DIAGNOSTIC EFFICACY OF A RADIOIODINATED CHLOROQUINE ANALOG IN PATIENTS WITH MALIGNANT MELANOMA

Charles M. Boyd, Lionel M. Lieberman, William H. Beierwaltes and Vijay M. Varma

University of Michigan Medical Center, Ann Arbor, Michigan

Confirmation of the diagnostic potential of a radioiodinated analog of chloroquine, ^{125}I-4-(3-dimethylaminopropylamino)-7-iodoquinoline (hereafter referred to as ^{125}I-NM-113),* was reported in a previous publication (*1*). The synthesis of this compound (*2*) and labeling with ^{125}I did not destroy the specificity for binding with melanin-containing tissue (*3*). The concentration of ^{125}I radioactivity from this compound in melanoma, eyes and liver was similar to the concentration of ^{14}C radioactivity after the administration of ^{14}C-labeled chloroquine (*3*). Investigation of the first three patients with malignant melanomas with the ^{125}I-labeled compound suggested that scanning techniques might be useful to delineate the extent of the primary lesion, to demonstrate whether palpable lymph nodes contain melanoma metastases and to determine whether or not metastases exist in nonvisible and nonpalpable nodes or in other metastatic sites (*1*).

Further evaluation in a wider variety of melanoma patients was obviously indicated in order to more fully evaluate the diagnostic potential of this radioiodinated chloroquine analog. This report summarizes the results of studies in the first 30 patients with nonocular malignant melanomas.

MATERIALS AND METHODS

Thirty consecutive patients with histologically proved, nonocular malignant melanomas were included in the study. Each patient was given orally approximately 2 mCi of ^{125}I or ^{131}I chloroquine analog containing 6–55 mg of the stable compound.

Patients were scanned at varying intervals after administration of the labeled compound with a 5-in. NaI(Tl) crystal scintillation scanner with 3-in. focal-length fine- and coarse-focus collimators. Scans were performed from 1 to 71 days after dose administration. We prefer to begin scanning on Day 4 or 5 after the dose when the general body background of radioactivity has decreased.

Six patients had daily blood, urine and stool collections for determination of concentration of radioactivity, obtained over periods of 14–21 days. Aliquots of specimens obtained were assayed in an automatic well counter. The urinary and fecal excretion were expressed as cumulative percent of administered dose. Blood concentrations were expressed as counts per minute per milliliter of whole blood.

Tissue specimens were obtained at surgery 2–46 days after dose administration in 15 patients and at autopsy 39 and 43 days after dose administration in two patients. Tissues in surgical specimens assayed included skin, fat, muscle, lymph nodes and liver. The concentrations of radioactivity in the specimens were analyzed in an automatic well counter.

RESULTS

Excretion studies. The radioactivity in blood fell to approximately one half of the original concentration in a period of 1–12 days with an average of 5 days (Fig. 1).

The cumulative urine and stool excretion of radioactivity ranged from 12.5 to 48.1% of the administered dose at Day 7, with an average of 27% (Fig. 2). Approximately two thirds of the radioactivity was in urine and one third in feces.

* Iodomethin, Mallinckrodt/Nuclear, St. Louis, Missouri.

Received Oct. 14, 1969; original accepted Feb. 5, 1970.

For reprints contact: Charles M. Boyd, Dept. of Internal Medicine (Nuclear Medicine), University Hospital, The University of Michigan Medical Center, Ann Arbor, Mich. 48104.

TABLE 1. POSITIVE SCANS CORRECTLY LOCALIZING MALIGNANT MELANOMA TISSUE

Patient	Drug	Primary	Scan	Clinical metastases	Scan	Comment
			Primary Lesions			
1	^{131}I	+	+	−	−	Intranasal melanoma
2	^{125}I	+	+	−	−	Radical neck dissection showed 30 of 30 nodes uninvolved
3	^{125}I	+	+	+	−	Satellites small and inguinal nodes partially amelanotic
			Metastatic Lesions			
4	^{125}I	−	−	+	+	Biopsy "positive" (autopsied)
5	^{125}I	−	−	+	+	Biopsy "positive" (autopsied)
6	^{125}I	−	−	+	+	Biopsy "positive"
7	^{125}I	−	−	+	+	Abnormal liver scan, confirmed at laparotomy
8	^{125}I	−	−	+	+	Biopsy "positive"
9	^{125}I	−	−	−	+	Liver scan positive 71 days post-dose
10	^{125}I ^{131}I	−	−	+	+	Biopsy "positive"
11	^{125}I ^{131}I	−	−	+	+	Clinically undetected axillary metastasis revealed
12	^{125}I ^{131}I	−	−	+	+	Intracranial metastases
13	^{125}I	−	−	+	+	Subcutaneous metastases
14	^{125}I ^{131}I	−	−	+	+	Subcutaneous metastases

