injections. After injection of trace quantities of selenium for a duration of several weeks, the serum proteins were isolated and hydrolyzed. The hydrolysates were separated by paper chromatography; the chromatograms were subsequently assayed for radioactivity. The results of the one-dimensional chromatographic studies suggest that some entity with chromatographic characteristics similar to selenocystine, but which was probably not selenocystine itself, was present in the purified hydrolysates. There was no indication of the presence of selenium analogues of cysteine or methionine.

Selenium becomes intimately associated with the serum proteins of rats after parenteral administration of sodium selenite. Radioassay methods were used to determine that only one-sixth of the radioselenium associated with the proteins in the crude serum could be removed by electrodialysis. Greater quantities of radioselenium were liberated during hydrolysis of the isolated serum proteins. The results of the experimentation indicate that the association probably occurs by more than one mechanism. The possible mechanisms for association are discussed.

Sixty-four per cent of the radioselenium in the purified hydrolysates of the rat serum proteins could be recovered in a precipitate of cystine and the basic amino acids. The hydrolysates used were prepared from the acetone precipitable serum proteins, which were subsequently hydrolyzed, and then purified by centrifugation and electrodialysis. Chromatographic studies of these precipitates were made; the results did not indicate the presence of selenocystine.

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