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Selenite (^{75}Se) as a Tumor-Localizing Agent in Man[1,2]

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INTRODUCTION

Radioisotopic agents which are selectively concentrated in tumors are potentially useful in the diagnosis and localization of neoplasms by scintillation scanning. Observations made by one of us (K.G.S.) on rats bearing transplantable sarcoma suggested that ^{75}Se selenite ($SeO_3^=$) might be such an agent. Twenty-four hours following the injection of this ion into the rats, radioactivity was concentrated in the tumor and virtually excluded from skeletal muscle and normal brain. Tumor-to-brain concentration ratios from 10 to 13 to 1 were obtained.

Using ^{75}Se selenite in humans we have successfully localized intracranial neoplasms as well as tumors in the chest and abdomen. This report presents the preliminary results of a clinical evaluation of this agent together with data on its distribution and biological fate in man.

METHODS

Selenium-75 as selenious acid, specific activity 20 to 50 mC per mg, was obtained from Oak Ridge National Laboratory, and, recently, from Nuclear Science and Engineering Corporation, Pittsburgh, Pennsylvania. Each shipment contained 0.5 N HCl. The pH was adjusted between six and seven with 0.1N sodium bicarbonate. Nonpyrogenic physiological saline was added to yield a final concentration of 50 to 100 μC per ml. Terminal sterilization was done by autoclaving in multiple-dose, rubber-capped glass vials. Radioactivity was assayed by using a calibrated well detector and scintillation spectrometer set at the 0.269 and/or

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the 0.405 MeV photo-peak of ^{75}Se with a two per cent window. The observed count rate was compared with that from a calibrated source of ^{131}I, with adjustments made for differences in decay schemes. In order to determine the chemical purity of the material, samples of ^{75}Se were subjected to paper electrophoresis in barbital buffer, pH 8.6. Assays of samples kept in glass at 4° C for as long as three months showed no loss of radioactivity and no oxidation of selenite to selenate.

Doses of ^{75}Se ranging from 1.5 to 4.0 μC per kg body weight were given intravenously to patients. Scans were performed at intervals from 4 to 96 hours following the administration of the dose. A Nuclear-Chicago Pho-Dot instrument was used with a 19-hole focusing collimator at a scanning speed of 45 or 60 cm per minute. Brain scans were routinely done first with ^{197}Hg chlormerodrin (10 μC per kg) four hours after the dose. Immediately following the ^{197}Hg scan, ^{75}Se was injected. With the pulse-height discriminator set at 0.269 MeV for ^{75}Se, essentially all counts from residual ^{197}Hg were eliminated.

In order to determine the biological T½ of ^{75}Se selenite, three patients were given 3.0 μC per kg intravenously and total-body radioactivity was measured at frequent intervals over total periods from 91 to 204 days using the two by three inch sodium iodide crystal of the scanner with the collimator removed. The detector was positioned six feet from the seated patient. Counting time was five to ten minutes. The statistical error of counting was less than three per cent. Complete urine collections were obtained from these subjects for 72 hours following administration of the dose.

Specimens of tumor and other tissues, as well as blood, were obtained at autopsy or at operation. Samples of tissue were weighed and homogenized in saline. Aliquots were assayed for ^{75}Se in a well-type counter. The concentration of ^{75}Se was expressed as a fraction of administered dose per gram of tissue or per ml of plasma, using an aliquot of the injected dose as a standard.

RESULTS

Brain Scans

Eight patients with proven intracranial neoplasms have been scanned with both ^{197}Hg chlormerodrin and ^{75}Se selenite. In every case, the tumor was correctly localized with both agents. Figure 1 (A–D) shows representative scans using ^{75}Se. Positive scans were obtained as early as four hours following injection of ^{75}Se, but highest target-to-nontarget counting ratios were found at 24 to 72 hours. Twenty-four hours following the dose, the maximum counting rate over the tumor in various patients was two to five times the counting rate over normal brain areas. The eight cases included one astrocytoma grade III, one meningioma, and six cases of metastatic lesions.