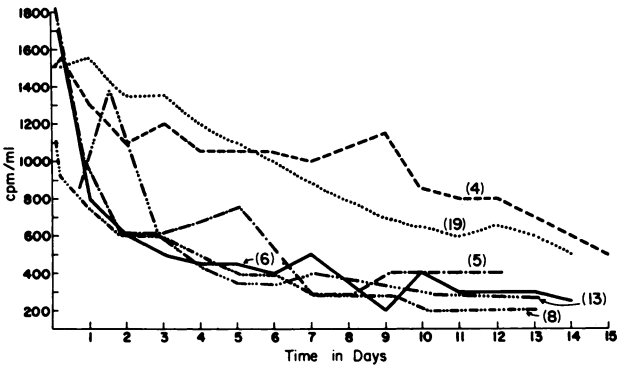

FIG. 1. Decreasing radioactivity in blood of six patients over 15-day period. Radioactivity concentration in blood fell to one-half original concentration in average of 5 days.

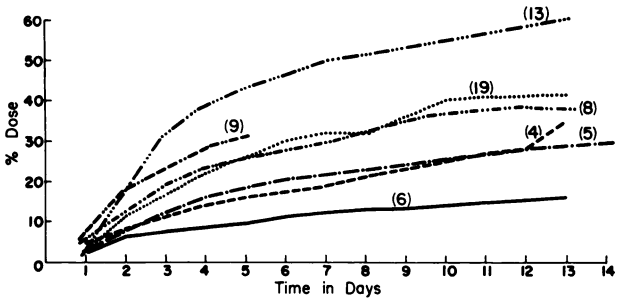

FIG. 2. Cumulative urine and stool excretion of radioactivity in seven patients. Average of 27% of administered dose was excreted in 7 days.

Tissue assays. The radioactivity-concentration ratio for melanoma compared to skin, muscle and fat ranged from approximately 7 to 60:1. Amelanotic tissue and areas of necrosis in melanotic tissue concentrated the compound poorly, if at all, compared with other tissues.

Correlation of scintiscans with histopathology.

Positive scans. Fourteen patients had positive scans correctly localizing the malignant melanoma tissue: three with primary lesions still present and 11 with

FIG. 3. Metastatic malignant melanoma of left hepatic lobe. Left: ^{198}Au liver scan. Middle and right: Isolated left upper quadrant abdominal scans with ^{125}I-NM-113 at Day 10 after dose showing positive uptake of radioactivity from ^{125}I-NM-113 in region of tumor where colloidal-gold scan shows a lack of radioactivity concentration.

metastatic lesions only. The pertinent clinical details of these 14 patients are presented in Table 1.

Figure 3 shows that metastatic melanoma to the liver presents as a cold area on the ^{198}Au liver scan and a positive area of uptake in the left lobe with ^{125}I-NM-113 (Patient 11).

Figure 4 demonstrates radioactivity uptake from ^{125}I-NM-113 in melanoma metastases to lymph nodes (Patient 11). Right and left axillary scans showed a positive uptake of radioactivity extending upward from the lung apices bilaterally. Each node was approximately 1 cm in dia. Bilateral axillary-node dissections showed metastatic malignant melanoma.

Figure 5 (Patient 12) shows scans of the brain with ^{125}I- and ^{131}I-NM-113 demonstrating a lesion in the parieto-occipital region in a patient with clinical intracerebral metastases. No substantial difference was seen in delineating the abnormality with the use of ^{125}I-NM-113 compared with the ^{131}I-labeled compound. The ^{99m}Tc scan was also positive for this abnormality. In patients with no evident intracerebral abnormality, a diffuse area of isotope localization was usually seen in the lower-hemispheric regions with ^{125}I-NM-113 and throughout the cerebral areas with ^{131}I-NM-113 (Fig. 6). Attenuation of the lower-energy gamma by the skull may account for the difference in appearance. The exact location of this radioactivity is under investigation.

In Figure 7 (Patient 3), a primary lesion on the ankle is demonstrated as a large area of positive uptake of the ^{125}I-NM-113. Satellite lesions measured less than 0.7 cm in dia and were not visualized on this scan. The femoral area below the right inguinal ligament showed no abnormality on the scan. Radioactivity in the bowel precluded adequate visualization of the pelvic nodes. Pelvic exploration during isolated-limb perfusion revealed melanoma-involved nodes, which were partly amelanotic.

