JOURNAL OF NUCLEAR MEDICINE 8:197-208, 1967

Uptake of Selenomethionine by Mouse and in Human Lymphomas, with Observations on Selenite and Selenate[1,2]

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Determination of the extent and activity of the lymphomatous diseases has been a difficult problem. Tomography (1) and lymphangiography (2) have offered some assistance; however, the former procedure has limited applicability and the latter is time-consuming and technically difficult. The report by Herrera and co-workers (3) that lymphomas often concentrated sufficient ^{75}Se-selenomethionine to be detected on external scanning, suggested that an additional procedure might be available for following these disorders. The present study was undertaken to evaluate ^{75}Se-selenomethionine as a scanning agent in the lymphomas, to compare it with lymphangiography, and to evaluate factors concerned with the uptake of the radiolabel by these tumors. In addition, since Cavalieri and associates (4) reported that selenite was taken up by a number of human tumors, uptake of both selenite and selenate have been compared with that of ^{75}Se-selenomethionine in a mouse lymphoma.

METHODS

Thirty-five patients with lymphomas were studied. The procedure was to administer 150 μC of ^{75}Se-selenomethionine intravenously (E. R. Squibb Co., specific activity about 300 mc/mg selenium). Abdominal scans were performed 30 minutes and 24 hours later and at repeated intervals when possible. If neck masses were present, these were also scanned. A Picker Magnascanner with three-inch NaI crystal was employed (240 to 320 keV window, 0.5 cm line spacing, conventional 19-hole collimator, focal point three inches). Patients voided just prior to scanning, in order to avoid activity in the bladder. If significant activity was found in the left upper quadrant on the 24-hour scan, a lateral scan was attempted in an effort to determine the anteroposterior depth at which the radioisotope had accumulated. In thirteen of the patients with Hodgkin's disease, lymphangiography was performed within three weeks of the radioisotope scan, so that the two procedures could be compared.

[1]Supported by Grants CA05094 and CA06519 from the U.S. Public Health Service and by an Institutional Grant (IN-31-G) from the American Cancer Society.

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^{75}Se-selenite (100 μC) was also given intravenously to a terminally ill 83-year-old man thought to have a lymphoma. At autopsy a week later, the palpable abdominal tissues were found to be liposarcomas.

^{75}Se-selenite, selenate, and selenomethionine were studied for entry into tumor, liver, intestine and muscle in mice at a dose of 6 μC (injected via the tail vein). Animals were sacrificed at various time intervals, tissues quickly removed, weighed and counted in a gamma-detecting crystal at constant geomerty. Two distinct mouse populations were utilized. The first was a group of hybrid mice (BC $\times$ B_{57}-designated PC_1A) bearing a transplantable lymphoma that had originated in a 23-month-old donor (747 PC_1A-5). Mice bearing tumors from the 1st and 3rd generation of transplantation were used (mice were provided by W. U. Gardner, Ph.D., Department of Anatomy). The second group consisted of normal BDF 1 male mice as controls, and similar animals (20 to 25 gm) one-to-two weeks after the injection of 10^5 to 10^6 L5178Y lymphoma cells subcutaneously into the abdominal wall.

To study the incorporation of ^{75}Se-selenomethionine into trichloroacetic acid insoluble fractions, a small fragment of mouse tissue was dissected out, homogenized in 1 ml cold Krebs-Ringer bicarbonate buffer (pH 7.4) and then an equal volume of 5% trichloroacetic acid added. After standing at 4°C. for 30 minutes, the suspension was centrifuged at 10,000 rpm for 10 minutes. The supernatant was decanted. The insoluble fraction was rewashed, centrifuged and the super-