Using both ^{197}Hg and ^{75}Se, given 24 hours apart, scans were made from five patients with cerebrovascular disease, as established by cerebral arteriography and/or craniotomy. The ^{197}Hg scans were positive in two cases with recent occlusion of the middle cerebral artery or its branches. The ^{75}Se scan was negative in both of these cases. The scans from one of these patients are shown in Figure

1 (E, F). In three other patients, including one case of cerebral embolism and two of thrombosis, both the ^{197}Hg and the ^{75}Se scans were negative. In addition a case of brain abscess was studied. The ^{197}Hg scan was positive, at four hours, and

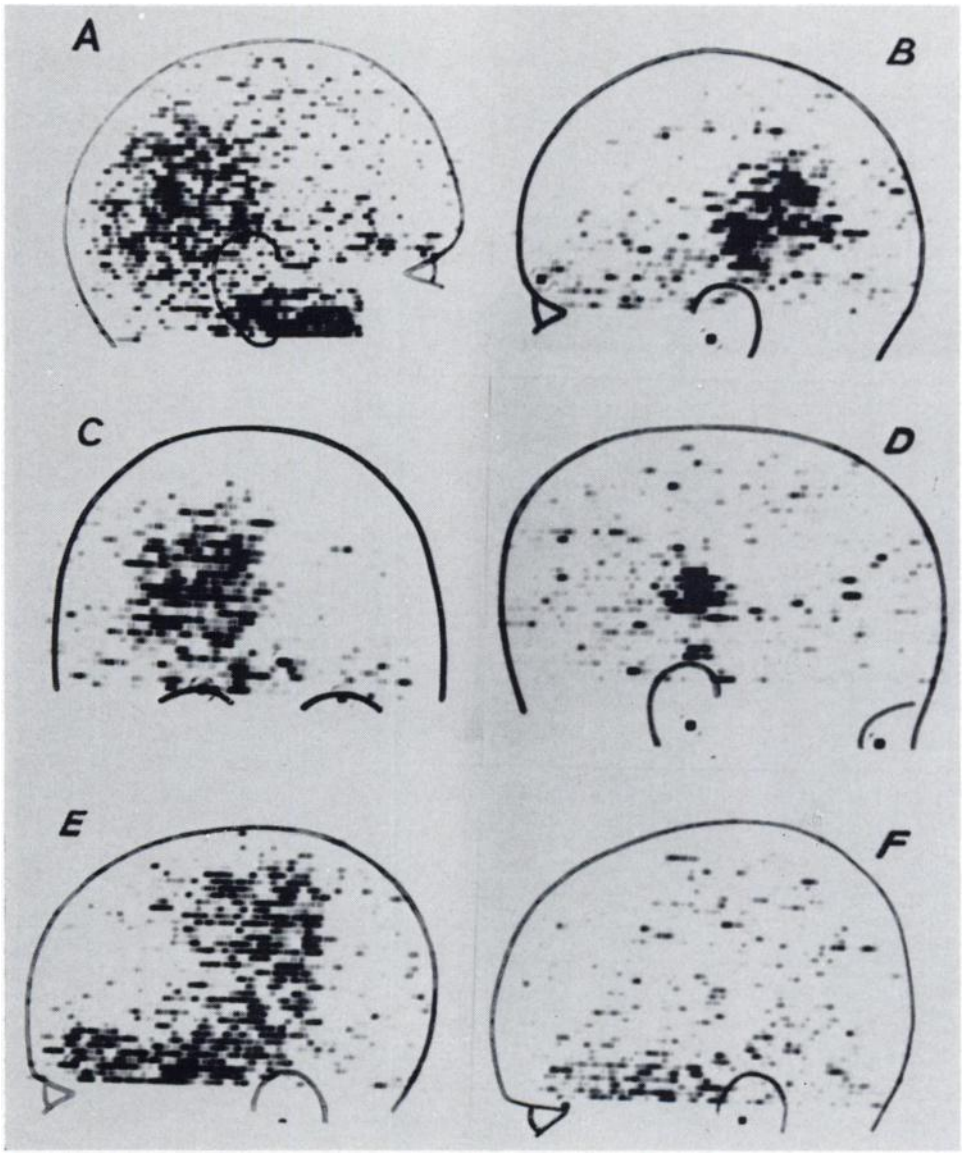

Fig. 1 (A-D). Brain scans performed 24 or 48 hours following the administration of ^{75}Se selenite, 4.0 μC per kg. Lesions are: (A) Astrocytoma, grade III. (B) Metastatic adenocarcinoma from colon. (C) Metastatic carcinoma from kidney. (D) Metastatic squamous-cell carcinoma from lung. *Scan (E) was obtained using ^{197}Hg chlormerodrin*, 10 μC per kg, in a patient with a recent cerebro-vascular accident, later shown by arteriography to be caused by an occlusion of the left middle cerebral artery. Scan (F) was done in the same patient as in (E) using ^{75}Se selenite, 4.0 μC per kg given immediately after the ^{197}Hg scan. The scan shown in (F) was performed 48 hours following the dose of ^{75}Se.

the ^{75}Se scan was negative at four hours, and questionably positive at 24 hours following administration of the dose.