There were no false-positive scans. In every case of *positive-abnormal* localization of the compound, the area was involved by melanotic malignant melanoma.

Correct-negative scans. Twelve patients with histopathologically proved malignant melanoma had no clinically evident metastases, and multiple scans of primary lymph-drainage areas (liver, lungs, brain, etc.) were negative. (The pertinent clinical details of these patients are presented in Table 2.) Biopsies

TABLE 2. NEGATIVE SCANS CORRECTLY SHOWING ABSENCE OF METASTATIC OR RECURRENT MELANOMAS

Patient	Drug	Primary	Scan	Clinical metastases	Scan	Comment
15	^{125}I	—	—	—	—	Re-excision of primary site negative
16	^{125}I	—	—	—	—	Re-excision of primary site negative
17	^{125}I	—	—	+	—	Suspected node negative on biopsy
18	^{125}I	—	—	+	—	Suspected node negative on biopsy
19	^{125}I	—	—	—	—	No clinical metastases
20	^{125}I ^{131}I	—	—	—	—	Post op scan of resected brain lesion negative
21	^{125}I	—	—	—	—	No suspected lesions
22	^{131}I	—	—	—	—	No suspected lesions
23	^{125}I	—	—	+	—	^{99m}Tc-sulfur colloid liver scan positive, laparotomy negative
24	^{125}I	—	—	—	—	No evident metastases
25	^{125}I	—	—	—	—	No evident metastases
26	^{125}I	—	—	—	—	No evident metastases

TABLE 3. FALSE NEGATIVE SCANS

Patient	Drug	Primary	Scan	Clinical metastases	Scan	Comment
27	^{125}I	—	—	+	—	Cervical node amelanotic on biopsy
28	^{125}I	—	—	+	—	Axillary node biopsy (pre-scan) showed amelanotic lesion
29	^{125}I	—	—	+	—	Amelanotic lesions of axilla and neck (huge metastases)
30	^{125}I	—	—	+	—	Brain lesion not visualized. Lung lesion amelanotic on biopsy
3	^{125}I	—	—	+	—	Small satellite lesions. Pelvic node dissection positive but nodes relatively amelanotic

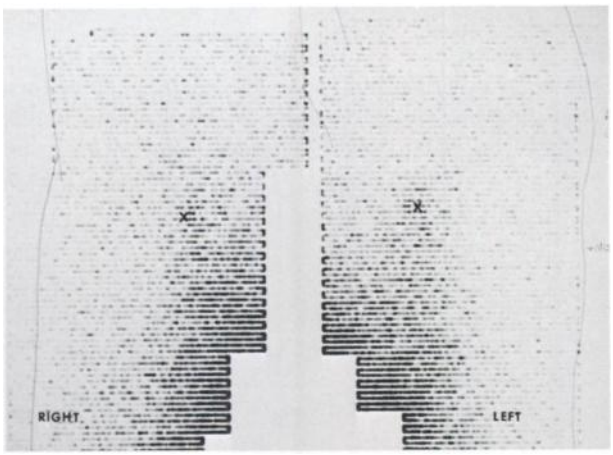

FIG. 4. Scans of both axillae in patient with axillary node metastases. Radioactivity in adjacent lung is seen in lower portions of scans.

of suspicious masses in Patients 15, 16, 17 and 18 confirmed the negative studies; they showed that no nodes or other tissues sampled were involved by metastatic disease.

False-negative scans. Five patients had negative scans despite clinically evident metastatic disease.* (The pertinent clinical details of these patients are presented in Table 3.) In all five of these patients the histopathology revealed that the metastases showed amelanotic anaplastic change. In addition to these relatively amelanotic lesions, Patient 3 had black-satellite skin lesions all less than 7 mm in dia at the time of the scans.

A summary of all the scan results is shown in Table 4.

Radiation doses. Total-body radiation dose calculated from tissue distribution and excretion studies was approximately 1 rad/mCi with the ^{125}I-labeled compound. The critical organ was the eye. We calculated from tissue-radioactivity studies in excised human eyes that a tracer dose of 2 mCi of the ^{131}I-labeled compound would deliver 50 rads to the choroid. The choroid dose with ^{125}I-NM-113 was 10–20% less (40–50 rads). Studies showed that the compound bound rapidly to melanin of the choroid and was released only very slowly (*4–6*); thus, the effective half-life was assumed to be the physical half-life.

