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Labeling of Human Globulin With I^{124} For Positron Scanning of Neoplasms[1,2]

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The present study is part of a continuation of laboratory and clinical investigations designed to determine the physicochemical properties and functions of the serum proteins in neoplastic diseases. Should any characteristics exist for a given protein fraction distinguishing its nature or behavior from a similar fraction in normal subjects (or those with various non-neoplastic diseases), a basis might be established for a diagnostic test for cancer. In studies of the metabolic behavior of radioactively labeled proteins (5, 6, 7, 8, 10) the turnover or degradation rate has been determined from serum and urine concentration curves as well as by using the whole-body gamma spectrometer. In a small series of patients with metastatic carcinoma of the breast, turnover studies of gamma globulin prepared from healthy normal donors and of aberrant gamma and beta globulins from patients with multiple myeloma have been initiated. The mean half-life for normal gamma globulin is far longer than that for the aberrant proteins in the same patient. This differential metabolic recognition of two types of globulin in the same individual may have significant application to the diagnostic problem if the site of accelerated catabolism should prove to reside within the growing neoplastic mass.

To determine the sites of globulin degradation *in vivo*, one may employ a sensitive external detector capable of determining the exact locus of the gamma ray emission from the administered radioisotopically-labeled protein. Instrumentation based on the principle of positron scanning appeared to offer the best possibility. Such a device utilizes the physical principle that radioactively emitted positrons (positive electrons) quickly interact with normal negative electrons

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with mutual annihilation, converting their masses to two gamma rays emitted in opposite directions; *i.e.*, 180° apart. Two detectors placed on opposite sides of the radiation source feeding appropriate electronics can identify these gamma rays in time coincidence and establish a line along which the radioactivity is located. This makes it possible to localize varying amounts of radioactivity in relatively small tissue volumes. The distribution of the radioactivity indicates the area in the tissue or organ scanned where the greatest number of disintegrations occur as the radioactive label is carried along with the protein being used in a metabolic process. This should then help to establish the site or sites of globulin catabolism.

The positron emitter selected for labeling the globulin was I^{124}. Since it and I^{131} are isotopes it was anticipated that it could be as readily used as the latter for satisfactory labeling of the globulins. I^{124} has been produced in England but no known producer of it existed in this country. After consultation with members of the staff of the Hot Laboratory Division and Physics Department of the Brookhaven National Laboratory it was found that they would undertake the arduous task of preparing and processing I^{124} in the amounts needed. The extensive experience of the group at the Massachusetts General Hospital (1) with the use of positron emitting copper and arsenic for scanning brain neoplasms *in vivo* made it desirable to undertake this joint study with them. The plan evolved was: (1) the I^{124} would be produced at the Brookhaven 60-inch Cyclotron and processed in the Hot Laboratory; (2) the normal and aberrant globulins would be fractionated, characterized as in our previous studies (8, 10), and labeled with I^{124}, following which (3) the labeled globulins would be tested in patients who had already been scanned with either radioactive copper or arsenic. Subsequent studies with a device capable of scanning other areas of the body for determining sites of catabolism for possible tumor localization in various parts of the body constitutes a separate long term research project. The portion of the study reported in this paper is concerned with the demonstration that human fractionated globulin may be satisfactorily labeled with I^{124} and that such a preparation when injected intravenously concentrates in sufficient amount in a brain neoplasm to be identified with positron scanning.

PATIENTS AND METHODS

The methods of isolation of the normal gamma globulin as well as the multiple myeloma globulins have been given in a previous publication (8). The electrophoretic and ultracentrifugal characteristics of the iodinated globulins were determined by methods also previously described (5). Iodination was carried out by two different technics: that of Hughes and Straessle (4) and of Helmkamp, Goodland, Bale, Spar and Mutschler (3). Both methods employ Oak Ridge I^{131}. The iodinating agent employed in the former technic is in alkaline medium, and in the latter technic greater efficiency of I^{131} incorporation is presumably achieved with iodine monochloride as the iodinating agent. The labeled globulins were sterilized by passage through an asbestos filter of the Seitz type, and sterility was proved by culture. Possible uptake of free iodine

label by the thyroid was blocked by administration of Lugol's solution to the patients before and during the study. The three patients studied had proven glioblastoma multiforme and all had been scanned at about the same time with copper or arsenic with positive localizations of the positions of the neoplasms in the brain.