Chest Scans

Three patients with primary lung tumors were scanned using ^{75}Se selenite (4 μC per kg body weight). In one case (Fig. 2) the tumor was visualized as late as fourteen days following administration of the ^{75}Se. Thoracotomy was done on the eighteenth day and revealed a bronchogenic carcinoma. The concentration of ^{75}Se in the tumor, measured *in vitro,* was 4.6 times the level in plasma. Positive scans were also obtained in one case of a plasma-cell tumor of the fourth anterior rib, and in another patient with a metastatic tumor of the scapula.

A patient with an acute, left, lower-lobe pneumonia, and another with a *Klebsiella* abscess in the right upper lobe, were also scanned. In neither was the lesion visualized with ^{75}Se selenite.

Abdominal Scans

Two patients with tumors of the gastrointestinal tract were scanned. In one, ^{75}Se was concentrated in an area later shown to correspond to a large mass of retroperitoneal lymph nodes replaced by tumor (Figure 3). In the other case, a carcinoma of the large bowel was visualized. Concentration of ^{75}Se in the liver was noted in all patients studied.

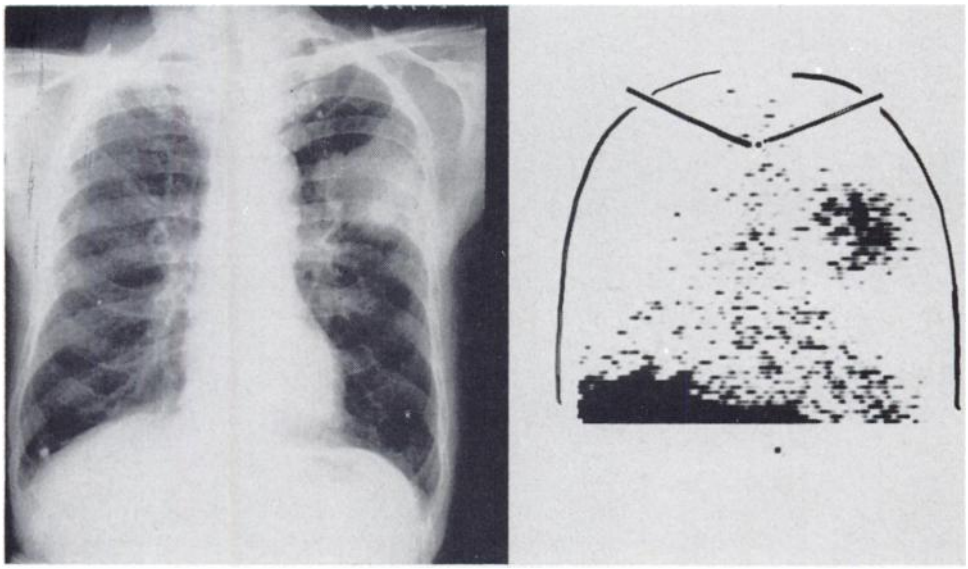

Fig. 2 ***Left:*** **Roentgenogram of chest (PA) in a patient with squamous cell bronchogenic carcinoma, left upper lobe.** ***Right:*** **scan of chest (supine) 48 hours following the administration of ^{75}Se selenite (300 μC) showing abnormal deposit of radioactivity in area corresponding to the tumor. Also note normal concentration of ^{75}Se in the liver.**

