duce potentiation of indirectly induced twitch tension by an effect on the motor nerve terminal resulting in repetitive discharge following a single stimulus(2,3). In the current experiments indirect stimulation of the muscle occasionally resulted in trains of transmembrane potentials but these were transient and were observed immediately following impalement of the cell. Although some irregularities occurred in the after potential, such trains of action potentials were not observed as an effect of PMA at a time when potentiation of muscle twitch was evident.

Summary. The potentiating effect of piperidylmethylandrostane on twitch response of intact, striated, anterior tibial muscle was shown to occur following both direct stimulation of the muscle and indirect stimulation *via* its motor nerve. By use of the microelectrode technic evidence was obtained which indicates that the potentiating effect is accompanied by an increase in conduction time of indirectly induced cell depolarization, and an increased duration of transmembrane and surface potentials of single muscle cells.

The advice and assistance of Dr. T. C. West and the technical assistance of Miss Jeanne Nelson is gratefully appreciated.

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Received July 11, 1962. P.S.E.B.M., 1962, v111.

Biosynthesis of Seleno-Compounds from Inorganic Selenium by Sheep.*† (27887)

IRENE ROSENFELD
Division of Agricultural Biochemistry, University of Wyoming, Laramie

Substitution of selenium for sulfur in biosynthetic processes has been postulated for a considerable period. The effects of these seleno-substituted compounds on the biological system and their function in the metabolic pathways are obscure. It is well known that small amounts of selenium may produce acute or chronic selenosis and death by its toxic effects(1), and that trace amounts of selenium prevent various disease syndromes in different species of animals(2).

Monogastric animals do not utilize inorganic sulfur compounds to any extent for synthesis of the essential sulfur amino acids and sulfur amino acids must be supplied by the diet. Evidence of microbial biosynthesis of cystine from S^{35} (elemental and sulfate sulfur) in the rumen of sheep and the presence of radioactivity in wool proteins has been reported(3). Highly significant correlations were found between the number of distorted fibers and selenium content of the wool(4).

Ruminants could supply the evidence whether selenium can substitute for sulfur in the biosynthesis of amino acids and to what extent this substitution can take place. The protein selected for investigation was wool fiber. Wool is particularly well suited for the study of protein synthesis because the catabolic breakdown encountered in other tissues is negligible in this protein.

* This work was supported in part by a research grant from AEC.

† Approved for publication by the Director of Experiment Station Journal No. 193.

Method. The sheep was clipped before the start of the experiment so that the new protein would be synthesized during administration of radioactive selenite. The animal was placed in a specially designed metabolism cage to prevent the contamination of the wool with the excretions which contained high concentrations of selenium. The Se^{75}-selenite was given orally, as indicated in the results. The increase of Se^{75} in the wool was followed by sampling the wool at 14-, 23-, and 60-day intervals with continued administration of the isotope up to 60 days. At termination of the experiment (96 hours after the last oral dose of Se^{75}) wool was clipped from the dorsal part of the animal in order to obtain wool fibers free from extraneous radioactivity. The wool was washed with water and detergent, and extracted in a Soxhlet for 8 hours. The clean wool was hydrolyzed with 3N and 6N HCl as indicated in the results and the hydrolysates were filtered to remove the humin. Excess HCl was removed by repeated evaporation on a steam bath and dissolved in water and decolorized with Darco G-60.

Total cystine in the wool hydrolysates was determined by the method of Sullivan and Hess(5). The cystine was isolated from 50 g of hydrolyzed wool protein by the method of Schmidt(6). The separation and isolation of amino acids on ion-exchange resins by carrier displacement chromatography was carried out with some modifications of the method of Buchanan(7). The amino acids separated by the resins were estimated by one- and 2-dimensional paper chromatography on Whatman No. 1 paper. The solvents were phenol saturated with 10% sodium citrate followed by sec. butanol formic acid and water (7:1.5:1.5). Determinations for amino acids were made according to Moore and Stein(8). Radioactivity was determined by a well-type Scintillation gamma detector. All samples were corrected for decay.

Results and discussion. The postulated selenium substitution for sulfur in the biosynthesis of selenium-containing proteins by microorganisms has been demonstrated. A methionine-requiring mutant of *E. coli* was able to utilize selenomethionine for normal exponential growth. Selenite-Se^{75} partially replaced sulfur in the presence of the glutathione sulfur reserve or with traces of sulfate in the biosynthesis of bacterial proteins(10).

