

⁷⁵Se Methionine as a Diagnostic Agent In Malignant Lymphoma¹

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INTRODUCTION

The detection of intraabdominal lymphoid tumors is a difficult clinical problem. Conventional radiographic techniques used currently for this purpose rely primarily on indirect evidence, such as alterations produced by compression or deviation of opacified viscera, which permit the examiner to infer their presence. The introduction of lymphangiography offered a more sophisticated means of radiographic visualization of lymphoid tumors, but not without some difficulty and hazard (1). Thus, a safe and simple method of direct demonstration of intraabdominal malignant lymphomas would be of value in diagnosis and management of these neoplasms.

In the course of obtaining a pancreatic scan using ⁷⁵Se-L-Methionine as part of the investigation of a patient with jaundice, we were able to demonstrate, on photoscan, a large, intraabdominal, well demarcated area of uptake. That the area was a lymphosarcoma was proved by needle biopsy, and later by complete autopsy. This chance observation prompted us to apply selenomethionine administration to a group of patients with proven malignant lymphomas. Patients undergoing pancreatic scans for other reasons and patients with known epithelial tumors, served as controls for this study. Our observations on these cases form the basis of this report.

¹A Preliminary Communication

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TABLE I
MALIGNANT LYMPHOMAS

<u>Case No.</u>	<u>Name</u>	<u>Age</u>	<u>Sex</u>	<u>Final Diagnosis</u>	<u>Region</u>	<u>Se⁷⁵ Scanning</u>
1.	R.A.	91	M	Recurrent Lymphosarcoma	Abdomen	Positive
2	P.E.	44	F	Generalized Lymphosarcoma	Abdomen	Positive
3	S.H.	61	F	Hodgkin's disease	Inguinal	Positive
4	P.M.	47	F	Generalized Lymphosarcoma	Neck	Positive
5	T.G.	73	M	Hodgkin's disease	Abdomen	Positive
6	L.A.	53	M	Hodgkin's disease	Axilla	Positive
7	M.B.	67	M	Possible retic cell sarcoma	Neck	Positive
8	R.E.	76	F	Lymphosarcoma	Abdomen	Negative ^x
9	R.J.	51	M	Lymphosarcoma & Seminoma	Pelvis	Negative ^x
10	P.P.	25	F	Reticulum cell sarcoma of breast	Chest Axilla	Negative ^x
<u>OTHER NEOPLASTIC DISEASES</u>						
11	MacC.M	72	F	Carcinomatosis (adenocarcinoma of rectal origin)	Abdomen	Negative
12	D.F.	67	M	Hepatoma	Liver	Negative
<u>CONTROL GROUP</u>						
<u>NON-NEOPLASTIC DISEASES</u>						
13	M.E.	34	F	Partial intestinal obstruction	Abdomen	Negative
14	O.R.	42	F	Thrombocytopenic purpura Auto immune hemolytic anemia	Axilla Neck	Negative
15	C.F.	55	F	Functional G.I. tract disease	Inguinal	Negative
16	S.L.	47	F	Observation-no disease found	Neck	Negative

^x Cases 8, 9 and 10: Recent surgery and heavy Xray therapy preceding the scan. No known disease at time of study.

METHODS AND INSTRUMENTATION

The technique of pancreatic photoscanning using ^{75}Se -L-Methionine described by Bender and Blau (2), Sodee (3), and recently modified by Rodriguez-Antunez (4) was used in this study. The scanning instrument was a Picker Nuclear Magnascanner with a five inch crystal fitted with a five inch 31 hole collimator. The voltage of the photocathode tube was set on the basis of the values obtained over the lesions, when they were palpable, or, in the case of non-palpable intraabdominal tumors, in areas of maximum count other than the liver and pancreas (which presumably corresponded to the tumors). The count

rate differential was set according to standard procedure (5). In small lesions, such as lymph nodes in the neck and inguinal region, a count rate differential of 30 was consistently employed. This was based on a 2:1 ratio of uptake in these small lesions as compared to a noninvolved area within the region taken to be representative of the body background.