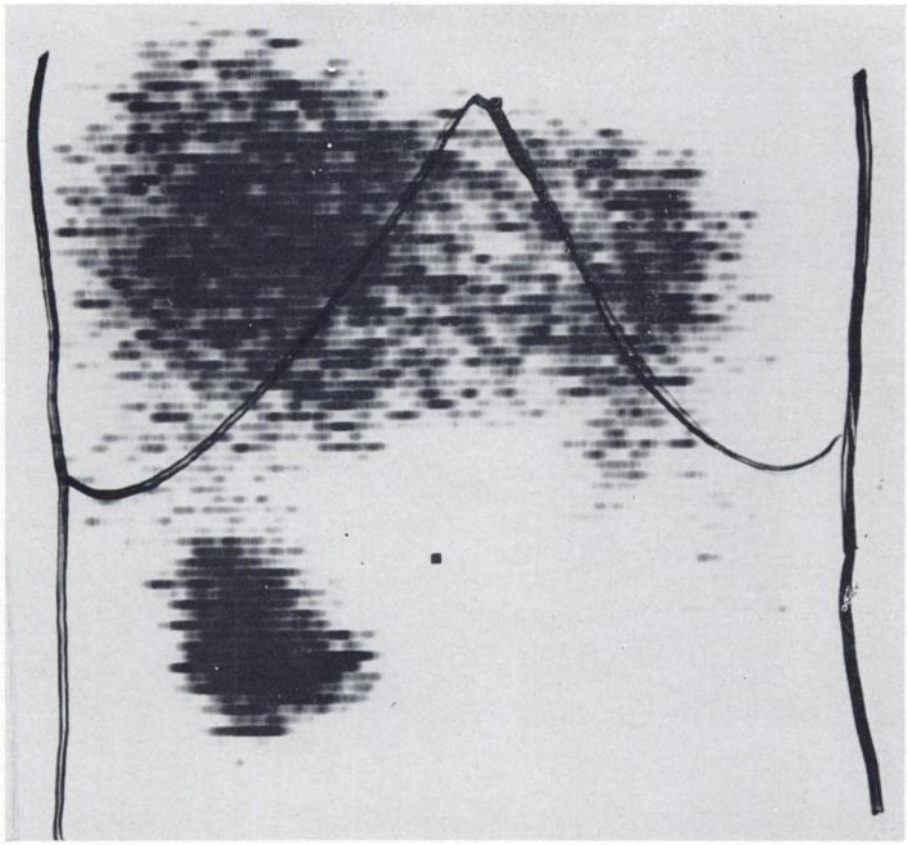

Fig. 3. Scan of upper abdomen in a patient 24 hours following injection of 200μC ^{75}Se selenite. The concentration in liver is normal, but radioactivity is also seen in left upper quadrant and right lower quadrant. These areas were later shown at autopsy to correspond to a large carcinoma in the stomach and a tumor mass replacing retroperitoneal lymph nodes.

Tissue Analyses

Specimens of tumor were obtained at autopsy or operation in six cases at intervals ranging from one to 54 days following administration of ^{75}Se selenite. Table I presents the results of the assays for ^{75}Se in these specimens and the concentrations of radioactivity in plasma obtained at the same time. In three cases intracranial tumors were obtained at surgery; in two, pulmonary neoplasms were sampled; and in one case the primary tumor was removed from the abdomen at autopsy. The concentration of ^{75}Se in the tumor ranged from 0.00085 to 0.0049 per cent of the dose per gram of tissue. The tumor-to-plasma concentration varied from 0.8 to 4.6.

In two cases (McD and Sta) specimens of tumor were homogenized in normal saline and the proteins precipitated with an equal volume of 20 per cent trichloroacetic acid (TCA). In both cases more than 95% of the total ^{75}Se was recovered in the precipitate. Treatment with hot TCA and repeated washing

TABLE I

^{75}SE IN TUMOR AND OTHER TISSUES

Patient	*Diagnosis*	*Interval after dose (days)*	*^{75}Se Concentration (Per Cent of Dose per Gram)#*					*Tumor/Plasma ratio*
			Tumor	*Plasma*	*Liver*	*Kidney*	*Skeletal muscle*	
Mul[1]	Meningioma	1	0.00244	0.0030	—	—	0.00033*	0.80
Lev[1]	Brain Tumor, metastatic	7	0.00265	0.0010	—	—	—	2.6
Wil[1]	Astrocytoma III	12	0.00082	0.00067	—	—	—	1.2
Fel[1]	Bronchogenic Ca. (primary)	18	0.0049	0.0011	—	—	—	4.6
McD[2]	Adenoca, Colon	37	0.0021	0.00082	0.0046	0.0084	—	2.6
Sta[2]	Bronchogenic (primary)	54	0.0014	0.00041	0.0025	0.0041	0.00064**	3.3

[1]Specimens obtained at operation.
[2]Specimens obtained at autopsy.
*Temporal muscle.
**Psoas muscle
#Corrected for radioactive decay.

of the precipitate failed to extract more than six per cent of the radioactivity in the TCA-precipitable fraction. In another experiment, homogenates of tumor were dialyzed overnight at 4° C against large volumes of normal saline. Only eight per cent of total ^{75}Se was found in the diffusate. This result agreed closely with that obtained by TCA precipitation.

