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The Tissue Distribution of Se^{75}-selenouracil and Se^{75}-selenourea*

By

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With 3 Figures in the Text

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The development of techniques of photoscanning has made it possible to visualize lesions in many areas that could not be studied by previous radiologic techniques. In order to permit the visualization of an organ or lesion by this scanning technique, a substance must be labeled with a radioisotope having a gamma, x-ray or positron emission and must, of course, concentrate in the organ to be studied in such a way that abnormalities of the organ can be seen as areas of increased or decreased uptake, relative to the rest of the organ. The concentration of the isotope must be low in surrounding organs that come within the field of the scan. As the distribution of a radioisotope depends upon the chemical form in which the isotope is given, further developments in the field of scanning will very probably be critically dependent upon basic pharmacologic work. It is a medical axiom that advances in therapeutics depend heavily upon basic pharmacologic investigations. But with the advent of photoscanning clinical medicine now has a diagnostic technique in which further advances will also depend heavily upon basic pharmacologic work. Moreover, the concern of pharmacologists in this area must lie more investigations of structure-distribution relations than structure-activity relations.

The diagnostic localization of a radioisotope to permit scanning is similar to the therapeutic localization of a radioisotope to permit irradiation of a neoplasm or hyperplastic organ in that both situations require high uptake in the targest tissue relative to the uptake in non-target tissue. But there is a significant difference in that the critical non-targest tissues in diagnostic localization are those anatomically adjacent to the target tissue whereas the critical non-target organs in therapeutic localization are those highly susceptible to radiation effect, such as bone marrow.

* Dedicated to Professor OTTO KRAYER, with gratitude and respect, on the occasion of his sixty-fifth birthday.

The development of labeled compounds to permit scanning of various organs has been slowed by the fact thas many of the common constituents of biologically important compounds, e.g., nitrogen, carbon, sulfur, etc. do not have radioisotopes with suitable emission and half-life. However, BLAU[2,3] substituted selenium for sulfur in methionine in order to take advantage of the gamma emission of selenium-75 in scanning the pancreas. WILLIAMS[6-8] had previously studied the distribution of unlabeled thiouracil and thiourea in experimental animals and man. He had found relatively high concentrations of these compounds in several organs of the body, including the adrenal glands. We therefore undertook a study of the selenium analogs of thiouracil and thiourea, labeled with Se^{75}, in order to determine whether such compounds might permit the visualization of the adrenal glands or other organs by scanning.

Methods

Selenouracil and selenourea, as supplied, had specific activities of 45 to 67 μc/mg and 1000 μc/mg, respectively*. To prevent oxidation, each compound was supplied in sealed vials, each vial containing 1 mc. All vials were kept at approximately $-18°$ C. until use and exposure of the compounds to light and air was kept as brief as possible during the experiments. Selenouracil was dissolved in dimethylacetamide just before injection. Selenourea was received as an aqueous solution. Just before use it was mixed with a solution of sodium chloride to bring it to isotonicity and to adjust the radioactivity to the desired value. The activity of the labeled selenouracil and selenourea solutions, as injected, was 80 to 384 μc/ml and 95 to 1300 μc/ml, respectively.

Thirty-five male rabbits, weighing approximately 3 kg each, were used. Of these, 21 received Se^{75}-selenouracil in doses of 40 to 250 μc intravenously. *Four* of the 21 died 1—12 minutes after injection. *Eight* were sacrificed, at times ranging from 1 hour to 30 days after injection of the isotope, by an excessive dose of pentobarbital sodium in isopropyl alcohol**. Directly after death, tissue samples (See Fig. 1) were taken and weighed and their radioactivity was determined in a well scintillation counter. *Two* rabbits were used only for scanning (see below) and tissues were not taken. *Three* received 6 to 8 doses of adrenocorticotropic hormone (ACTH)*** prior to receiving Se^{75}-selenouracil. Each dose was 3 clinical units, given intramuscularly at 12 hour intervals, the last dose being given on the morning the rabbit was to receive the radioisotope. *Two* rabbits received thiouracil, dissolved in their drinking water, for 2 days before the Se^{75}-selenouracil was given. One of these was sacrificed 1 hour after the injection of the isotope; this rabbit had received approximately 250 mg of thiouracil. The other was sacrificed 5 days after injection of the isotope and continued to receive thiouracil in his drinking water until sacrifice; he had received a total of 380 mg of thiouracil. As NaOH was required to dissolve the thiouracil, *two* other rabbits served as controls, drinking water of the same pH (8.0—8.2) without thiouracil.