In some of the cases, assay procedures to estimate radioactivity content within the tumors and other tissues have been carried out. Table I and II summarize these reports.

Agent: ^{75}Se -L-Methionine (Stethotope)[®] was employed throughout the study at a dose of 250 μC for a 60 kg body weight.

Patient Material: The clinical group consisted of 16 cases. Table I summarizes the data on these patients.

Results: For simplicity of presentation we have summarized part of our results in Table I.

Since the initiation of the study three patients from this group have expired (Cases 1, 11 and 13). None of the deaths appeared to be related in any way to the procedure. All three cases had complete post mortem examinations.

TABLE II

Results of assay procedure in tissues of Case 1 and Case 12.

Samples in Case 1 & 12 were obtained two weeks after the injection of the isotope. Note similarity of results in tumor and liver tissue, tissue in Case 1 obtained at time of autopsy and that of Case 12 at operation.

A.R. - Tumor Tissue

Sample Weight in Grams	Background Corrected Count per Minute	Total μC in Sample	$\mu\text{C}/\text{gram}$ of Tissue
#1 0.9	1958	0.083	0.0092
#2 1.05	2514	0.110	0.0105
#3 1.02	2286	0.098	0.0096
#4 0.9	2234	0.095	0.0106
#5 <u>0.86</u>	<u>1938</u>	<u>0.083</u>	<u>0.0097</u>
Average 0.95	2186	0.0938	0.0099

Average 0.0099 $\mu\text{C}/\text{gm}$ of tissue

F.D. - Liver Tissue

#1 0.29	0.01
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Assay Reference Standard

0.25 μC on 26th March 1965

Background Corrected C F M 59,000

Case 1 died as a result of recurrent lymphosarcoma which produced mechanical obstruction of the small intestine and common bile duct.

Case 11 died as a result of extensive carcinomatous involvement of abdominal viscera and abdominal lymph nodes.

Case 13 died as a result of gangrene of the small bowel secondary to ileo-ileal intussusception, seemingly related to the presence of a villous adenoma of the ileum. At autopsy there was no evidence of recurrent or metastatic disease.

In view of the seemingly long residence time of the isotope in the tumor tissue in our first case, two weeks, (Table II), we have attempted to establish the residence time in the tumor in another case (case 2). The value obtained was a biologic half life of approximately 24 days.

Assay of radioactivity in samples of blood in two of our patients gave results similar to those obtained by Digulio and Beierwalters (6) and Penner (7) (8), who found that plasma values at 24 hours, range from 50-75% of the values of the sample obtained two minutes after injection of the radioisotope.

In an effort to localize the radioactive selenium within the tissues, autoradiography was attempted on case 1 with negative results.

TABLE III

These figures represent approximate values derived by comparing counts obtained with the scanner with five inch collimation at three inches from skin. Reference standard was counted in the same manner. The results, therefore, represent approximate values for the area of interest.

<u>Patient</u>	<u>Site</u>	<u>Time After Injection</u>	<u>μc</u>
H.S.	Left Inguinal	30 Minutes	0.30
"	Left Inguinal	24 hours	0.33
A.L.	Left Axilla	30 minutes	0.12
M.P.	Left Neck	30 minutes	0.15
"	Right Neck	30 minutes	0.15
"	Left Neck	24 hours	0.15
"	Right Neck	24 hours	0.18

Assay Reference Standard

0.08 μc in 8 ml of water

0.08 μc/gm

immersed in fluid

CASE REPORTS

The following five cases are thought to be of sufficient clinical interest to warrant presentation in some detail.

Case 1: A.R.: A 91 year old white male was readmitted to the Danbury Hospital for evaluation of progressive weakness, anorexia, weight loss, and increasing jaundice of two months duration. Six months earlier the patient underwent a subtotal gastrectomy for a lymphosarcoma of the stomach. His subsequent course was uneventful until two months prior to his readmission, when he noted the insidious onset of the above symptoms.