Distribution of ^{75}Se in Man

Figure 4 shows the time course of the counting rate recorded over the liver and mid-thigh, and the concentration of radioactivity in the plasma in three subjects given ^{75}Se selenite intravenously at time zero at a dose level of 3.0 μC per kg body weight. In each case the curves were normalized to the zero-time value estimated by extrapolation of the initial portion of the curves. The mean values are plotted, with ranges indicated.

During the first hour following injection radioactivity over the liver rose rapidly to a maximum of 1.7 times the initial value. After two hours the hepatic curve fell gradually. The concentration of ^{75}Se in the plasma declined rapidly during the first hour to 0.34 of the initial level, reached a plateau, and from four to twenty-four hours fell gradually. The counting rate over the thigh declined more slowly than the plasma level and remained nearly constant from four to twenty-four hours.

By dividing the values for plasma into those for liver and those for thigh, one can obtain a measure of the rates of change in distribution of the isotope. The liver/plasma ratio was 4.8 (arbitrary units) at one hour and remained unchanged. The thigh/plasma ratio, however, increased from 2.2 at four hours to 2.9 at twenty-four hours following injection. These results indicate that during the initial twenty-four hours ^{75}Se leaves the vascular compartment, rapidly enters the liver, where uptake is virtually complete by 60 min, and more slowly penetrates into the tissues of the thigh.

Analysis of the plasma at various intervals following administration of ^{75}Se indicates that from 55% to 70% of the total ^{75}Se is protein-bound (TCA precipitable) at 60 min, 84% to 90% at four hours, and more than 95% is protein-bound at twenty-four hours.

In one case 200μC ^{75}Se selenite was preincubated at 37° for 60 min in ten ml of the patient's own plasma before injection. The rate of clearance from plasma, and the appearance of radioactivity over liver and thigh were measured. There was no significant difference in any of these parameters from the results obtained after direct intravenous injection of ^{75}Se selenite. In another experiment, normal plasma was incubated at 37° for two hours with ^{75}Se selenite (0.5 μC per ml). After incubation between 10% and 30% of total radioactivity was TCA precipitable.

During the first few days following the administration of ^{75}Se selenite very little of the radioactivity is bound to erythrocytes. At twenty-four hours, less than five per cent of the total blood ^{75}Se is associated with red cells. After the first week an increasing proportion of the isotope is bound to the erythrocytes.

Scans of the abdomen were performed at intervals ranging from four hours to four days following injection. Radioactivity was distributed uniformly through