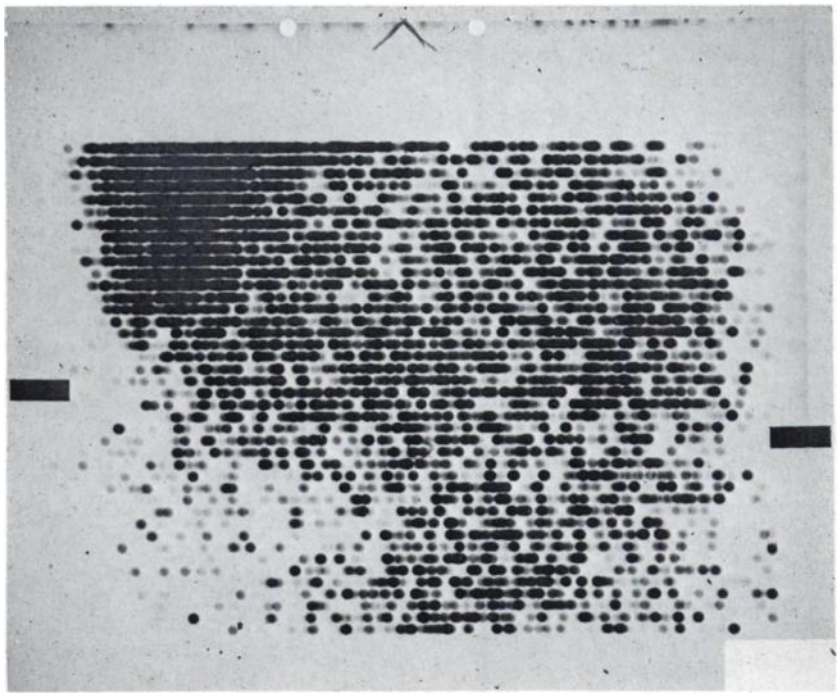

Fig. 1. Abdominal scan in patient 16, one-half hour after ^{75}Se-selenomethionine. There was widespread uptake, with a photodensity nearly equal to that of the liver. The picture was little changed at 24 hours. The umbilicus and costal margins are marked on the film.

natant added to the first supernatant (a third washing was noted to add less than 1% activity to the supernatant and was abandoned). The activity in the insolubles, divided by the activity in the insolubles plus supernatant, was used as the fractional incorporation into protein (experiments have yet to be done, however, as to the site of incorporation—whether peptide-bound or non-specifically attached). In an attempt to block entry of ^{75}Se-selenomethionine into protein, mice were given 1.5 mg of puromycin and 100 μg of actinomycin-D intraperitoneally at hourly intervals for a total of four injections. Immediately after the last injection, animals were injected with the ^{75}Se-selenomethionine.

An attempt was made to demonstrate ^{75}Se-selenomethionine entry against a concentration gradient into lymphoma cells *in vitro*. In this series of experiments, 50 to 250 mg segments of the L5178Y lymphoma were incubated in 250 mg of buffer (Krebs-Ringer or Fischer's medium) (15) containing 2 μC ^{75}Se-selenomethionine. Incubation was at 37°C. after gassing with 95% 0_2 + 5% CO_2. By the decrease in the counts in the external solution, and the assumption that 80% of the tumor tissue was water, the uptake/gm tissue water could be determined at 15, 30, 60, 90 and 120 minutes.

RESULTS

On the basis of pancreatic scans performed in this laboratory, we defined a normal abdominal scan as follows. Uptake in the liver or pancreas was normal, as was a minimal patchy uptake in the left side (possibly representing the kidney). Activity in the small bowel did not form a definite pattern, and

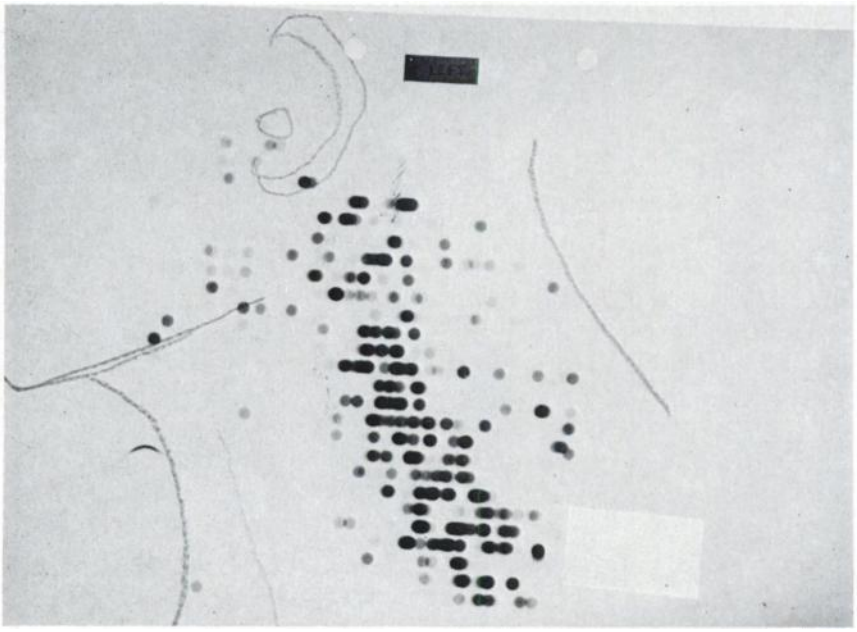

Fig. 2. Scan of the left side of the neck of patient 34, one-half hour after administration of the radiolabeled selenomethionine. Uptake corresponded to palpable nodes.