The I^{124} was produced in the Brookhaven National Laboratory 60-inch Cyclotron by bombarding an antimony target with an alpha beam (40 MeV). For example, a target receiving 275 μah of an alpha beam over an 8.5 hour period resulted in a sample which after processing (2) yielded 2.94 mC of I^{124}.

RESULTS

In this study it was first necessary to obtain a suitable I^{124} preparation for labeling the globulins. In order to determine whether the chemically processed I^{124} was satisfactory a comparison was made of the per cent of globulin labeled by I^{131} and by I^{124}, utilizing in each instance the same technic for iodination. To further clarify this point two different technics for iodination were tested first with I^{131} and then the more efficient method was selected for comparing the binding capacity of the two radioisotopes. The results of all binding experiments are shown in Table I. The method of Hughes and Straessle (4), when applied to I^{131}, gave yields of 4 to 11.2 per cent binding, whereas that of Helmkamp, Goodland, Bale, Spar and Mutschler (3) gave a range from 65 to 70 per cent (Series I and II). In the original series of bombardments it was not known with certainty that the subsequent processing would produce I^{124} without residual impurities. For that reason, as seen in Series III, I^{131} was processed in a manner similar to that for the I^{124} after bombardment. It was then used for

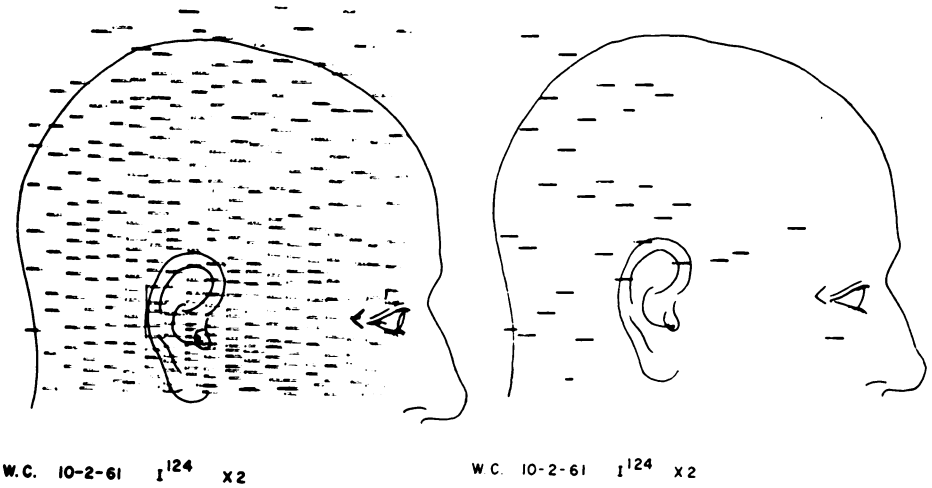

Fig. 1. Lateral scan of patient with left frontal-parietal glioblastoma multiforme injected with 260 μc of I^{124} labeled gamma globulin. Scan, performed at double normal speed, shortly after injection, appears normal; 1a is coincidence scan, 1b is simultaneous unbalance scan.