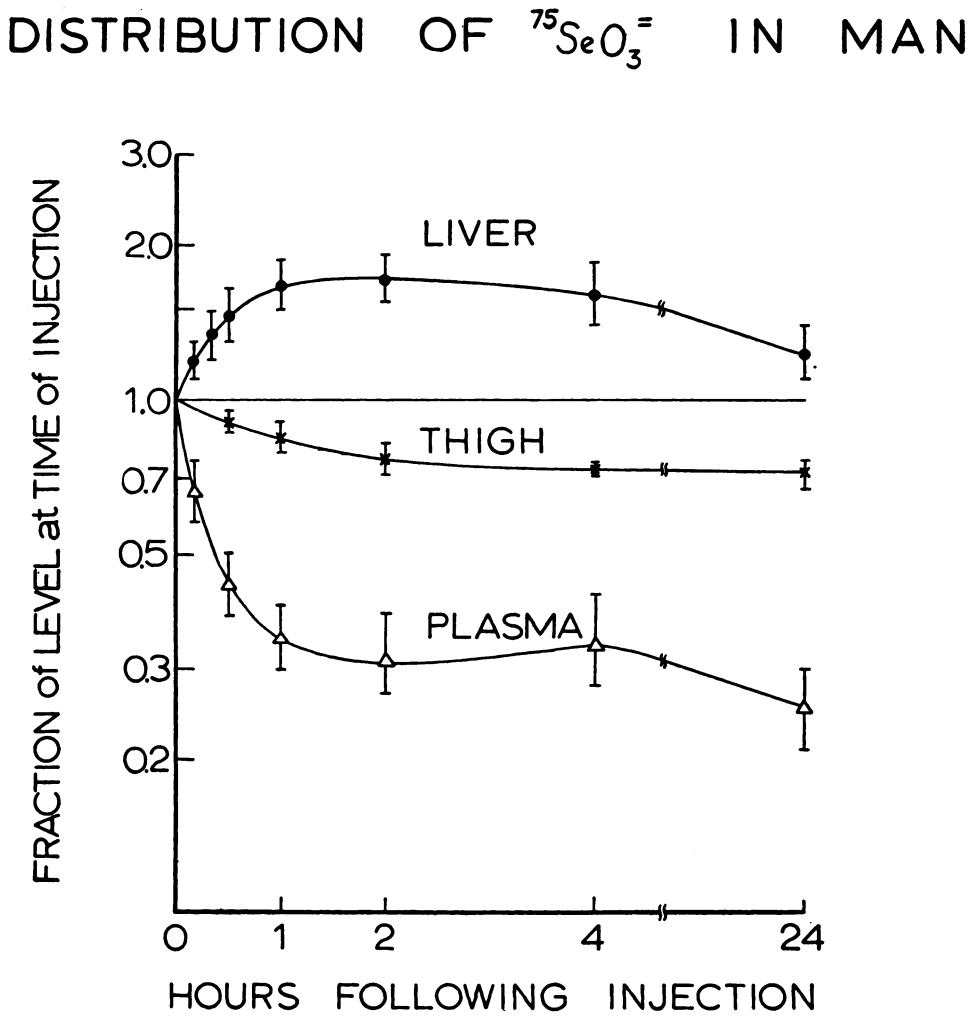

Fig. 4. Distribution of ^{75}Se selenite in three subjects. A dose of 200μC was given intravenously at time zero. Counting rates were recorded over the liver and the mid-thigh (using a two-inch sodium iodide detector and a single-hole collimator) and concentration of total radioactivity in plasma was measured *in vitro*. Each curve was normalized to the time zero value, estimated by extrapolation of the initial portion. The average for the three subjects is shown, and the range is indicated by brackets. The ordinate is logarithmic.

the liver at all times. Neither the gall bladder, intestine, or pancreas were visualized. In some patients the spleen and left kidney were visualized after twenty-four hours.

In Table I are listed concentrations in liver, kidney, and skeletal muscle in a few patients on whom these tissues were sampled. The concentration in kidney exceeded that in liver in both cases studied. Assuming that both kidneys weigh 300 grams and the liver 1500 grams, then at 37 days the kidneys contained 2.5 per cent and the liver 6.9 per cent of the injected dose. The ^{75}Se concentration

in skeletal muscle in the patient biopsied at twenty-four hours was only 0.00033 per cent per gram, one-tenth of the plasma level. Because of the large total mass of muscle (30,000g), the ^{75}Se content of skeletal muscle at twenty-four hours is approximately 10 per cent of the dose.

Excretion

Cumulative excretion of ^{75}Se in the urine of three subjects during the initial twenty-four hours following injection averaged 11% of the dose, and during the first 72 hours, 16.5 per cent.

Total-body Retention

Figure 5 shows that total-body content of radioactivity, not corrected for radioactive decay, in three subjects studied for intervals ranging from 100 to 204 days following injection. The count rate recorded at one hour after injection was used as the reference (100%) value. During the first three days, from 20% to 25% of the dose was excreted. Thereafter, the total-body retention curve followed a single exponential rate of decline with a T½ ranging from 39 to 51 days (average 43 days). In the subject followed for the longest period, less than three per cent of the injected dose remained in the body at the end of 204 days. The biological T½ calculated from the physical T½ of 127 days and the average T½ effective of 43 days, is 65 days (for approximately 75% of the dose).

Radiation Dosimetry of ^{75}Se Selenite in Man

In calculating the total-body radiation exposure from a single dose of ^{75}Se selenite we have used the effective T½ values obtained above, *i.e.*, 1.5 days for 25% of the dose and 43 days for seventy-five per cent. For an administered dose of 4.0 μC per kilogram body weight, assuming uniform distribution, the cumulative total-body exposure is 1.14 rad.

In order to estimate the dose given to the liver and the kidneys, we have assumed the uptake in liver to be 12% and in the kidneys six per cent of the dose, and the effective T½ in both organs to be the same as the total-body T½. Thus, the liver would receive 4.78 rads and the kidneys would get 6.12 rads from an administered dose of 4.0 μC per kg.