differed in the 30-minute and 24-hour scans. If there was any question as to whether activity represented radiolabel in the gut lumen, or in nodes or abdominal masses, scans were repeated (as the pattern in the bowel lumen will change with time).

Thirteen patients with Hodgkin's disease had both lymphangiograms and ^{75}Se-selenomethionine scans of the abdomen (Table I). The two procedures agreed in ten patients (no abnormal nodes or masses in six, abnormal uptake in four). In one case, the lymphangiogram was not entirely satisfactory (too little dye in the abdomen) while the ^{75}Se-selenomethionine scan was grossly positive (there were no unsatisfactory scans in this group of 13 patients). In two cases, the lymphangiograms were reported as showing small para-aortic nodal enlargements, while the ^{75}Se-selenomethionine scans were interpreted as being within normal limits.

In 22 other patients with lymphomas, lymphangiography was not performed in temporal proximity to the radioisotope scans (Table II). Of these patients, 13 had abnormal uptake over the abdomen (seen on the scan at 30 minutes as well as at 24 hours). Seven patients (who did not have lymphangiograms) with negative scans had slight uptake in the left upper-to-middle quadrants. Using the criteria that the spleen, unless palpably enlarged, was anterior and lateral to the kidney and most frequently ended at the upper half of the third lumbar vertebra (17), an attempt was made to assign activity to either the spleen

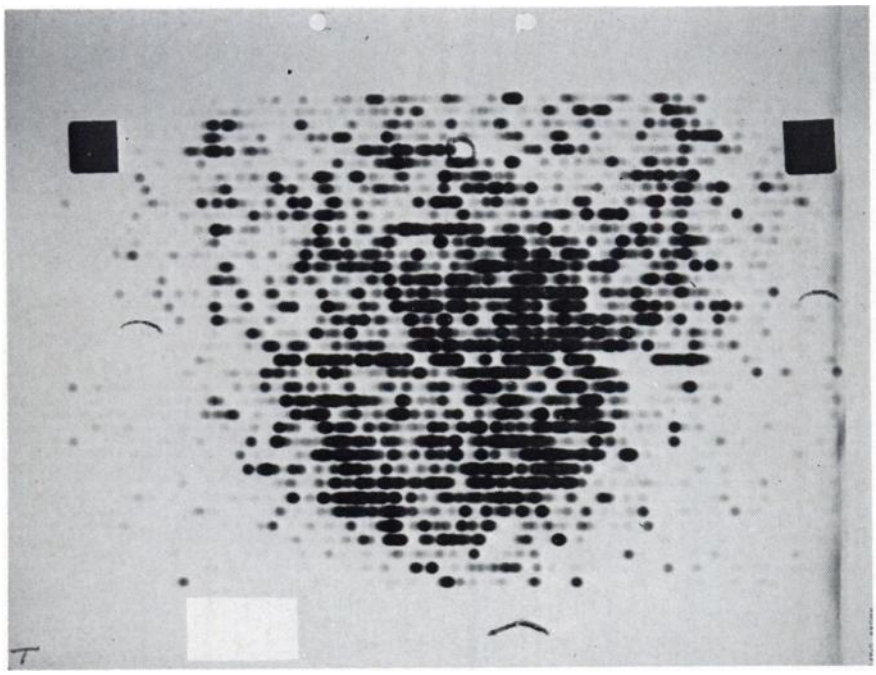

Fig. 3. Abdominal scan in patient 24, done one-half hour after giving the radiolabeled compound. The uptake in the lower abdomen persisted with an approximate half-time of four days. The patient had voided just prior to the scan. The umbilicus, pubic bone and iliac crests are marked.

or kidney. On lateral scans in three of these patients, the uptake appeared to be in the kidney. Concomitant kidney or spleen scans have not yet been attempted. Uptake of ^{75}Se-selenomethionine likely also occurred in the right kidney, but its observation was obscured by the large quantity of radioactivity in the liver. Selected scans are presented in Figures 1 through 4.

