from another plate for a blank. Silica gel washed with absolute methanol showed lower, and less deviation in, blank values than an unwashed one.

Recovery data are shown in Tables I and II. The method using washed silica gel gave excellent recovery and good relative standard deviation.

As mentioned above, our procedure of application of samples to the holes, has proved to be an excellent method.

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Production of ^{75}Se-selenocystine by neutron activation*

It has been noted that neutron activation can result in the labeling of selenium compounds[1]. This observation seemed surprising since it would be expected that the recoil energy, following neutron capture, would suffice to rupture carbon–selenium and selenium–selenium bonds. In view of the pronounced ability of mercaptans (and to a lesser extent disulfides) to act as free radical trapping agents[2], as well as the reported high scavenging activity of selenium[3], it seemed that the labeled molecules might be formed by the recombination of free radical fragments. For instance, it could be demonstrated that neutron activation of diphenylselenide yields both labeled diphenylselenide and labeled diphenyldiselenide[4]. In view of these observations, it appeared likely that neutron activation of symmetrical selenides and diselenides might be a useful method of preparing ^{75}selenium labeled compounds.

One hundred milligrams of selenocystine were irradiated for 30 h at a flux of $1 \cdot 10^{13}$ neutrons/cm^2/sec. If irradiation were uniform and there had been negligible self-shielding, the expected specific activity would be 5.8 μC/mg. Since the fractional abundance of ^{74}Se in nature is only 0.87 %, the specific activity could have been increased some 120-fold by utilizing the amino acid synthesized from pure ^{74}Se. The original amino acid was a light yellow, while the post-irradiation sample was distinctly red in color.

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Purification was accomplished by dissolving the material in water, centrifuging, and applying the supernatant to 0.20 mm cellulose thin-layer chromatographic plates (Analtech). The radioactive ^{75}Se-selenocystine was spotted alongside of the nonlabeled amino acid and cystine as well. When run in the ascending direction on these plates (butanol–acetic acid–water, 3:1:3), the red color was left behind at the origin. Placing the red impurity in boiling water resulted in the precipitation of a black material, most likely selenium.

TABLE I

ASCENDING CHROMATOGRAPHIC SYSTEMS USED IN IDENTIFYING ^{75}SE-SELENOCYSTINE

Medium	*Solvent*	R_F
Whatman No. 1 paper	Isopropanol–HCl–H_2O 1300:334:500	0.18
Whatman No. 1 paper	Water–butanol–acetic acid 1600:340:60 (lower phase)	0.79
Whatman No. 1 paper	Water saturated with phenol	0.83
Cellulose TLC	Butanol–acetic acid–water 3:1:3	0.60
Cellulose TLC	Isopropanol–ammonia–water 12:1:12	0.95
Cellulose TLC	Isopropanol–ammonia–water 48:1:12	0.50

At R_F = 0.6, there was a spot that appeared dark when exposed to ultraviolet radiation (2537 Å), gave a pink reaction to ninhydrin (0.1 % in butanol, air dried), and contained radioactivity as determined by scraping the area off the plate and performing gamma ray counting (200–400 keV, background corrected). The cold selenocystine had the same R_F (as did cystine), gave an identical ultraviolet absorption and reaction to ninhydrin as did the radioactive product. Spots at R_F 0.6 were collected, eluted into water, and rechromatogramed twice. No radioactivity was present on the final two runs at any point other than the single spot. After the final thin-layer chromatographic run, the water was flash evaporated and nearly 3 mg of a light yellow material obtained. This radioactive substance was indistinguishable from nonradioactive selenocystine in six chromatographic systems (Table I). The specific activity of the ^{75}Se-selenocystine was about 2.9 μC/mg (one-half of that predicted in a uniform flux with negligible shielding).

It thus appears that ^{75}Se-selenocystine can be produced in low yield by irradiation of the amino acid. The known scavenging ability of selenium may account for the recombination of symmetrical halves of the molecule (or their exchange with nonradioactive molecules) following cleavage by the (n,γ) reaction.

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