The chemical toxicity at the doses employed is negligible. A dose of 300 μC contains only 15 μg of selenium as selenite. This amount is less than the daily urinary excretion of total selenium of humans living in non-seleniferous regions (2).

DISCUSSION

The biochemical and nutritional aspects of selenium metabolism have been the subject of considerable study, especially in microorganisms and in animal species. This work has been reviewed elsewhere (1,2). Although selenium and sulfur are closely related chemically, there are important differences in the metabolism of these elements in rats and other animals (2). In the case of man, Nelp & Blumberg (3) have recently compared the fate of ^{75}Se selen*ate* with that

of ^{35}S sulfate. They found that during the initial minutes following intravenous administration of these ions, the apparent volume of distribution of selenate was significantly higher than that of sulfate. Plasma-protein binding of selenium was observed after 60 minutes.

Seleni*te* appears to be even more reactive than seleni*ate in vivo* (2). It has been shown that selenite is a potent oxidizer of sulfhydryl groups (4). This fact may explain, at least in part, the rapid fixation of ^{75}Se in tissues following the administration of labeled selenite. Results of *in vitro* experiments with sheep blood cells have indicated that the entry of ^{75}Se selenite into cells is an active process dependent upon oxidative metabolism (5). The chemical form of bound ^{75}Se is not known. We have some evidence suggesting that ^{75}Se is bound to tissue proteins, probably in covalent linkage (unpublished observations). It is unlikely that ^{75}Se is directly substituted for sulfur in methionine and cysteine, since mammalian cells, unlike bacteria, are not able to synthesize these amino acids *de novo*. The possibility exists, however, that a proportion of injected ^{75}Se becomes metabolized by intestinal microflora to ^{75}Se seleno-methionine, as has been suggested by Shrift (6).

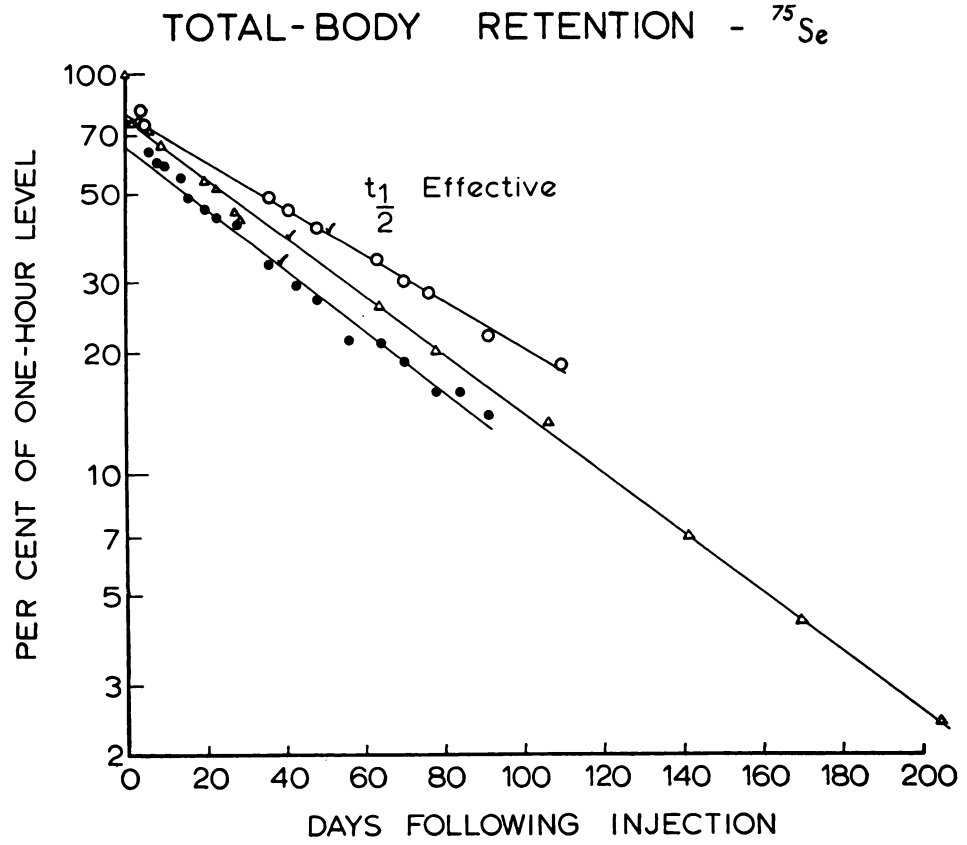

Fig. 5. Total-body retention of ^{75}Se following injection of labelled selenite in three subjects. The counting rate at one hour was used as the reference value, and the ordinate is logarithmic. See text for method of counting. *Counts are not corrected for radioactive decay.*