TABLE I

COMPARISON OF PER CENT OF I^{131} AND I^{124} GLOBULIN BINDING

Series	*Date*	*Radioisotope Used and Amount in mc*	*Type of Gamma Globulin Labeled and Amount in mg*	*Method*	*Per Cent Binding*
I	10/14/58	10.0 I^{131}	100. m.m. globulin	Hughes et al	6.0
	3/24/59	23.9 I^{131}	100. m.m. globulin	Hughes et al	10.0
	11/2/59	8.0 I^{131}	83.3 m.m. (B) globulin	Hughes et al	4.0
	2/15/60	17.0 I^{131}	120. m.m. (B) globulin	Hughes et al	7.5
	4/20/60	27.0 I^{131}	150. N globulin	Hughes et al	11.2
	6/9/60	14.0 I^{131}	25. N globulin	Hughes et al	6.0
II	1/12/61	5.25 I^{131}	50. m.m. globulin	Helmkamp et al	68.0
	3/16/61	4.2 I^{131}	100. m.m. globulin	Helmkamp et al	70.0
	3/28/61	6.8 I^{131}	100. m.m. globulin	Helmkamp et al	65.0
	6/15/61	50.0 I^{131}	4.2 N globulin	Helmkamp et al	66.0
	7/13/61	5.0 I^{131}	100. N globulin	Helmkamp et al	70.0
	11/27/61	1.25 I^{131}	60. N globulin	Helmkamp et al	70.0
III	9/19/60	0.1 I^{131}	100. m.m. globulin	Helmkamp et al	0.
	9/22/60	0.1 I^{131}	100. m.m. globulin	Helmkamp et al	10.0
	3/16/61	4.2 I^{131}	100. m.m. globulin	Helmkamp et al	0.0
	4/21/61	6.2 I^{131}	25. m.m. globulin	Helmkamp et al	31.0
	5/3/61	0.4 I^{131}	25. m.m. globulin	Helmkamp et al	45.0
	5/5/61	1.0 I^{131}	25. m.m. globulin	Helmkamp et al	0.5
IV	11/15/60	9.4 I^{124}	25. N globulin	Helmkamp et al	30.0
	11/15/60	4.1 I^{124}	25. N globulin	Helmkamp et al	6.0
	4/14/61	15.2 I^{124}	25. m.m. globulin	Helmkamp et al	.009
	5/8/61	5.5 I^{124}	25. m.m. globulin	Helmkamp et al	18.0
	9/12/61	1.08 I^{124}	25. N globulin	Helmkamp et al	0.3
	9/26/61	8.0 I^{124}	25. N globulin	Helmkamp et al	18.0
V	6/25/62	9.5 I^{124}	25. N globulin	Helmkamp et al	58.7
	6/25/62	9.7 I^{124}	25. N globulin	Helmkamp et al	59.0
	8/10/62	5.8 I^{124}	25. N globulin	Helmkamp et al	49.0
	8/10/62	5.8 I^{124}	25. N globulin	Helmkamp et al	52.5

Key: m.m. = Multiple Myeloma Serum Fractionation of Globulin
N = Normal Serum Fractionation of Globulin
B = Beta Globulin

iodination. The results for binding were highly erratic at that time with some complete failures and yet with a yield as high as 45 per cent. Presumably these results were due to incomplete separation of the I^{124} so that residual material interfered with the iodination.

It was decided that in spite of these erratic results the I^{124} obtainable at that time would be used (Series IV) for iodination. As would be anticipated, considerable variation occurred in binding capacity. However, one of the yields giving 18 and another 30 per cent constituted excellent preparations and were used for localization studies in patients. With further experience in processing I^{124} a satisfactory preparation was produced (2) and used in Series V. Independent checks were made in two laboratories utilizing the same material. Consistent results became readily obtainable with at least 50 per cent binding and the labeled protein met all of our required standards (8) for administration to patients (9).

Three patients with glioblastoma multiforme were scanned using I^{124} labeled gamma globulin as a localizing agent. The doses per 70 kilogram weight of patient were respectively 260, 360 and 695 microcuries with the total globulin not exceeding 10 milligrams. Scans were made immediately after intravenous injection and on successive days up to eight days. The I^{124} globulin scans showed some distinctive characteristics different from either arsenic or copper. Initially in the I^{124} scans the radioactivity was entirely in the blood. This was observed in the scans by uniform density over the head with slight increase over the temporal muscles and a distinct lack of concentration in the periphery of the head. The blood level was also confirmed by monitor counts over the body. The blood pool in the heart was higher than other areas. All three I^{124} scans showed uniform distribution on the first day; the second day's scans were also fairly uniform with perhaps a very slight indication of uptake in the tumor. By the third day the uptake in tumor was beginning to be distinct, becoming more so on successive days. Even as long as the eighth day post-injection the tumor was distinctly

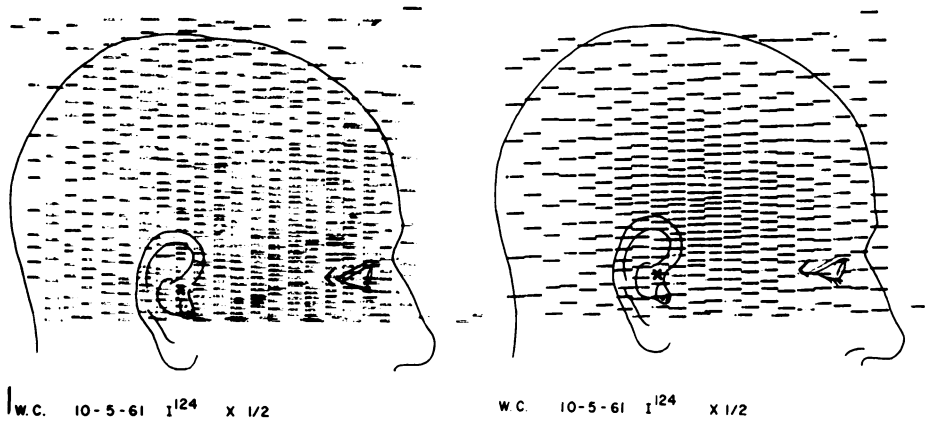

Fig. 2. Repeat scan 3 days post-injection for same patient as shown in Figure 1. Abnormality is clearly seen on coincidence scan (a). Unbalance scan (b) shows cluster of straight marks indicating concentration is on left. Speed reduced to ½ normal.