Physical findings revealed a chronically ill, cachectic, markedly jaundiced male with a large mass palpable in the right upper quadrant. A liver scan using ^{131}I Rose Bengal disclosed no evidence of metastatic tumor in the liver. The mass was not visualized on this study. A pancreatic scan using ^{75}Se -Selenomethionine was performed to confirm a suspected clinical impression of pancreatic pseudocyst. This study delineated the lesion quite satisfactorily. Needle biopsy demonstrated recurrent lymphosarcoma. The patient's course was progressively down-

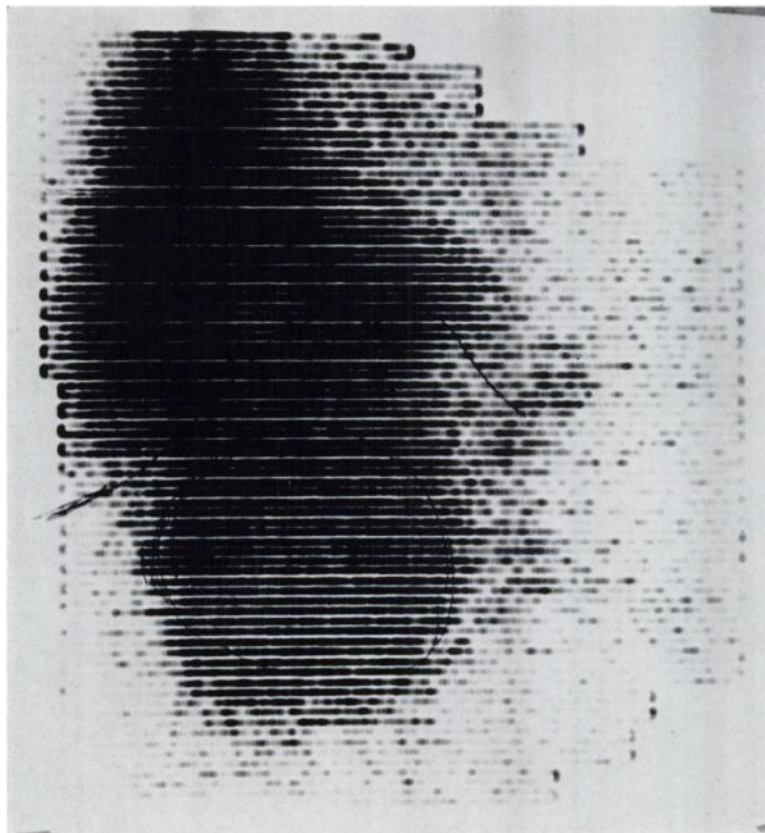


Fig. 1. ^{75}Se -Selenomethionine scan of the abdomen. Note the presence of the large tumor mass located below the inferior edge of the liver. A scan repeated at time of autopsy, 14 days later, disclosed essentially similar findings, (Case 1).