* These compounds were synthesized by the U. S. Nuclear Corporation, Burbank, California, under the direction of Dr. KARL AMLAUER.

** "Lethal" solution. Haver Lockhart Laboratories, Kansas City, Missouri, U.S.A.

*** Acthargel, Armour Pharmaceutical Company, P.O.Box 511, Kankakee, Illinois, U.S.A.

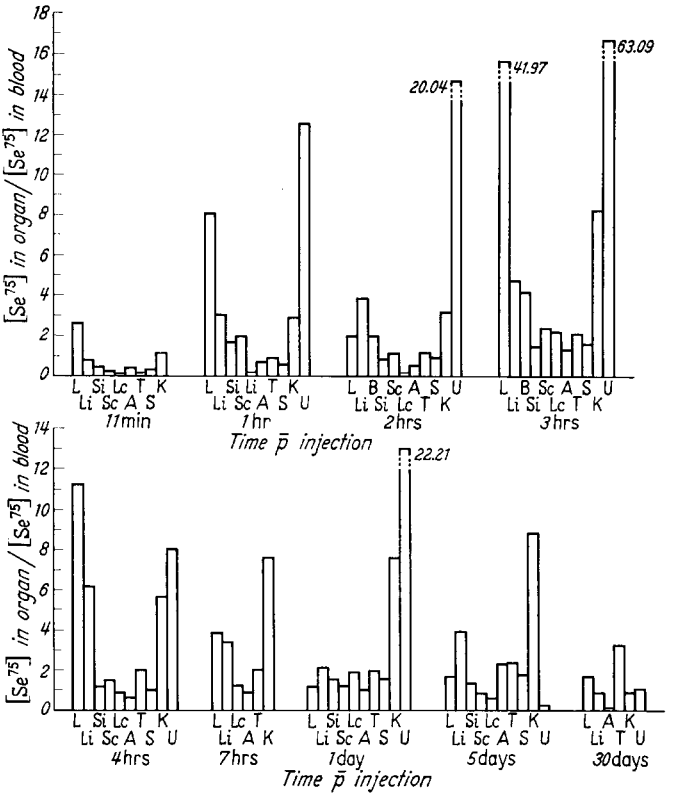

Fig. 1. Tissue: blood concentration ratios for Se^{75} in tissues of rabbits at various time after injection of Se^{75}-labeled selenouracil. Only the 11 tissues with highest concentrations of the radioisotope are shown in the figure. Other tissues and secretions studied included bone, bone marrow, fat, large intestine (i.e., the full thickness of the wall without the contents of the lumen), pancreas, skin, spleen, stomach, stomach contents, testes and pituitary. One rabbit was studied at each time indicated in the figure. *L* Lung; *Li* Liver; *B* Bile; *Si* Small Int; *Sc* Small Int cont; *Lc* Large Int cont; *A* Adrenal; *T* Thyroid; *S* Spleen; *K* Kidney; *U* Urine

Fourteen rabbits received Se^{75}-selenourea in doses of 40 to 200 μc intravenously. *Eight* of these were sacrificed and tissue samples were taken 20 minutes to 3 days after injection of the isotope (See Fig. 2). *Four* were used only for scanning. *Two* received ACTH before injection of the isotope. One of these received 7 and the other received 8 doses; the dose, route and schedule of injection were the same as those used prior to Se^{75}-selenouracil injection.

Scans were performed in 10 of the rabbits that had received selenouracil, at times ranging from 20 minutes to 25 hours after the administration of the isotope. A total of 10 scans was performed in 8 of the rabbits that had received selenourea, at times ranging from 25 minutes to 7 days after administration of the isotope. Scans were usually performed *before* sacrifice, with the rabbit under pentobarbital anesthesia. The rabbits were scanned in the prone or supine position, with legs extended. The field of the scan usually encompassed the thorax and upper abdomen. A commercially available photoscanner*, employing a 3″ ×2″ crystal and 19-hole

* "Magnascanner", Picker X-ray Corporation, White Plains, New York, U.S.A.