^{125}I versus ^{131}I-chloroquine analog. The ^{131}I-NM-113 was used alone in two patients. Both ^{125}I- and ^{131}I-NM-113 were used separately in five patients (see Tables 1 and 2).

* One of these patients (3) is also included in the group (Table 1) with demonstrable primaries.

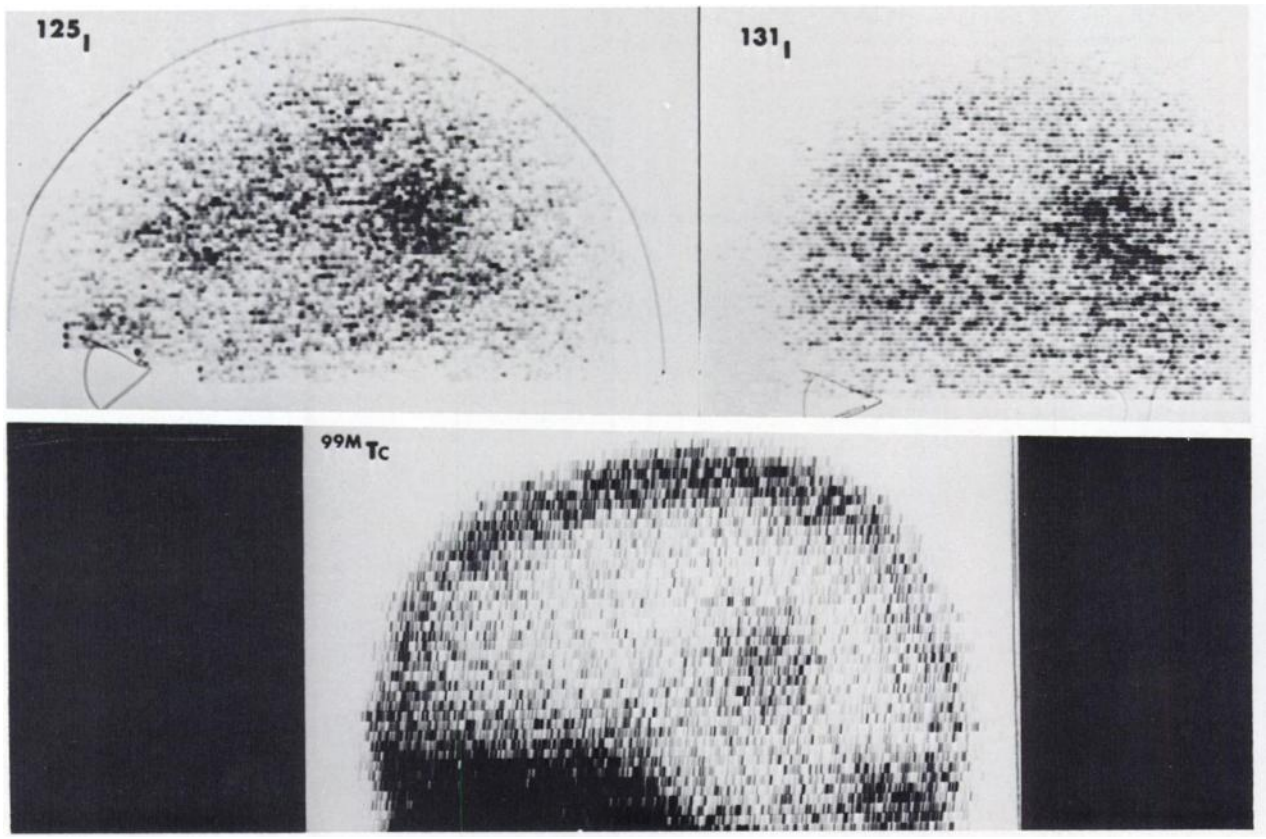

FIG. 5. Melanotic malignant melanoma metastases in parieto-occipital area as seen with ^{125}I-NM-113, ^{131}I-NM-113 and ^{99m}Tc.