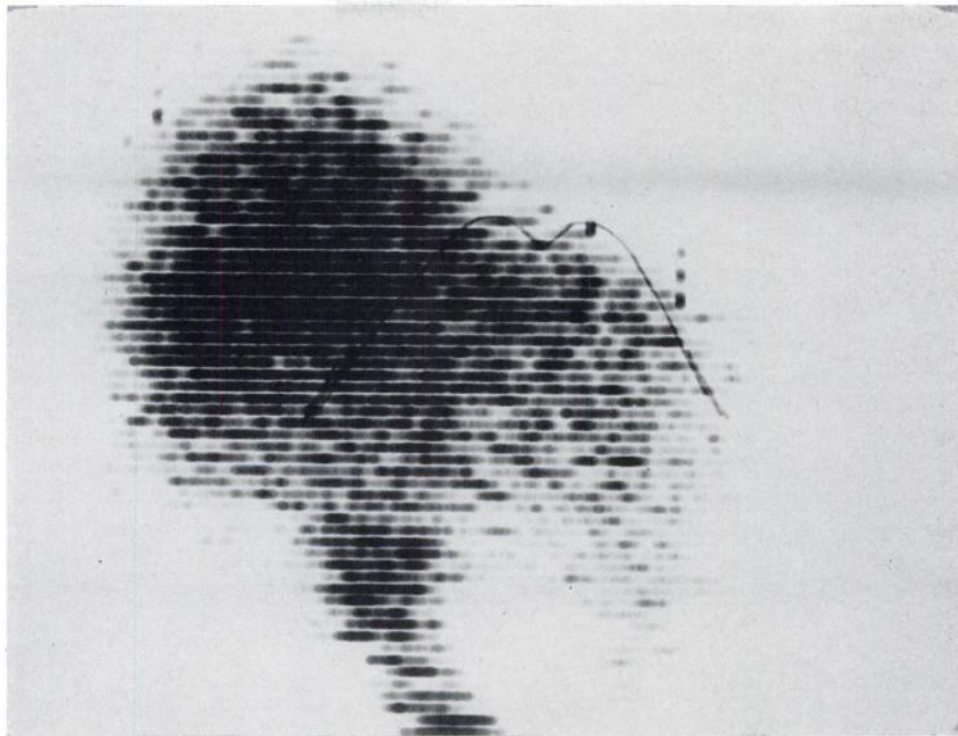


Fig. 2. Photo scan of liver with ^{131}I Rose Bengal. Note the solid vertical area of radioactivity extending downward from the inferior margin of the liver representing radioactive material in the duodenum. Superimposed on the upper portion of the duodenum is the gall bladder.

hill with death two weeks later. A repeat photoscan obtained at the autopsy table disclosed considerable residual activity in the tumor mass (Fig. 1).

Case 2: E.P.: This 40 year old white female presented for the first time in November 1962, because of a large abdominal mass discovered during routine examination prior to elective pelvic surgery. There were no systemic complaints associated with this finding. Investigation at another institution, including upper gastrointestinal tract, gall bladder, Ba enema and intravenous pyelogram x-rays, and blood chemistries, were all within normal limits.

Physical findings disclosed the presence of a tender moveable mass approximately eight cm in diameter in the mid left lower quadrant. At surgery a large mass involving the entire mesentery of the small bowel was encountered. Multiple periaortic lymph nodes were noted, and a biopsy specimen of one node proved to be lymphosarcoma. Over the ensuing 2½ years, this patient's course was characterized by metastatic recurrences involving the cervical nodes, ribs, sternum and right clavicle, with satisfactory remission obtained in each instance with intensive x-ray therapy to each area. At the time of this study, ^{75}Se -Selenomethionine clearly delineated, an abdominal tumor mass in her right upper quadrant (Figs. 2, 3).

Case 3: H.S.: This patient is a 61 year old white female who was admitted to the Danbury Hospital because of marked weight loss, lightheadness, unsteadiness of gait, and drenching sweats of nine months duration.

On physical examination, positive findings in this chronically ill white female included hyperemic patches over the buccal mucosa. There were fine, moist, inspiratory rales throughout both lung fields and multiple nodularities in the right lower quadrant with a slightly moveable, firm nontender mass in the right inguinal region. Shortly after admission, the liver became palpable and inguinal adenopathy became more marked. Laboratory data revealed moderate anemia, a normal total white count with a moderate number of atypical lymphocytes. Platelets were normal. Serum protein electrophoresis was abnormal though non-specific. Chest x-ray and intravenous pyelography were unremarkable. Lymph node biopsy obtained from the right inguinal area revealed Hodgkin's disease. The patient was treated with a conventional course of nitrogen mustard and a satisfactory remission was obtained. With a recurrence of her symptoms, she was started on Chlorambucil® therapy orally, with a poor response. Following this period the photoscan of the inguinal and iliac lymph nodes was obtained at one half, 24 and 48 hours following the intravenous injection of ^{75}Se -Selenomethionine (Fig. 4).

