^{75}Se-Selenomethionine in the Study of Protein and Amino Acid Metabolism of Adult Rats[1] (34696)

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^{75}Se-Selenomethionine has been used as a convenient tracer in studies of protein metabolism. It is incorporated into various proteins such as those of erythrocytes and hemoglobin (1, 2), plasma proteins (3, 4), intestines (5, 6), pancreas (7), pancreatic juice proteins (8, 9), chicken egg white proteins (10), fetal tissues (11), liver, kidney, and others (12, 13). Hansson (8, 9) found that pretreatment of the animals with methionine reduced the incorporation of selenomethionine into the pancreatic juice proteins indicating a competition between methionine and selenomethionine. Thus, there is abundant evidence, in short-term studies at least, that ^{75}Se-methionine is incorporated into proteins presumably replacing methionine. Rates of incorporation of ^{75}Se-methionine into tissue proteins must be some measure of protein synthesis.

Since ^{75}Se is a gamma emitter, the overall metabolism of ^{75}Se-selenomethionine might be conveniently followed by whole-body counting, and if selenomethionine serves as a tracer for methionine, one would expect the incorporation and loss from proteins to be influenced by the amounts of methionine and protein in the diet. This paper reports experiments designed to determine whether whole-body counting of animals labeled with ^{75}Se-selenomethionine might be useful in following protein metabolism or in measuring protein requirements.

Methods. Three experiments were carried out utilizing mature female Charles River albino rats (200–220 g). On arrival, they were fed a purified basal diet containing 6% lactalbumin for a standardization period. The basal diet contained (%): corn starch, 38.5; sucrose, 38.8; hydrogenated vegetable oil, 16.5; cod liver oil, 0.5; Hegsted IV salt mixture (14), 5; choline chloride, 0.2; vitamin mixture (15), 0.5. The protein or amino acid mixtures were substituted for corn starch and sucrose on an equal weight basis so the diets were isocaloric. After 1 week the rats were labeled with L-^{75}Se-selenomethionine, a product of the Radiochemical Center, Amersham, England, diluted in saline and injected intraperitoneally. Animals were kept on the 6% lactalbumin diet for a second week to allow incorporation of the selenomethionine into tissue proteins. Then they were divided into approximately homogeneous groups that were given the experimental diets. The standardization and the experimental diets were fed *ad libitum.*

Urine and feces were collected and the total radioactivity in the excreta was determined weekly using a Nuclear Chicago automatic gamma well counter. In Expts. 2 and 3 the radioactivity in the animals themselves was also determined in a whole-body counter (Packard ARMAC).

By the end of each experimental period the animals were sacrificed. Samples of blood and of several tissues were taken for determination of ^{75}Se radioactivity.

Expt. 1. Fourteen groups of 5 rats each were labeled with 3.375 μCi containing 1.0 μg of ^{75}Se-selenomethionine/rat. The experimental diets consisted of varying levels of either protein or amino acid mixtures added to the protein-free basal diet. One diet contained what we called the "complete amino

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acid mixture"[2] and the rest of the diets supplied either threonine, methionine, or lysine at ⅔ or ⅓ that of the complete amino acid mixture. To obtain threonine-, methionine-, or lysine-free diets, these amino acids were completely omitted from the mixture. Lactalbumin diets were also fed to provide zero (protein-free), 1.28, 2.57, or 3.85% protein ($N \times 6.25$). The animals were sacrificed after 6 weeks.

Expt. 2. Six groups of 4 rats each were labeled with 2 μg of ^{75}Se-methionine/rat. To determine the effect of dietary protein or methionine on the turnover rate of ^{75}Se, three groups received diets containing 0, 4, or 8% lactalbumin protein and the other 3 groups received diets containing 4% lactalbumin protein and supplemented to contain a total of 0.15, 0.20, or 0.40% of L-methionine. After 1 month all diets were supplemented with 1.22 ppm of sodium selenite and the animals continued for an additional 50 days until sacrifice.

Expt. 3. In order to determine the effect of dietary selenium on the loss of ^{75}Se from the animal's body, 3 diets were tested on 6 groups of animals, of 4 rats each, labeled with 2.9 μCi of ^{75}Se-methionine/rat. The dietary levels were: 0% protein, 4% lactalbumin protein, and 4% lactalbumin protein supplemented to contain a total of 0.40% methionine. Each diet was fed supplemented with sodium selenite, 1.22 ppm, and unsupplemented. The experimental period was 51 days.

Results. Expt. 1. The average rate of excretion of ^{75}Se gradually declined with time in all groups. The diets fed appeared to have minimal, if any, effect on the amount excreted. Similarly, the level of radioactivity in the tissues was little affected by diet. Somewhat higher levels in most tissues, especially plasma and blood, were found at low levels of amino acid or protein intake but these were not significant ($p > 0.05$).

Expt. 2. As in the previous experiment, loss of radioactivity was insignificantly affected by levels of protein or methionine provided ($p > 0.05$). In comparison to the other tissues studied, the kidneys showed maximum retention. When diets were supplemented with selenium, the excretion rates increased and retention decreased.

Expt. 3. As shown in Fig. 1, the rate of retention of the label was markedly affected by the presence or absence of the selenite supplement, but appeared to be independent of the level of protein or methionine fed. Similarly, Fig. 2 shows that the addition of selenite to the diet markedly affected the level of ^{75}Se in most of the tissues, particularly in the kidney, while dietary level of protein or methionine had little and inconsistent effects.