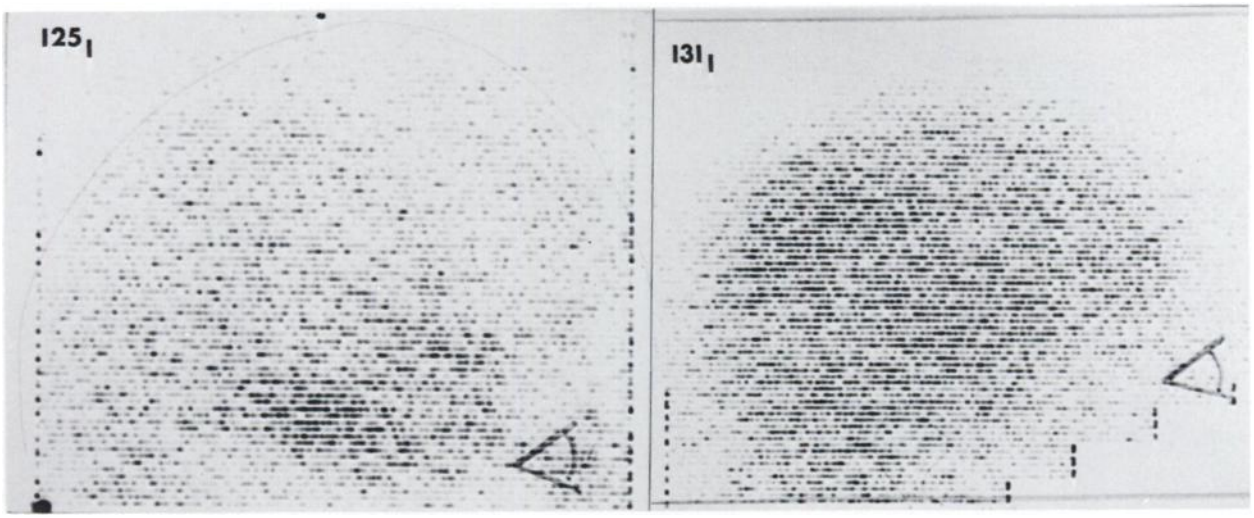

FIG. 6. Normal appearance of ^{125}I-NM-113 and ^{131}I-NM-113 brain scans in patients with no metastatic involvement.

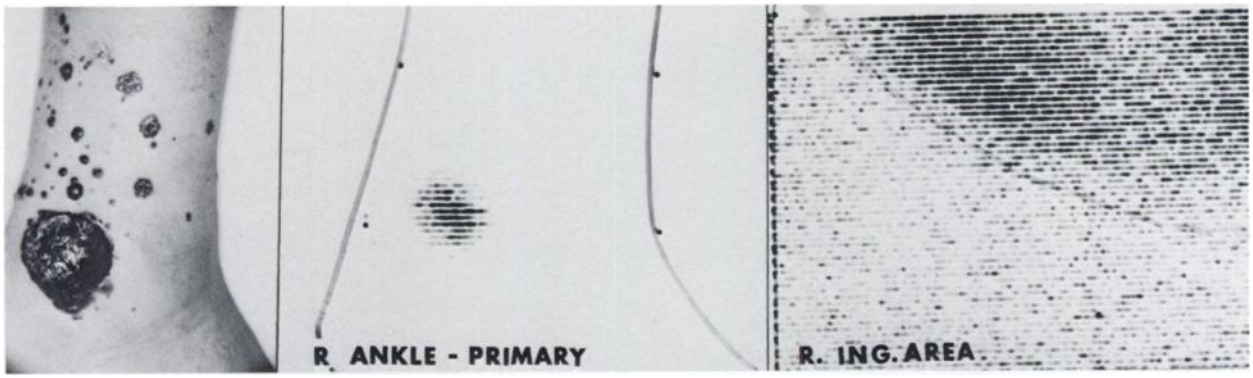

FIG. 7. Left: Primary ankle malignant melanoma with surrounding satellites. Middle: Scan of primary lesion. Right: Scan of right inguinal area showing radioactivity in bowel above inguinal ligament.

In no instance did the ^{131}I label disclose a lesion, superficial or deep, that was not visualized with ^{125}I. In superficial skin and subcutaneous lesions, ^{125}I was clearly superior in resolution to ^{131}I.

Toxic reactions. No reactions to the labeled drug were seen in any of these patients.

DISCUSSION

Clinical use of a positive scan. In all instances of palpable nodes, the scans were positive unless amelanotic change or extensive necrosis had occurred. Thus, when a positive scan was obtained in a patient with malignant melanoma and palpable lymph nodes, the clinical impressions of metastatic spread of a melanotic melanoma could be confirmed preoperatively. A positive scan when nodes were not palpable would further lend more accurate preoperative assessment as to the extent of disease. This situation was found in Patient 11.

Metastases to liver. Patients 7 and 9 had hepatic metastases which were shown on a ^{125}I-NM-113 scintillation scan. In Patient 7, the hepatic metastases were not detected until this scan localized the lesion and directed attention to the involved area. Subsequent laparotomy confirmed the presence of a malignant melanoma metastasizing to the liver. Systemic chemotherapy was instituted in Patient 9 with melanoma of the liver (shown by scanning with ^{125}I-NM-113, thus avoiding an unnecessary surgical exploration of the abdomen to confirm the diagnosis).