Case 4: M. P.: This patient is a 47-year-old white female who was admitted to the

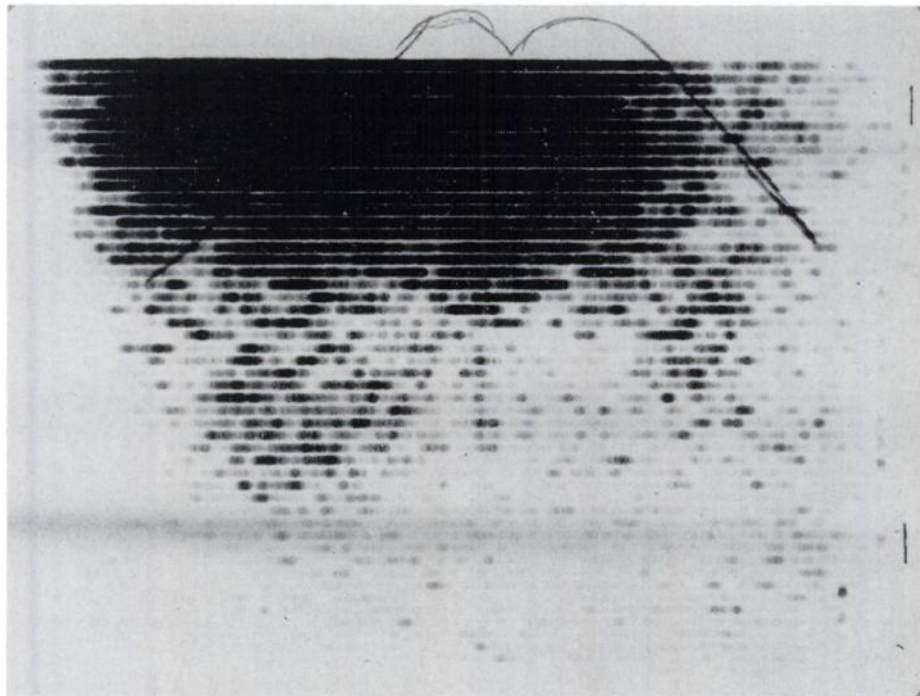


Fig. 3. ^{75}Se Methionine photo scan of case 2. Notice round area of activity in relation to, but distinct from, inferior edge of right lobe of liver. This corresponds to palpable mass in this region. Radioactivity in pancreas merges with that of left lobe of liver.

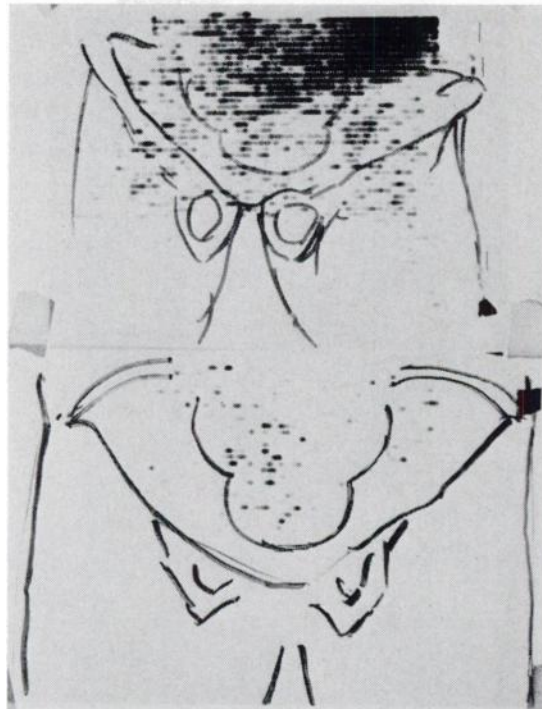


Fig. 4. *Upper part:* photo scan of case 3. Note areas of activity in the inguinal regions. Areas of activity correspond to palpable masses of lymph nodes. *Lower Part:* Negative scan obtained from case 15 for control purposes.