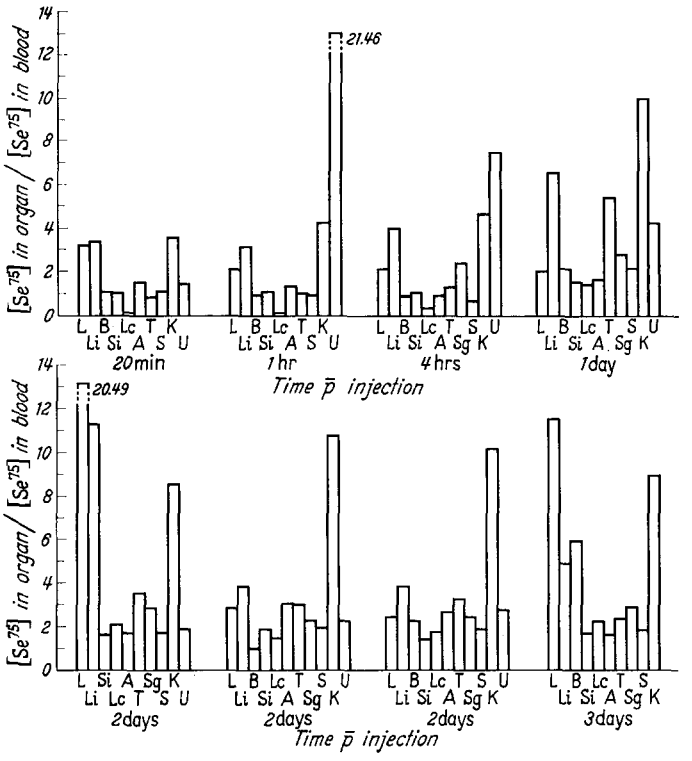

Fig. 2. Tissue: blood concentration ratios for Se^{75} in tissues of rabbits at various times after injection of Se^{75}-labeled selenourea. Only the 11 tissues with highest concentrations of the radioisotope are shown in the figure. Other tissues and secretions studied included bone, bone marrow, fat, large intestine, small intestine contents, skeletal muscle, pancreas, skin, spleen, stomach, stomach contents, testes and pituitary. Each of the 8 sets of data was obtained from a single rabbit; note that 3 rabbits were sacrificed 2 days after injection. *L* Lung; *Li* Liver; *B* Bile; *Si* Small Int; *Lc* Large Int cont; *A* Adrenal; *T* Thyroid; *Sg* Salivary Gland; *S* Spleen; *K* Kidney; *U* Urine

focusing collimator was used. Contrast amplification of scans was obtained by the use of closed circuit television, as described by BENDER and BLAU[1].

Results

Fig. 1 shows the organ:blood ratio of Se^{75} concentration, i.e., [Se^{75}] in the organ/[Se^{75}] in blood, after administrat'on of Se^{75}-selenouracil in the 8 rabbits sacrificed for tissue counts plus one rabbit that died 11 minutes after injection. Only tissues with relatively high concentrations of Se^{75} are shown in the figure; tissues with lower concentrations are noted in the legend. Renal concentration and urinary excretion of the isotope obviously play a prominent role after administration of Se^{75}-selenouracil. Hepatic concentration and biliary excretion also appear significant. The highest relative concentration in the adrenal glands appears at 5 days in this small series of animals, but even at this time