^{75}Se-selenomethionine entered lymphomas in both strain A and BDF 1 mice. After six microcuries were administered intravenously, the ratio of activity (per gram of tissue) in the tumor to that in the liver was slightly higher with selenomethionine than with the inorganic forms. Table III shows the results for the BDF 1 mice.

Figure 5 documents the entry of ^{75}Se-selenomethionine into the TCA insoluble form after its intravenous injection into mice. Uptake into the TCA insoluble ("protein-bound") form within the liver was partially suppressed by puromycin and actinomycin D. Uptake into the bound form by the lymphoma was considerably less, but suppression of conversion to the "protein-bound" fraction also occurred (from about 40% conversion in controls to 10% in the treated mice).

In vitro segments of the L5178Y lymphoma accumulated ^{75}Se-selenomethionine against a concentration gradient to a slight extent (Table IV). Because the uptake was too small to adequately test the effect of inhibitors, these experiments were not pursued and a statistical analysis is not given.

TABLE I

RESULTS OF THE LYMPHANGIOGRAM AND ^{75}SE-SELENOMETHIONINE ABDOMINAL SCAN IN 13 PATIENTS WITH HODGKIN'S DISEASE

Patient	*Age*	*Sex*	*Approx. Duration of Disease (mos.) Prior to Test*	*Lymphangiogram*	*Scan*
1	42	M	34	Pos. (para-aortic)	Neg. (splenic uptake)
2	13	F	1	Neg.	Neg. (kidney uptake)
3	35	F	86	Neg.	Pos. (abdomen and pelvis)
4	30	F	61	Pos. (para-aortic, esp. left)	Neg.
5	19	M	72	Pos. (right)	Pos. (right and lower)
6	54	M	1	Neg.	Neg. (kidney uptake)
7	48	M	132	Pos. (pelvis and right aortic)	Pos. (pelvis)
8	34	M	5	Neg.	Neg.
9	66	M	3	Neg.	Neg.
10	18	M	9	Pos. (diffuse)	Pos. (diffuse abdomen and pelvis)
11	35	M	1	Neg.	Neg. (kidney uptake)
12	17	M	3	Neg.	Neg.
13	46	M	7	Pos. (left and para-aortic)	Pos. (left)

DISCUSSION

While there can be little question that the lymphangiogram offers a detailed view of the abdominal lymph nodes, the method is often difficult and time-consuming. The selenomethionine scans cannot give the resolution possible with lymphangiography. However, the ^{75}Se-selenomethionine approach is rapid, and relatively comfortable for the patient. Additional data are needed as to the correct interpretation of the scans. Small lesions may well be missed because of lack of resolution and because of activity in the small bowel. Rounded, well-defined, persistent uptake, however, should raise the suspicion of uptake by a lymph node or mass.

On the basis of the agreement in this study in 10 out of 13 cases between lymphangiography and the ^{75}Se-selenomethionine scans (and further positive uptake in one case scanned, with a technically unsatisfactory lymphangiogram), further evaluation appears warranted. What other abdominal lesions might also cause uptake of the radiolabel can only be speculated upon at present. Additional studies are required before possible "false positives" can be identified. The entry of radioisotopes into tumors because of altered vascular permeability has been noted (16). With ^{75}Se-selenomethionine, the situation is likely complicated by additional factors, since the compound can be incorporated into protein.