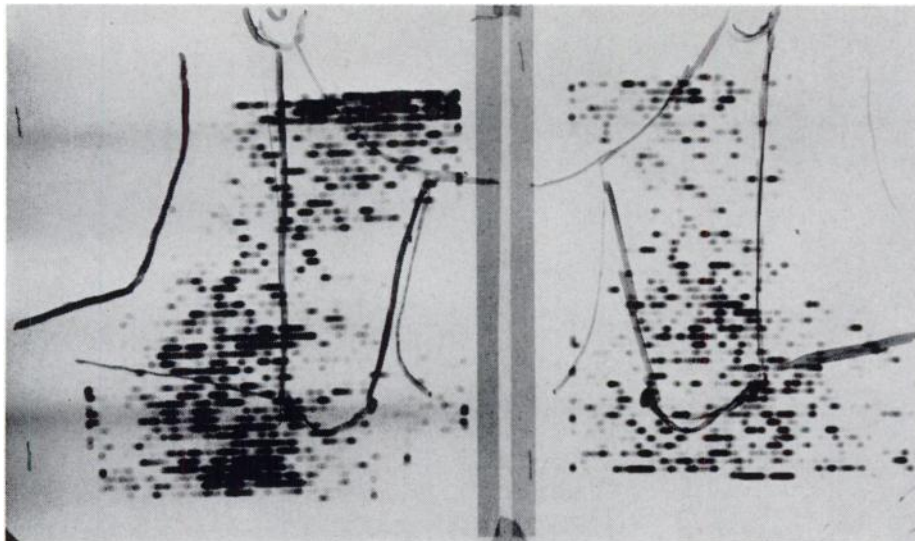


Fig. 5. *Left:* Photo scan obtained 30 minutes after injection of ^{75}Se -Selenomethionine. Note area of activity in the right supraclavicular area corresponding to mass of palpable nodes. *Right:* Control scan in case 16.

hospital for evaluation of exertional dyspnea, fatigue, dizziness, fulness of the abdomen and swelling of her legs for four months.

Pertinent findings on physical examination included generalized lymphadenopathy, more marked in the posterior cervical areas, bilaterally, and in the axillary region. Marked splenomegaly extending to below the level of the pelvic brim was noted, and there was an enlargement of the liver four cms below the right costal margin.

Laboratory studies revealed hypochromic microcytic anemia with normal total white count and platelets. Bone marrow aspiration revealed a hypocellular marrow with no evidence of malignant disease. Plain films of the abdomen confirmed the clinical finding of splenomegaly. Biopsy of one of the axillary glands was interpreted as characteristic of lymphosarcoma. A scan was obtained in the abdominal region using ^{75}Se -Selenomethionine and considerable uptake of the isotope by the spleen was noted. In addition, a dense scan was recorded over the cervical lymph node area, 30 minutes after injection, and again on a repeat study 24 hours later (Fig. 5).

Case 5:G.T.: This 73-year-old white male was admitted because of painless jaundice and fever of an unknown origin. On the basis of x-ray studies, he had