TABLE I. Increase of Se^{75} in Wool after Oral Administration of the Isotope.

Se^{75} given, No. days	Total dose Se^{75}, mc	c/m/g wool $\times 10^{4}$*	Se, ppm
14	12†	1.67	
23	24†	2.16	
60	88‡	147.4	1.8

* Radioactive measurements were carried out in Scintillation Well Counter. Measurements were corrected for decay.

† Se^{75} was given twice a week as sodium selenite and contained 7 mg of selenium/2 mc.

‡ Se^{75} was increased to 3 and subsequently 5 times/wk. The animal also received 20 mg of nonradioactive selenium as sodium selenite on days when no Se^{75} was administered.

Incorporation of inorganic Se^{75} in yeast protein and biosynthesis of Se^{75}-selenomethionine and Se^{75}-selenocystine with elution characteristic from resin similar to S^{35}-cystine and S^{35}-methionine was reported(11). The over-all yield of amino acids was approximately 20-40% based on the starting Se^{75}.

The dose, duration of experiment, and rate of increase of Se^{75} in the wool fibers are given in Tables I and II. It is evident from Table I that there was a rapid apparent increase of selenium in the wool. The data in Table II indicate that not all of the radioactivity was bound to the protein. Treatment with various solvents removed a large amount of Se^{75} from the wool. In the sample collected on the 14th day, over 99% of the radioactivity was removed from the wool

TABLE II. Rate of Increase of Bound Se^{75} in the Wool Fibers.

Se^{75} given, No. days	Treatments*			Se^{75} bound to wool
	Water	1% detergent	Alcohol and ether (1:1)	
	%			
14		99.4	.5	.03
23	53.3	20.0	4.2	22.6
60	39.4	5.6	.4†	59.4

* Each treatment was repeated until no more radioactivity was detected in solvent.

† 50 g wool was extracted in Soxhlet for 8 hr followed by distilled water washings until wash water was free of radioactivity.

TABLE III. Effect of Acid Hydrolysis* on Bound Se^{75} in the Wool.

	3N HCl†	6N HCl†
	%	
Se^{75} in humin	5.1 ± .2‡	3.2 ± .5‡
Se^{75} on Darco G-60	37.0 ± .5	56.9 ± 2.0
Se^{75} in filtrate	58.2 ± 4.0	37.8 ± 5.0
Total Se^{75} recovered	100.3 ± 4.7	97.9 ± 5.5

* Hydrolysis was carried out for 3 hr in an autoclave at 110°.
† Results are avg of triplicate samples.
‡ Percent variation in the 3 samples.

with solvents and only a trace of Se^{75} was bound with the wool fibers. The solubility of this radioactivity substance in water suggests that probably some seleno-compound(s) was secreted through the sebaceous glands of the skin. This is the first recognition of selenium elimination through the skin glands by ruminants. At termination of the experiment, 50% of the radioactive selenium was bound with the wool protein. This radioactive wool was used for separation and isolation of the seleno-compounds.

The distribution of Se^{75} in wool hydrolysate is indicated in Table III. It is quite evident from the recovery that either not all of the selenium in the wool protein was combined with the amino acids or that the selenium compounds are far more susceptible to cleavage and volatilization than their sulfur analogues. The 6N HCl filtrate contained somewhat less radioactivity than the 3N HCl; however, there was also greater loss of cystine when stronger acid was used.

The humin and charcoal were washed until no further ninhydrin tests were obtained, indicating that the residual radioactive compounds were not amino acids, or if they were, they behaved differently from the S-amino acids.

The isolated cystine by a chemical method from 50 g of wool protein hydrolysates weighed 605 mg and contained 5.4×10^6 c/m Se^{75}. The cystine-Se^{75} contained a small amount of tyrosine and aspartic acid which could not be separated by repeated crystallization. The crystallized cystine-Se^{75} (100 mg) was separated on a Dowex-1 succinimide resin column. The position of the amino acids was established by uni- and di-dimensional paper chromatograms. Tubes 82-115 contained one single compound and most of the radioactivity was present in these tubes. The R_f value on paper chromatograms of the isolated compound was compared with known selenocystine and cystine and they were identical. The compound eluted from the spot with dilute HCl was radioactive, thereby confirming the biosynthesis of Se^{75}-selenocystine. Total radioactivity recovered was about one-fourth of the original radioactivity present in the crystalline cystine. The probable explanation may be that during crystallization the radioactive Se^{75} adhered to the crystals since no significant changes occurred in the radioactivity of the compound by repeated crystallization, or the loss of radioactivity may have been due to the decomposition of the compound.