Discussion. These experiments are based upon the hypothesis that ^{75}Se-methionine follows the same course in protein metabolism as methionine. If this is true, the turnover rate of selenomethionine from animals previously labeled should be influenced by the levels of protein or essential amino acids in

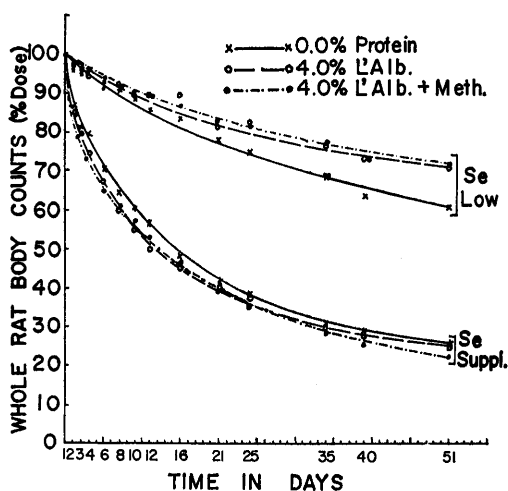

FIG. 1. Change in whole-body counts with selenium low and selenium supplemented diets of varying protein or methionine content (Expt. 3).

[2] The complete amino acid mixture contained *l*-amino acids and supplied 0.64 g of amino nitrogen/100 g of diet. Each 100 g of diet contained the following amino acids (mg): histidine HCl·H_2O, 112; arginine HCl, 134; cystine, 135; isoleucine, 219; leucine, 274; phenylalanine, 167; tyrosine, 119; tryptophan, 44; lysine HCl, 447; threonine, 203; methionine, 64; glutamic acid, 1051; glycine, 536; ammonium citrate, 808. The essential amino acids were supplied by the Ajinomoto Company of New York, Inc., New York, New York.

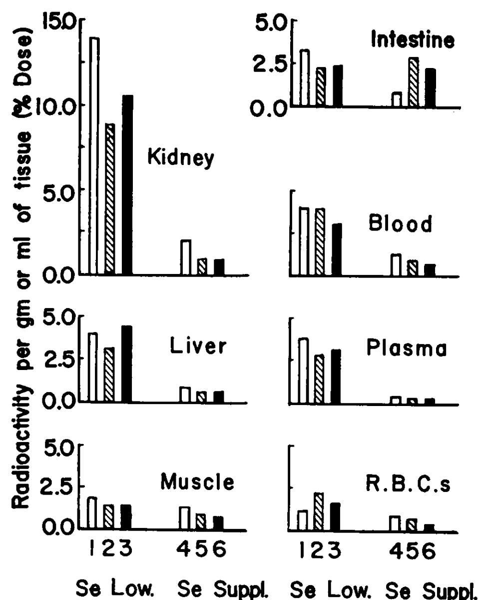

FIG. 2. Average level of ^{75}Se in different tissues in selenium low and selenium supplemented rats fed varying levels of protein or methionine (Expt. 3): (bars 1 and 4), 0% protein diet; (bars 2 and 5), 4% lactalbumin protein diet; (bars 3 and 6), 4% lactalbumin protein diet supplemented with methionine.

the diet due to variations of the "amino acid pool" or to differences in the rates of protein anabolism or catabolism. From studies in which ^{35}S-methionine was administered to young rats, Montavon *et al.* (16) concluded that excretion of ^{35}S was not markedly influenced by the dietary level of methionine until the level exceeded 0.5%. Thus, they considered that this was an estimate of the minimum requirement of methionine. This value agrees with the estimate of Rao *et al.* (17).

The present results show that feeding relatively large amounts of protein or methionine does not appreciably change the rates of ^{75}Se excretion or retention after ^{75}Se-methionine labeling. In most tissues the retention of ^{75}Se did not appear to be related to the protein or amino acid content of the diet. It is uncertain how much of the activity may represent the amino acid analogue and how much may represent other forms of selenium. This jeopardizes the underlying assumption of these experiments. Excretion of ^{75}Se was clearly related to the selenium content of the diet. These data are in accord with the statement of Schwarz (18) that "while it is likely that selenium when given in large amounts may travel with sulfur in its metabolic channels, available evidence suggests that it follows pathways of its own when supplied at small, physiological levels," and the conclusion of Burk *et al.* (19) that selenium levels of tissues and excreta in rats did not vary with changes in protein intake.

Since it is reasonable to assume that selenomethionine labels the methionine pool, we conclude that much of the selenomethionine is destroyed when it is released from the tissue proteins. The selenium presumably follows a number of pathways and much is retained in certain tissues, especially the kidney, if the animal is fed a diet relatively low in selenium. In any event, the data presented show that the excretion of ^{75}Se from the body after the tissues are labeled with ^{75}Se-methionine is little influenced by the methionine or protein content of the diet and probably cannot be used to evaluate overall changes in protein metabolism.

Summary. There is abundant evidence that selenomethionine is an analogue of methionine. We have utilized whole-body counting of ^{75}Se after the administration of ^{75}Se-selenomethionine to determine whether this technique might be used to evaluate overall changes in protein metabolism in animals fed different amounts of protein or methionine. Changes in overall body counts were not sensitive to changes in these dietary variables, but the excretion of ^{75}Se was greatly affected by the selenium content of the diet. Thus, whole-body counting is apparently not a useful technique for following the metabolism of tissue proteins.

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