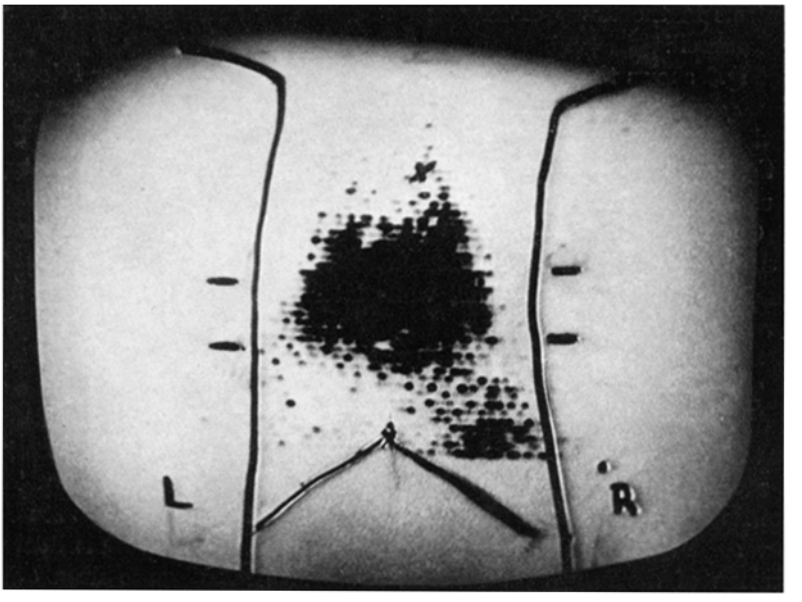

Fig. 3. Scintigram of a rabbit after administration of Se^{75}-selenouracil. Dorsal view. One hundred μc was given intravenously $5^1/_2$ hours before scanning, which was carried out under pentobarbital anesthesia. The outlines of the body wall and lower costal margins have been projected onto the scan. After completion of the scan the rabbit was sacrificed while still in scanning position. The lungs, diaphragm and liver were transfixed and held in position with long needles inserted through the skin. The thorax and abdomen were opened for inspection. The upper horizontal lines drawn at each side of the body wall show the highest level reached by the liver under the curve of the diaphragm in expiration. The lower lines show the lowest level reached by the lungs curving over the diaphragm. The upper "*X*" shows the lowest cervical vertebra. The lower "*X*", at the apex of the inferior costal margins, shows the lowest thoracic vertebra

the concentration in the liver is greater than in the adrenal gland. A remarkable finding is the high concentration of Se^{75} in the lung, noted throughout all the early hours after injection.

Fig. 2 shows the [Se^{75}] in organs/[Se^{75}] in blood in 8 rabbits after administration of Se^{75}-selenourea. Renal and hepatic excretion of the isotope are important after injection of Se^{75}-selenourea. The highest [Se^{75}] in adrenal glands/[Se^{75}] in blood after injection of Se^{75}-selenourea is found at 2 days, among the times measured. A high pulmonary concentration of Se^{75} is found after administration of labeled selenourea; although a larger number of animals would obviously be required to establish the precise time of peak concentration, this appears to come at a later time after injection of selenourea than after injection of selenouracil.

Prior administration of ACTH did not significantly influence adrenal uptake of Se^{75} in these experiments. The rabbits that received stable thiouracil did not show significant differences from their

controls in the tissue distribution of Se^{75} after administration of Se^{75}-selenouracil.

Fig. 3 shows the scan of a rabbit obtained $5^1/_2$ hours after injection of 100 μc of selenouracil. The outline of the lungs is visible, most evident in the lower lobes, where the density due to the concentration of Se^{75} in the lungs is superimposed on that due to concentration of radioisotope in underlying liver.

Of the two compounds, the use of labeled selenouracil allowed better scans. The lungs were best demonstrated in scans taken early after administration of selenouracil, while later times proved better in the case of selenourea.

Discussion

The concentration of radioisotope in each organ is reported as concentration relative to that in blood rather than absolute concentration, for the organ : blood ratio is more helpful than the absolute concentration in predicting the usefulness of a labeled compound for scanning. The distribution of Se^{75} after administration of the two compounds studied here suggested the following: these compounds should permit demonstration of the lung by scanning; no serious interference from other intrathoracic organs should be expected; the principal interference would be expected to come from the liver; the lungs would probably be best demonstrated in scans taken early after administration of selenouracil, while later times would be better in the case of selenourea; the former compound would permit better visualization of the lungs. These predictions were confirmed by actual scans.

The concentration of radioisotope in the lung after administration of these compounds is of interest. Our experiments do not permit us to say in what form the radioisotope is present in the lungs, or whether it is present in significant concentrations in expired air, but it should be noted that individuals poisoned with selenium excrete the metal in expired air, presumably in the form of methyl selenide. Hepatic concentration and biliary excretion, renal concentration and urinary excretion of radioisotope after administration of these compounds are not surprising. In one rabbit receiving selenourea, a high concentration in the wall of the large intestine was observed twenty-four hours after injection. Although colonic excretion of metals is well-known, this single observation, not found in rabbits studied at other times in our experiments, must be accepted with caution. Similarly, the demonstration of an unusually high concentration of radioisotope in the heart of one rabbit 30 days after administration of selenouracil is of interest, but it must also be accepted with the caution that a single observation deserves.

In view of the potential importance of pulmonary scans in demonstrating certain lesions (e.g., pulmonary infarcts) not easily detected by