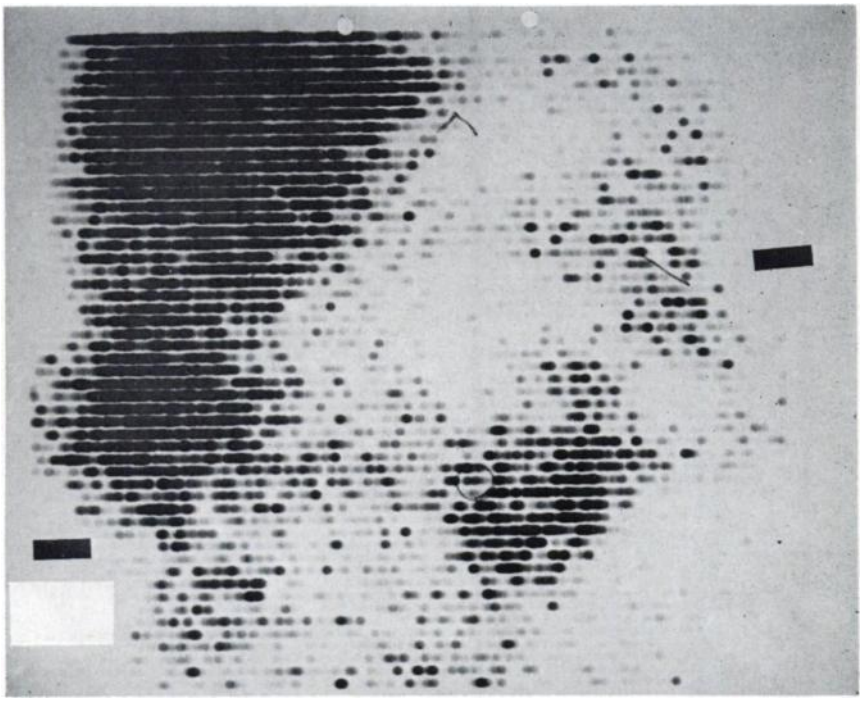

Fig. 4. Abdominal scan in elderly man, one-half hour after ^{75}Selenite. The mass below the liver, and that near the umbilicus, proved to be a liposarcoma at autopsy. The costal margins and umbilicus are marked.

TABLE II

^{75}SE-SELENOMETHIONINE SCANS IN PATIENTS WITH LYMPHOMAS, PLUS THE RESULTS OF A SELENITE SCAN

Case	*Age*	*Sex*	*Approximate Duration of Illness Prior to Scan (mos.)*	*Diagnosis*	*Scan*
14	46	F	1	Reticulum cell sarcoma	Positive in left lower abdomen
15	19	F	12	Hodgkin's disease	Negative; splenic uptake
16	45	F	48	Lymphosarcoma	Uptake over large areas of abdomen
17	78	M	10	Reticulum cell sarcoma	Uptake in palpable left abdominal mass
18	26	M	6	Hodgkin's disease	Uptake in midabdomen (and chest)
19	50	M	9	Reticulum cell sarcoma	Negative. Uptake in spleen or kidney
20	61	M	2	Lymphoma, ? type	Uptake in LUQ and center abdomen
21	31	M	60	Hodgkin's disease	Negative. LUQ uptake
22	52	F	48	Lymphosarcoma	Negative. LUQ uptake
23	23	F	12	Hodgkin's disease	Positive; uptake over palpable sternal mass epigastrium
24	23	F	2	Reticulum cell sarcoma	Positive; uptake in lower abdomen
25	22	M	1	Hodgkin's disease	Positive; uptake over mediastinum and left axilla
26	19	F	36	Hodgkin's disease	Negative
27	66	M	40	Hodgkin's disease	Negative. LUQ uptake
28	59	M	48	Reticulum cell sarcoma	Positive; uptake in area below liver
29	46	M	12	Malignant and lymphoma	Uptake in mass LUQ
30	18	F	1	Hodgkin's disease	Negative. LUQ uptake
31	43	F	72	Reticulum cell sarcoma	Negative
32	31	M	96	Hodgkin's disease	Negative; LUQ uptake
33	21	F	48	Hodgkin's disease	Positive; uptake in midabdomen
34	24	M	6	Hodgkin's granuloma	Uptake over left side of abdomen and in cervical nodes

TABLE II (CONT'D.)

^{75}SE-SELENOMETHIONINE SCANS IN PATIENTS WITH LYMPHOMAS, PLUS THE RESULTS OF A SELENITE SCAN

Case	*Age*	*Sex*	*Approximate Duration of Illness Prior to Scan (mos.)*	*Diagnosis*	*Scan*
35	56	M	14	Hodgkin's disease	Paramedian and left lower quadrant uptake
				Selenite	
	83	M	6	Liposarcoma	Positive; large discrete abdominal uptake in 2 areas

LUQ = left upper quadrant.