Metastases to brain. Metastatic melanoma to the brain was shown by a ^{125}I-NM-113 scan in Patient

TABLE 4. SUMMARY OF 30 PATIENTS

		Primary disease	Metastatic disease
"Positive" scans	Correct	3	11
	Incorrect	0	0
"Negative" scans	Correct	0	12
	Incorrect	0	5*

* One patient recorded in primary disease group also.

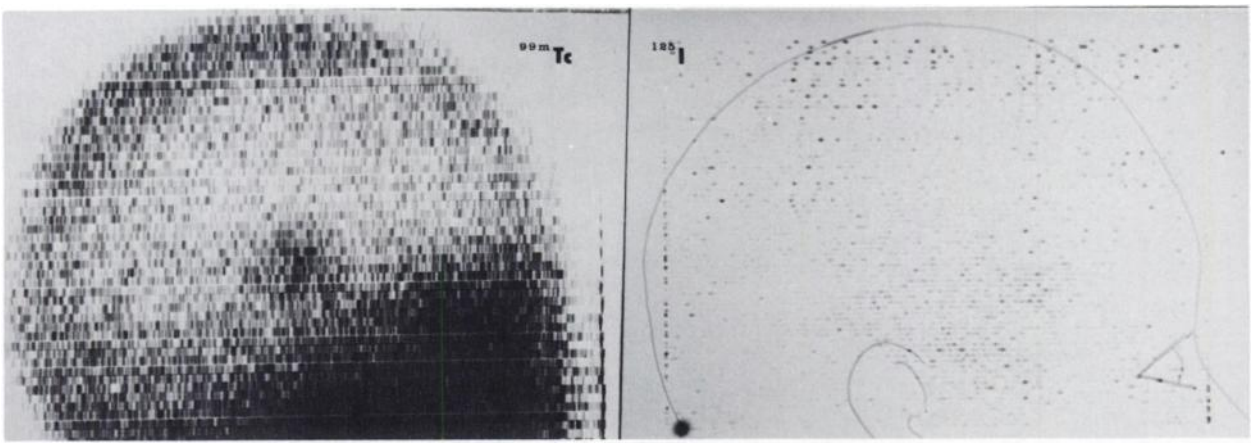

FIG. 8. Left: ^{99m}Tc brain scan showing parietal abnormality. Right: ^{125}I-NM-113 false-negative brain scan suggesting amelanotic change. "Normal" concentration is seen in low frontoparietal area of brain scan.