The amino acid composition of wool protein was separated by carrier displacement chromatography on ion-exchange resins. Fig. 1 gives the amino acid composition of the wool protein and the distribution of the radioactive selenium in the fractions as they were eluted from the resins. By using this method for separation of total amino acids, the concentration of some amino acids differed from that reported by Gillespie and Simmonds(9). They reported higher values of proline, serine and threonine, all of which were above 1,000 μm per gram of wool. By estimating the number of residues they estimated the molecular weight of protein in

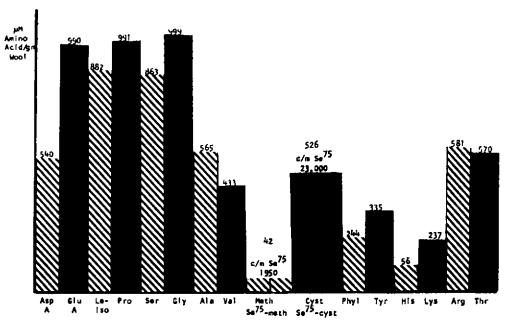

FIG. 1. Separation of amino acids from wool proteins. Asp A, aspartic acid; Glu A, glutamic acid; Le-iso, leucine isoleucine; Pro, proline; Ser, serine; Gly, glycine; Ala, alanine; Val, valine; Meth, methionine; Se^{75}-meth, selenomethionine; Cyst, cystine; Se^{76}-cyst, selenocystine; Phyl, phenylalanine; Tyr, tyrosine; His, histidine; Lys, lysine; Arg, arginine; and Thr, threonine.

wool to be over 16,000. By estimating the number of residues from present data, using their method, the estimated molecular weight of wool protein was over 19,000.

The amount of Se^{75} recovered as Se^{75}-selenocystine and Se^{75}-selenomethionine from the resins was low when compared with the original radioactivity in the clean wool. The resin columns retained large amounts of Se^{75} which would suggest that the chromatographic behavior of the seleno-compound(s) in wool protein was not identical with the normal components.

Se^{75}-selenomethionine and Se^{75}-selenocystine separated by the ion-exchange resins and known selenocystine and selenomethionine had the same R_f values as their sulfur analogues, again indicating that the selenium sulfur analogues cannot be resolved by the present methods.

Numerous studies in monogastric animals indicate that far greater percentage of selenium is "bound" by the tissue proteins(1), than by the administration of sulfate sulfur. Whether in monogastric animals the protein bound selenium is a substitution of selenium for sulfur in the sulfur amino acids or is produced by biosynthetic processes is difficult to resolve. In experimental animals the concentration of selenium could never approach that of sulfur; therefore, the biosynthesis of the sulfur compounds will always exceed that of the selenium analogues, as was indicated in the present data. Even before sulfur-selenium equilibrium could be reached the animal would die of selenium intoxication.

Following subcutaneous injection of Se^{75} into dogs, between 15 to 18% of Se^{75} was found in S-sulfokerateine of the hair and between 1 to 7% Se^{75} was in the cystine fraction(12), suggesting again that selenium is incorporated in the proteins of monogastric animals; however, the mode of binding is still obscure.

Summary. The biosynthesis of seleno-analogues of sulfur amino acids has been studied in the ruminant (sheep) by repeated *per os* administration of $H_2Se^{75}O_3$. In the biosynthetic processes of the rumen selenium can replace sulfur to form seleno-analogues of the sulfur compounds. Se^{75}-selenocystine with cystine was chemically separated from wool hydrolysate. Chromatographic separation of the amino acids in wool is reported. Se^{75}-selenocystine and Se^{75}-selenomethionine in the wool hydrolysate was demonstrated. Neither selenocystine nor selenomethionine can be separated by present methods from their sulfur analogues.

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