Scans may also be taken over any palpable masses, in addition to the abdomen. We have found it advisable to have the patient void prior to scanning, to eliminate activity in the urine (this is usually negligible at one-half hour after injection). Scans during at least two intervals are needed to delineate activity due to pooling of the radiolabel in the gut lumen. Radioactivity in the pancreas is usually greater than that of surrounding masses, but has not presented a problem. Scanners with five or eight-inch NaI crystals will likely do a superior job in detecting deep abdominal lesions, as compared with the three-inch scanner we had available. We have begun looking at posterior scans in an effort to detect small para-aortic nodes that are difficult to discern on conventional anterior scans. Activity in the left upper quadrant may pose a problem. Some activity may normally be present in spleen or kidney. However, malignant lymphoma can occur in the spleen (5), kidneys (6) or stomach (7) (and lateral scans should be investigated in an effort to separate these structures).

Studies remain to be done on the turnover rate of the radiolabel in abdominal locales in patients (some data are available in animals) (8), as well as the effect of agents which alter protein synthesis. Certainly abnormal proteins occur in the lymphomas (9,10), but their relationship to ^{75}Se-selenomethionine uptake is unknown. Following injection of ^{75}Se-selenomethionine into animals (11,12), the amount in the protein-bound fraction increases with time. The same apparently occurs, on the basis of data processed here, in mouse lymphomas. Binding of the radiolabel into liver protein could be blocked by actinomycin D and puromycin; despite the lower uptake of the label in the mouse lymphoma, inhibition of entry into protein by use of actinomycin D and puromycin could also be demonstrated. It is known that actinomycin D (an inhibitor of protein synthesis by blocking the DNA → RNA transcription) is an inhibitor of L5178Y lymphoma cells (14).

^{75}Se-selenomethionine is an amino acid and likely participates in transport into cells by the systems that handle monoamino-monocarboxylic acids. Minimal uptake was demonstrated here in an *in vitro* system. If the technique could be perfected, it would provide a useful tool for following the effect of agents which alter amino acid transport or protein production. The uptake of methotrexate has been shown to be a useful indicator of the response of mouse lymphomas to methotrexate (13). Whether ^{75}Se-selenomethionine uptake will have any prognostic significance is uncertain. To answer this, it will be of importance to follow the uptake of this compound in patients with lymphomas both before and after therapy.

The entry of ^{75}Se-selenite into a liposarcoma, when coupled with Cavalieri's report (4) of uptake of this label by brain tumors, makes additional evaluation of selenite as a scanning agent appear of interest.

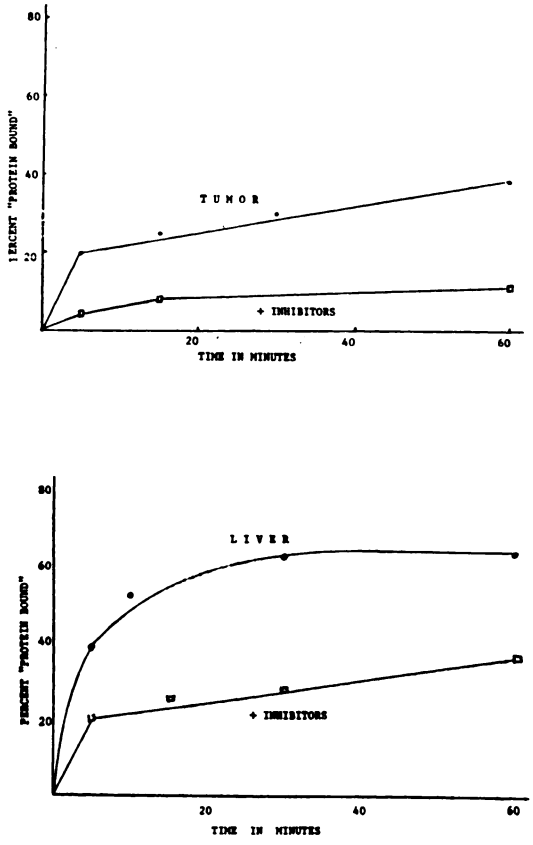

Fig. 5. The trichloroacetic acid insoluble and possible "protein bound" radioactivity after intravenous administration of ^{75}Se-selenomethionine in strain BDF 1 mice. Each value is the mean of three animals (standard deviations were about ± 5%).

SUMMARY

^{75}Se administered intravenously as selenate or selenite entered mouse lymphomas. The specific activity in these tumors, as compared with that in the liver, was not as favorable as following ^{75}Se-selenomethionine. ^{75}Se-selenite was noted to accumulate in a human liposarcoma. This observation, plus Cavalieri's report of selenite uptake by human tumors, indicates the need for further evaluation of selenite as a radiopharmaceutical.