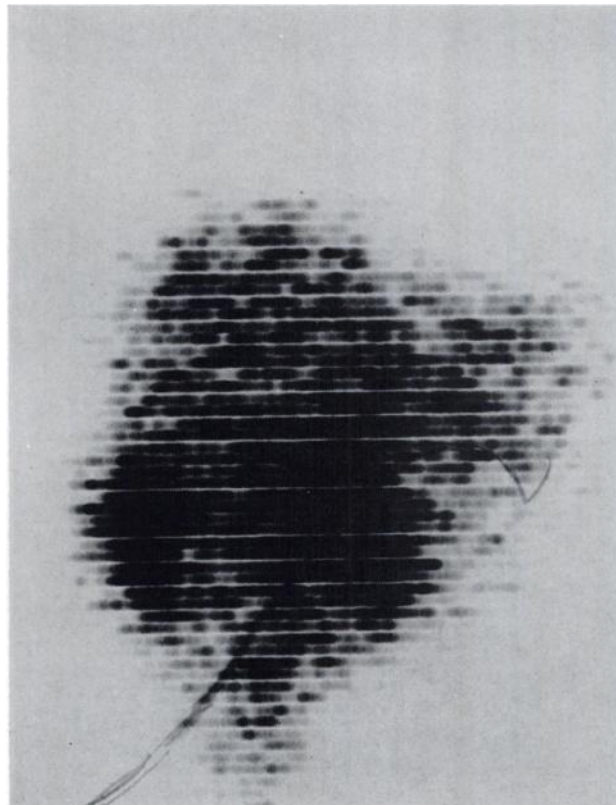


Fig. 6. Early scan, notice well delineated "black" area of leakage in relation to the lateral margin of the right lobe. The second "black" area in relation to the midportion of the inferior edge corresponds to gall bladder activity.

been diagnosed at this hospital six months before as having a bleeding duodenal ulcer. At that time, splenomegaly was discovered. He was placed on an ulcer regime. He did well until five days prior to present admission, when he developed generalized malaise, chills and fever. On admission, positive physical findings were limited to hepatomegaly and splenomegaly with tenderness. Laboratory investigations disclosed chemical and enzymatic blood serum changes suggestive of hepatitis. X-ray studies of the gastrointestinal tract confirmed the presence of chronic duodenal pathology.

Needle biopsy of the liver was performed. It showed histologic changes of a nonspecific reactive nature. The patient began to complain of discomfort in the right upper quadrant. The day after biopsy, a ^{131}I Rose Bengal liver study was performed. The early scan (30 minutes after injection of 150 uc) disclosed leakage of radioactivity into the subphrenic area (Fig. 6). The late scan (24 hours) disclosed that the leakage extended along the right gutter to the right pelvis (Fig. 7), while the bulk of the excreted isotope delineated the colon. The patient, in the meantime, had developed signs of paralytic ileus. At exploration of the area of leakage, bile was found to be leaking retroperitoneally. Mesenteric lymph nodes were found to be enlarged. A biopsy disclosed Hodgkin's



Fig. 7. Late scan showing the ascending, transverse and proximal descending colon to be well delineated. Note, in addition, a vertical area of activity to the right of the ascending colon, corresponding to the area of leakage.

disease. ^{75}Se -Selenomethionine scan approximately one week after the operation demonstrated excellent uptake by neoplastic nodes (Fig. 8).

DISCUSSION

Bender and Blau first synthesized ^{75}Se -L-Methionine for use as an agent for pancreatic scanning. They reasoned that due to the chemical similarity of selenium and sulphur, the selenium analog of L-Methionine would resemble natural methionine sufficiently to take its place in protein synthesis. Since their original publication, many (3)(9) have used this agent successfully for pancreatic scanning and for study of pancreatic secretion. Potchen (10) and later Beierwaltes and Diguilio (11), have used this compound to demonstrate parathyroid tissue. The first of these authors noted that, in addition, the material is also picked up by the thymus. Spencer (12) and Blau demonstrated that ^{75}Se -L-Methionine and ^{35}S -L-Methionine were transported through the intestinal wall at the same rate, suggesting that the ^{75}Se analog possesses biologic properties similar to those of the natural amino acid. Bender and Blau (2) have also used ^{75}Se -Methionine to scan prostate and parotid. Our chance observation (in our initial first case) led us to use this agent in a variety of malignant diseases of lymphoid origin: lymphosarcoma, Hodgkin's disease, and reticulum cell sarcoma. It is noteworthy that all the cases with clinical evidence of active disease had enough uptake to