The apparent affinity of ^{75}Se for tumors of various types, as demonstrated in the present study, may be a reflection of the high rate of protein synthesis in actively growing cells. Alternatively, ^{75}Se may gain entry into neoplastic cells in the form of labeled plasma proteins. More than half of the circulating total ^{75}Se is precipitable, that is protein-bound, one hour after injection of labelled selenite. Studies of tumor-bearing rats (7) and mice (8) have clearly shown that neoplasms take up larger amounts of unaltered plasma proteins from the circulation than do any other tissues. The process may involve pinocytosis. Studies are in progress to investigate the mechanisms by which selenium is incorporated into mammalian cells growing in tissue culture. It is hoped that such experiments may shed light on the more complex *in vivo* events.

The apparent specificity of ^{75}Se selenite for tumors deserves some comment. In two patients with proven cerebrovascular lesions and in one case of a brain abscess, ^{75}Se scans were negative and ^{197}Hg chlormerodrin scans were unequivocally positive. In each case both agents were administered and scans were done within a period of less than 48 hours. Furthermore, ^{75}Se has permitted the visualization of tumors in the chest and abdomen. In two cases inflammatory pulmonary lesions gave negative ^{75}Se scans. Only continued experience with this agent will answer the question of tumor-specificity.

On the basis of the preliminary results with ^{75}Se selenite the following advantages of this agent appear to include: 1) the gamma-ray spectrum with its 0.269 MeV photon is easily collimated with commercial available equipment and allows high detection efficiency; 2) ^{75}Se selenite is relatively inexpensive; 3) the material is radio-chemically stable for at least three months; 4) the long physical T½ permits scans to be repeated at daily intervals after a single dose; 5) uptake by skeletal muscle is low, which may allow the detection of posterior-fossa lesions; 6) the radiation dose to the kidneys from ^{75}Se is much lower than that from ^{203}Hg in brain scanning; 7) ^{75}Se appears to be useful in detecting intrathoracic and intra-abdominal neoplasms.

The main disadvantage of ^{75}Se selenite is the relatively long biological T½ and high cumulative total-body dose. In view of the uncertainty regarding the radiation dose to the gonads, it is not recommended for routine use in patients under the age of forty. It is worth noting, however, that the biological T½ of ^{75}Se seleno-methionine in man is 140 to 150 days (9) which is considerably longer than the estimate of 65 days which was found in the present study for ^{75}Se selenite.

If further experience with this agent enhances its promise of high tumor specificity, then the relative disadvantage of radiation exposure would be outweighed by its great diagnostic value.

SUMMARY

To a degree which permits visualization of the tumor by scintillation scanning, ^{75}Se labelled selenite ($SeO_3^=$) has been shown to localize in intracranial, intrathoracic, and intraabdominal neoplasms of humans. Assays of specimens of tumors obtained at operation or autopsy yielded concentrations of ^{75}Se ranging

from 0.8 to 4.6 times the level in plasma. The results of distribution studies showed relatively high concentrations of label in liver and kidney and low levels in skeletal muscle. The calculated total-body radiation exposure based on a diagnostic dose of 4.0 μC per kg body weight and an average effective T½ of 43 days (for 75% of the administered dose) is 1.14 rads. Preliminary clinical experience indicates that ^{75}Se selenite may offer specific tumor-localizing properties not found in other currently used agents.

ADDENDUM

Since submission of the manuscript for publication further experience with ^{75}Se selenite has been gained. Six additional patients with brain tumors and one with granuloma have given positive scans with ^{197}Hg chlormerodrine and ^{75}Se selenite. One low-grade astrocytoma was missed with both agents. Among four other patients with recent cerebro-vascular accidents and positive ^{197}Hg scans, three were negative with ^{75}Se. Although ^{75}Se may not distinguish between neoplastic and inflammatory lesions, this agent appears to be useful in differentiating cerebro-vascular from other types of intracranial lesions.

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