present roentgenologic techniques, the question of practical clinical use of Se^{75}-selenouracil arises. These compounds do not seem to give sufficiently *good* scans to make them of practical importance. The scan shown in Fig. 3 is greatly inferior in quality to the scans of such organs as liver, kidney, heart (myocardium), etc. that we can now routinely obtain in experimental animals and in man by the use of other labeled compounds. A second disadvantage is the toxicity of the compounds used in this study. Among the 4 rabbits dying after injection of selenouracil, *two* received an estimated dose of 1.5 mg, *one* received 3.5 mg and *one* received 8.8 mg of the compound. All of the rabbits that died after injection showed considerable dyspnea before death. No rabbits died after selenourea injection. There may be a real difference in the toxicity of the two compounds, but the higher specific activity or greater solubility of the selenourea may very well have accounted for its greater safety. In any event, the problems of solubility and toxicity with either compound could be greatly ameliorated by the production of compounds of still higher specific activity. A third problem, hepatic interference, is a much more difficult one. This is a well-known phenomenon with scans of several other organs. This interference is due to an unfortunate combination of circumstances,—the large mass of the liver, its "strategic" position in relation to thoracic and abdominal fields and its capacity to concentrate an extraordinary variety of substances. Although tissue absorption cannot be entirely ignored, organs of large size will in general, of course, emit more radiation than smaller organs when the concentration of isotope per gram of each organ is the same. This puts the lung at a serious disadvantage, as much of the lung volume in vivo is air rather then tissue. Taking all three disadvantages into consideration, it should be definitely stated that neither of the two compounds studied here is recommended for a trial of scanning in human patients. Other techniques of pulmonary scanning, which are being developed by other workers, seem to offer more promise.

Finally, the relation of our experiments to the possibility of demonstrating the adrenal gland by scanning deserves mention. With the limitations of resolution of our present scanning apparatus, it is unlikely that the adrenal glands of rabbits could be demonstrated with great accuracy by scanning but the human adrenal glands are large enough to permit such a demonstration, provided the proper labeled compound to concentrate in the adrenals is found. In view of the limitations of other methods of study of the physiology and pathology of the adrenal gland, the development of adrenal scanning would probably represent a greater advance than the development of pulmonary scanning. Although appreciable adrenal: blood ratios were found in our experiments with selenourea and selenouracil, the ratios were not sufficiently high,

especially in comparison with the ratios in surrounding organs, to make these compounds useful in adrenal scanning. It is possible that a return to the sulfur-containing compounds themselves, instead of their selenium analogs, might be more fruitful. For the sulfur compounds, another method of labeling will be needed. 5-iodo-2-thiouracil (iodothiouracil) has been synthesized and actually used in clinical medicine. If this compound were labeled with radioactive iodine, it might permit adrenal scanning. We have initiated preliminary work to investigate this possibility.

More significant is the demonstration of CHENOWETH[4] that certain anesthetics are preferentially concentrated in the adrenal gland. Although Chenoweth's experiments did not employ labeled compounds, the adrenal concentration of halothane, $CF_3CHBrCl$, and methoxyflurane, $CH_3OCF_2CHCl_2$, are noteworthy. We believe that several members of the series of halogenated hydrocarbons, if properly labeled, may offer significant aid in the development of adrenal scanning. Although ordinarily given as inhalation anesthetics, some of the compounds in this series have already been used by other workers[5] in injectible emulsions for anesthesia. Using compounds of high specific activity, it should be possible to inject emulsions sufficient for a trial of adrenal scanning without anesthetic effect.

We consider that compounds of the following structure deserve a trial in adrenal scanning:

$$RCF_2I \quad \text{and} \quad RCF_2CXYI,$$

where R = CH_3O, CH_3CH_2O, H or any halogen and X and Y = H or any halogen.

Simpler halogenated hydrocarbons, not containing fluorine, and compounds of longer chain length than those shown here are also of interest, as are symmetrical substituted ethers. We are now carrying out preliminary investigations with both fluorinated and non-fluorinated hydrocarbons of the type described above, the radioactive label being the iodine atom(s) in the molecule. The presumable fat solubility of such compounds also suggests possible usefulness in brain scanning, though our preliminary results with these compounds have been more encouraging with respect to concentration in the adrenal glands than in the brain.

Summary

With the development of photoscanning clinical medicine now possesses a diagnostic technique in which further advances will depend heavily upon pharmacologic investigations of structure-distribution relations. In the present study the distribution of the selenium analogs of thiouracil and thiourea, labeled with Selenium-75, was studied in

rabbits. After administration of either compound, high concentrations of Se^{75} were found in the lung, liver and kidney. The concentration of Se^{75} in the lungs after administration of these compounds, especially Se^{75}-selenouracil, permitted visualization of the lungs in the living, intact animal by photoscanning. However, neither Se^{75}-selenouracil nor Se^{75}-selenourea permit pulmonary scans of high quality and these particular compounds are not recommended for trial in man. The possible relation of labelled compounds of this type, as well as certain other series of labeled compounds, to the problem of adrenal scanning is also discussed.

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