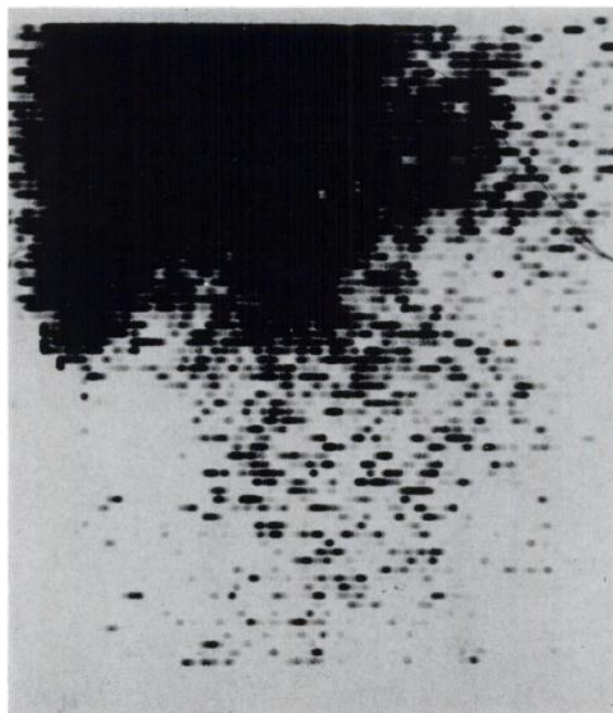


Fig. 8. ^{75}Se -Methionine scan showing liver and pancreas well delineated in the upper portion of photograph. Note sharply demarcated small areas of activity corresponding to groups of neoplastic mesenteric nodes.

be demonstrated by photoscans obtained at 30 minutes, 24 hours, and in some cases, as late as two weeks. Specific attempts to demonstrate normal lymph nodes in five cases with this technique were unsuccessful. This may, however, be a reflection of the limitations of the method used rather than a true lack of uptake. Further studies will attempt to elucidate the degree of uptake by normal and inflamed lymph nodes.

Blau (13) has used the agent in a variety of epithelial neoplasms with negative results. His findings are in agreement with ours in the two cases studied. This number is too small to be of any significant value. However, it suggests that the uptake may be limited specifically to tumors of lymphoid origin. Considerably more cases of epithelial neoplasia must be evaluated before a valid conclusion, concerning this specificity, can be considered as established.

At this stage of our knowledge, it is not known whether ^{75}Se -L-Methionine is incorporated in the amino acid form for protein synthesis by the tumor, or as a result of phagocytic property of these tumors. The long residence time within the tumor, although not conclusive, would appear to suggest that the first possibility is likely. Studies now in progress will attempt to provide an answer to this problem.

Why lymphomas should exhibit this uptake, however, remains purely speculative. At this time, the possibility of use for "resident" protein synthesis must be seriously considered in the light of the work of Warshawsky, Leblond and Droz (9) concerning synthesis of "sedentary" proteins by the pancreas. These authors employed Leucine H3, glycine and methionine H3 in their studies in experimental animals.

Penner's (7) electrophoretic studies of plasma after injection of ^{75}Se -Methionine showed the radioactive tag in relation to serum globulins, this may partially explain the affinity of reticuloendothelial tissue derivatives for the amino acid.

In view of the absence of really significant uptake by normal lymph nodes with the method employed in this study, it is possible that uptake by the tumors reflect a faster rate of protein synthesis related to the rapidity of growth.

SUMMARY AND CONCLUSIONS

A group of malignant lymphomas exhibited a degree of uptake of ^{75}Se -L-Methionine which permitted their demonstration by means of photoscanner. This property appears, at this time, not to be shared by epithelial neoplasms, either in original sites or lymph node metastases. Normal lymph nodes could not be demonstrated in this study. Malignant lymphomas which have been recently treated with surgery and/or exradiation failed to exhibit the above property. Of interest, was the lack of clinical evidence of recurrent activity in this last group.

If these preliminary observations can be confirmed by further studies, a valuable and safe technique will be available for the diagnosis of malignant lymphomas.

ADDENDUM

After the completion of this manuscript, Dr. R. P. Spencer, Yale New Haven Hospital, has informed us that he has been able to obtain photoscans using our technique on four cases of malignant lymphomas.

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