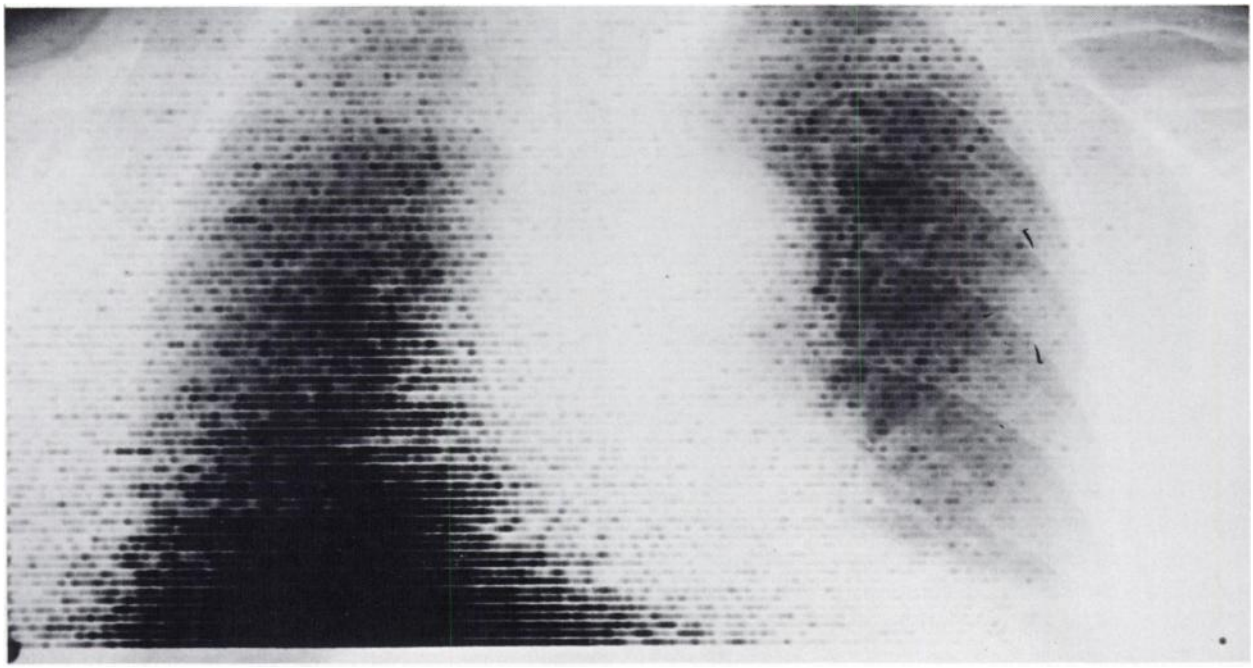

FIG. 9. "Normal" lung scan with ^{125}I-NM-113 superimposed on chest roentgenogram.

12 (Fig. 5). The histologic finding of malignant melanoma was predicted preoperatively in this patient. Patient 30 (Fig. 8) with intracranial metastases shown on a ^{99m}Tc brain scan had a negative brain scan with ^{125}I-NM-113, suggesting amelanotic transformation. Amelanotic melanoma was found on biopsy of a lung lesion in this patient. Thus, ^{125}I-NM-113 appeared to be a "specific" localizing agent for melanotic malignant melanoma metastases as compared to other "nonspecific" compounds used in brain scanning.

Metastases to lung. Limitations in scanning with this compound were encountered in lung metastases. Patient 4 had pleural implants of malignant melanoma which were not seen on lung scans with ^{125}I-NM-113, presumably because of interfering uptake in the adjacent normal lung tissue (Fig. 9). An ordinary chest roentgenogram appeared to offer better diagnostic efficacy here. In no instance were we able to demonstrate a positive uptake of ^{125}I or ^{131}I-NM-113 in melanoma metastases seen in the chest roentgenogram.

Significance of a negative scan. Negative scans in the face of clinically evident malignant melanoma were found in lesions which were amelanotic or showed extensive necrosis and lesions which were

too small for resolution. The ability to detect a melanoma depended on the concentration and total amount of melanin in the lesion, the volume and the diameters of the lesion and the depth of the lesion in the tissue. Flat-satellite lesions in skin measuring less than 7 mm in dia were not visualized in Patient 3. Studies are now under way to explore further the variabilities of cross-sectional area, total counts, concentration of counts and background influence on the ability to visualize these lesions. Thus a negative scan does not mean that metastatic disease is absent.

^{125}I versus ^{131}I-NM-113. Generally, resolution was better with ^{125}I than with ^{131}I-NM-113. The longer half-life of the ^{125}I-labeled compound allowed greater versatility in scanning procedures, lower radiation dose, lack of associated beta dose and no loss of diagnostic ability.

Mechanism of binding to melanin. The mechanism of binding of chloroquine and its analogs to melanin is speculative. Allison, O'Brien and Hahn have reported the intercalation of the molecule into deoxyribonucleic acid (*7*). Our studies have shown poor, if any, concentration of this compound in amelanotic melanoma metastases (usually metastatic from a previously excised melanotic lesion). Thus, the affinity of this compound for melanoma appeared to depend almost solely on the presence of melanin granules and not on some other function of the melanocyte.

Benign and malignant black moles. In Patients 7, 8, 10, 11 and 12 (on whom scans were being performed for proved malignant melanomas), scans were done on one or more black moles up to 1 cm in dia that appeared to be benign. The scans showed no uptake in these lesions. Scans of melanotic melanoma metastases of less than 1 cm in the same patients were also negative. Quantitative tissue-radioactivity concentration in benign moles showed no increase in activity above that of normal skin.

Ocular melanomas. We have studied ocular melanomas which will be the subject of a separate communication (Boyd, unpublished data). Obviously, the ocular melanoma adds counts to the eye background counting rate. The question to be solved is: at what total mass of the ocular tumor do the added counts in the affected eye exceed the differences found between two normal eyes in patients without ocular melanomas. We are currently determining the normal range of percent differences between the two eyes by point counting the two eyes of every patient receiving a tracer dose for a proved dermal malignant melanoma.

Radiation dose to total body. The estimated radiation doses with this compound delivered to the total body by the tracer doses used here were considered to be within that allowable in patients with suspected or known malignant melanoma.