Studies on the uptake of ^{75}Se-selenomethionine revealed that within a few minutes after intravenous administration to mice with the L5178Y lymphoma, a portion of the radiolabel was in trichloroacetic acid insoluble form in the liver and the tumor. Binding could be partially blocked by actinomycin D and puromycin. Entry of the selenomethionine radiolabel could also be demonstrated following *in vitro* incubation with slices of mouse lymphoma tissue.

Experiences with the distribution of ^{75}Se-selenomethionine in 35 patients with Hodgkin's disease and other lymphomas were outlined. Of particular note was uptake in the left upper quadrant (likely kidney or spleen). The necessity of emptying the bladder and of repeat scans (to identify the radiolabel in the small gut lumen) was emphasized.

In 13 of the above cases of Hodgkin's disease, lymphangiograms were performed as well as the ^{75}Se-selenomethionine abdominal scans. The two procedures agreed in ten cases (six negative, four positive). In two cases, small para-aortic nodes were detected by lymphangiography but not by scanning. In one case, massive abdominal uptake was detected by scanning, while the lymphangiogram was not satsifactory. ^{75}Se-selenomethionine, while not specific for lymphomas, appears worthy of further evaluation in following the course and distribution of these lesions.

TABLE III

ENTRY OF ^{75}SE (AS SELENITE, SELENATE AND SELENOMETHIONINE), GIVEN INTRAVENOUSLY, INTO SELECTED TISSUES OF REPRESENTATIVE BDF 1 MICE BEARING THE L5178Y LYMPHOMA. EACH VALUE IS THE MEAN OF THREE MICE. THE STANDARD DEVIATION WAS ABOUT 10% OF THE MEAN.

	Counts/mg of Wet Tissue					
	Selenite		*Selenate*		*Selenomethionine*	
	30 min.	*60 min.*	*30 min.*	*60 min.*	*30 min.*	*60 min.*
Tumor	28	35	57	57	94	98
Liver*	260	207	280	368	282	302
Intestine	87	128	312	417	213	287
Muscle	11	13	17	15	21	15
Ratio: $\frac{\text{Tumor}}{\text{Liver}}$	0.11	0.17	0.20	0.15	0.33	0.32

*There was some replacement of hepatic tissue by tumor.

ACKNOWLEDGEMENT

We are grateful to Dr. A. C. Sartorelli for the L5178Y lymphoma cells.

TABLE IV

UPTAKE OF ^{75}Se-SELENOMETHIONINE BY SEGMENTS OF L5178Y MOUSE LYMPHOMA INCUBATED *in vitro* AT 37°C (95% O_2 + 5% CO_2). OBSERVE THAT AT 120 MINUTES, IN KREBS-RINGER BUFFER, THE ACTIVITY IN THE TISSUE APPEARED TO BE DECREASING. EACH VALUE IS THE MEAN OF SEPARATE DETERMINATIONS ON TWO MICE. SINCE THE UPTAKE WAS SO MINIMAL, ADDITIONAL STUDIES WERE NOT PERFORMED.

	Time of Incubation (Min.)				
Incubation Fluid	15	30	60	90	120
Fischers' Medium	0.9	1.3	—	1.6	2.1
Krebs-Ringer Bicarbonate	—	0.9	1.3	1.4	1.1

Results are expressed as: (counts/gm tissue water)/(counts/ml initial standard)

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CORRECTION NOTICE

The article entitled "Guidelines and Criteria for a Committee Authorizing the Use of Radioactive Isotopes in Humans," by Joseph P. Kriss, Raymond Barrall, Robert Greenberg, Gerald Hanks, Charles E. McLennan and Edward Siegel was inadvertently omitted from the January 1967 Table of Contents. It is suggested that the reader cut out the listing below and paste it into the Table of Contents of the January 1967 issue of the JOURNAL OF NUCLEAR MEDICINE.

JOSEPH P. KRISS, RAYMOND BARRALL, ROBERT GREENBERG, GERALD HANKS, CHARLES E. MCLENNAN AND EDWARD SIEGEL. Guidelines and Criteria for a Committee Authorizing the Use of Radioactive Isotopes in Humans 70