localized, in spite of the fact that the counting level was extremely low because two half-lives had gone by. Scanning speed was changed to compensate for decay and to produce uniformly dense scans. It is important to note that these I^{124} scans indicated definitely that the gamma globulin had been taken up in the tumor and it was not merely a question of relative amounts of blood in the tumor compared to normal brain, for if it were the latter, the concentration should have been apparent on the first day's scan.

Figures 1, 2, and 3 show 3 of 5 scans performed on a patient who had had a partial removal of a left frontal-parietal glioblastoma multiforme on 8/3/61. The first scan was performed ½ hour after injection, with repeat scans on the 2nd, 3rd, 4th, and 7th days. The first scan is typically normal, indicating uniform distribution of isotope in the blood. The 3rd day scan, while greatly reduced in density (speed changed by factor of 4) clearly shows the abnormal area. The unbalance scan shows a cluster of straight lines, indicating a left unbalance. The 7th day scan, while still less dense, still indicates the abnormality. The other two scans in the series are similar to the 3rd day scan, and all of these closely resemble a scan made with As^{74} the following week.

COMMENT

This is the first time that I^{124} has been used to label a protein, in this instance, gamma globulin. Three cases with brain neoplasms were studied and the series is not large enough to make a comparison between I^{131} and I^{124} labeled normal and abnormal globulin. The purpose in using these cases was simply to show that good scans could be made. The objective in this developmental program is to determine with a properly devised positron scanner whether an antigen-antibody reaction occurs at tumor sites(s) when an I^{124} labeled aberrant globulin (carrying antibody to neoplastic tissue) is injected into the patient. If this occurs, whereas with I^{124} labeled normal globulin there is no antigen-

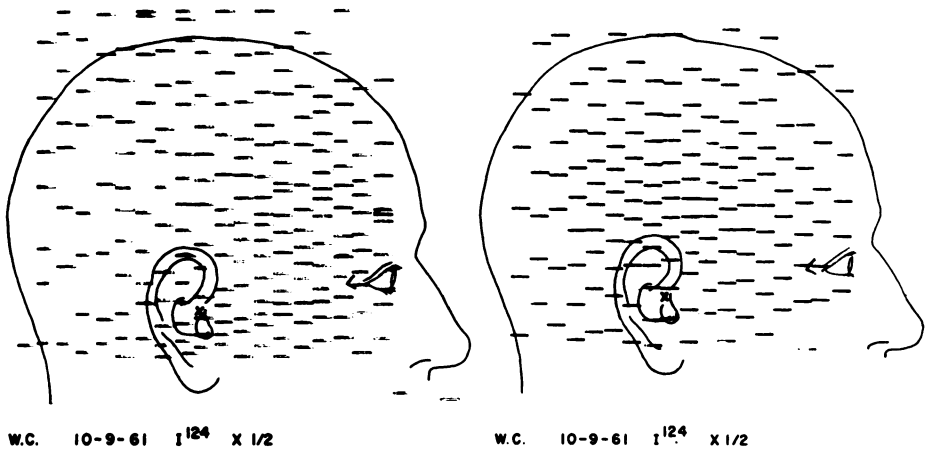

Fig. 3. Repeat scan 7 days post-injection for same patient. Density is reduced because of isotope decay, but abnormality still visible in frontal region (a); unbalance (b) shows left-sided concentration; speed ½ normal.

antibody reaction then in a given patient at a given site the counting rates for the two globulins (administered at different times) should be different. Presumably with the aberrant globulin the counts would be considerably higher due to increased catabolism. The localization with the positron emitter (two gammas coming off at virtually 180°) would permit, by use of collimated beams, a much more precise localization of the lesions than would be possible by the usual scanning for random gamma rays from I^{131}.

SUMMARY

Normal and aberrant gamma globulin have been labeled by I^{124} produced by alpha particle bombardment of antimony in a 60-inch cyclotron. Localization of the labeled globulin in primary brain neoplasms has been demonstrated by positron scanning. It appears possible by this procedure to investigate in various tissues and organs in man sites of catabolism of similarly labeled proteins.

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