Radiation dose to eye. The radiation dose of about 23 rads/mCi delivered to the eye was well below that reported to produce damage to the eye. No permanent harmful effects were noted in the retina of rabbits receiving from 3,000 to 5,000 rads of x-radiation to the eyes. Merriam saw no harmful effects in the retina in patients receiving up to 5,000 rads to the eye when having radiation therapy to the head and neck for malignant disease (oral communication, 1968). The minimal cataractogenic dose in humans has been found to be about 450 rads (*8*).

Radiation dose to thyroid. Significant, although reduced, thyroidal uptake was found despite routine use of Lugol's solution before dose administration. Patient 11 was on Lugol's solution for 15 days prior to dose administration and had been taking two grains of desiccated thyroid for several years (neither of which prevented uptake of ^{125}I-NM-113 by the thyroid) (Fig. 10). However, routine use of Lugol's solution was suggested in view of some reduction in uptake and to prevent uptake of radioiodine from subsequent deiodination.

CONCLUSION

In conclusion, this radioiodinated chloroquine analog has been helpful in diagnostic use in many patients with malignant melanoma. Therefore, ^{125}I-NM-113 is of significant therapeutic value in the evaluation of melanoma patients by providing the surgeon with valuable preoperative information and

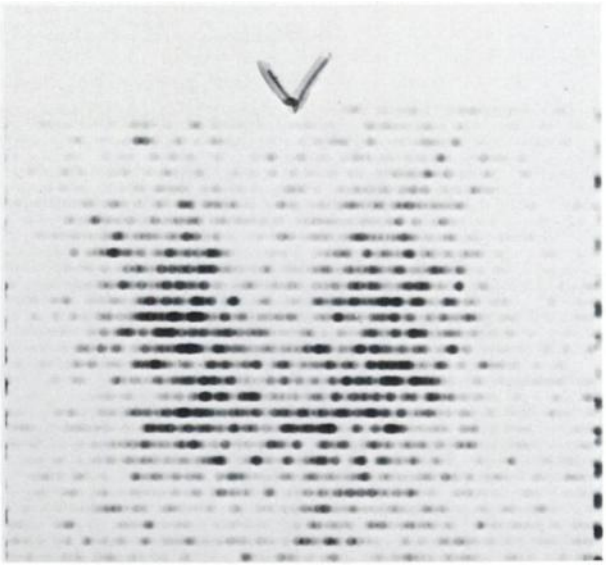

FIG. 10. Thyroid scan with ^{125}I-NM-113, despite administration of exogenous thyroid and Lugol's iodine solution.

by helping to direct methods of therapy. It may be of use in following patients with known disease to delineate areas of spread previously unsuspected, thus guiding further treatment.

ACKNOWLEDGMENTS

This work was supported in part by USPHS Grants CA-05134-06 and CA-05134-07, U.S. AEC Grant AT(11-1)-2031, the Elsa U. Pardee Foundation and the Nuclear Medicine Research Fund.

REFERENCES

1. BEIERWALTES, W. H., LIEBERMAN, L. M., VARMA, V. M. AND COUNSELL, R. E.: Visualizing human malignant melanoma and metastases. *J. Am. Med. Assoc.* **206**:97, 1968.

2. COUNSELL, R. E., POCHA, P., MORALES, J. O. AND BEIERWALTES, W. H.: Tumor localizing agents. III. Radioiodinated quinoline derivatives. *J. Pharm. Sci.* **56**:1,042, 1967.

3. BEIERWALTES, W. H. *et al:* Scintillation scanning of malignant melanomas with radioiodinated quinoline derivatives. *J. Lab. Clin. Med.* **72**:485, 1968.

4. BERNSTEIN, H., ZVAIFLER, N., RUBIN, M. AND MANSOUR, A. M.: The ocular deposition of chloroquine. *Invest. Ophthal.* **2**:384, 1963.

5. SAMS, W. M. AND EPSTEIN, J. H.: The affinity of melanin for chloroquine. *J. Invest. Dermatol.* **45**:482, 1965.

6. POTTS, A. M.: The reaction of uveal pigment *in vitro* with polycyclic compounds. *Invest. Ophthal.* **3**:405, 1964.

7. ALLISON, J. L., O'BRIEN, R. L. AND HAHN, F. E.: Nature of the deoxyribonucleic acid-chloroquine complex. *Antimicrobiot. Agents Chemotherapy,* **5**:310, 1965.

8. MERRIAM, G. R., JR. AND FOCHT, E. F.: A clinical study of radiation cataracts and relationship to dose. *Am. J. Roentgenol.* **77**:759, 1957.