

## Radio-nuclides in surgery: Part 3

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*Using radio-labelled antibodies appears to be an innovative method of localising and treating tumours. Theoretically sound though the technique may be, there are many technical problems which need to be overcome before it becomes universally acceptable.*

This issue will lay emphasis on isotopes of iodine and their applications in the radio-immunology, thyroid gland etc. This member of the halogen family, besides being an essential element in the human body, also has many radioisotopes which can be easily attached to various substances as a radioactive label (tables 1a,b).

### Radio-nuclide immunology

#### Radio-immunoassay

Berson and Yalow were the first to develop the technique of radio-immunoassay (RIA) in 1958 using these isotopes. RIA can be used to quantify any substance in the body fluids against which an antibody can be raised, viz. all peptide hormones and many antigens (Hb<sub>s</sub>ag, oncofoetal antigen etc). It is a rapid (within a few hours) and sensitive method as compared to bioassay (which is time-consuming, expensive and less specific). RIA depends on quantitative interaction between an antigen (ag) and its specific antibody (ab). A known amount of <sup>125</sup>I-labelled ag competes with an unknown amount of unlabelled ag in the test sample, for a specific ab. Free and bound ag are separated by washing and a gamma counter analyses the radioactivity of the ag-ab complex.<sup>1</sup>

**Tumour diagnosis:** RIA is used for circulating oncofoetal ag (OFA) viz. (a) alphafoetoprotein (AFP) in hepatoma and germ cell tumours of testis (teratoma) and ovary; (b) pancreatic OFA (POA) in pancreatic cancer; and (c) carcinoembryonic ag (CEA) in colorectal and pancreatic cancer. RIA is not helpful in the early diagnosis of these tumours because circulating levels of these ag depend on tumour mass and they may not be raised in small tumours. However, RIA is useful in detecting early tumour recurrence during post-operative follow-up, when the circulating levels of OFA rise again, often pre-dating clinical evidence of recurrence by several months. The ab used in RIA are the conventional polyclonal antiserum (PAS) or the newer, more specific monoclonal ab (MAB).

**PAS:** The anti-sera produced by immunising rabbits, sheep, goats or horses with tumour ag preparations contain ab reacting to an array of tissue and tumour ag (hence the term polyclonal). Thus they are not specifically directed against any particular tumour. Purification procedures are required, and attempts to improve tumour specificity of these ab by absorption with normal tissues often results in loss of ab activity.

**MAB:** These specific (monoclonal) ab are produced from a single ab-producing cell line, by the *mouse hybridoma technique* (fusion of murine splenic β-lymphocytes and myeloma cells). Such a cell line can be maintained indefinitely in culture to produce large quantities of MAB (table 2). As is obvious from the table, hybridomas produce ab showing restricted but not necessarily 100% tumour-specific reactivity.

#### Radioimmunolocalisation

Ab may be labelled with radioisotopes without altering their ag-binding characteristics. Once these tagged ab attach on the tumour the same may be imaged with a gamma camera. Thus radioimmunolocalisation (RIL), a.k.a. tumour imaging (TI) or immunoscintigraphy (ISG), allows early detection and localisation of primary, secondary and recurrent tumours. (In RIA, labelled ag is used to detect circulating tumour ag; in RIL, labelled ab is used to detect the tumour itself).

The radioisotopes used are <sup>131</sup>I (most common), <sup>123</sup>I (table 1A), <sup>111</sup>Indium(In), <sup>67</sup>Gallium(Ga) and <sup>99m</sup>Tc-RBC (for image enhancement; described below). The false-negative results of RIL often result from limitations in resolution of the image, which in turn is dependent on the energy of gamma emission and other characteristics of the isotopes. <sup>111</sup>In appears to be the most suitable for RIL and also gives better tomoscintigraphic images (infra), though each isotope has its own disadvantages and uses (table 3).

The ab used in RIL may be PAS or MAB. The latter has the following advantages; (a) the amount of injected immunoglobulin is less; (b) the number of cross-reactions are few; and (c) there is increased tumour specificity. Table 4 gives a few examples of RIL/TI using <sup>131</sup>I-labelled PAS. The technique of imaging colorectal cancers (CRC) using <sup>131</sup>I-labelled MAB is described.

**Technique:** Human CRC was first imaged with <sup>131</sup>I-anti-CEA MAB Mach et al in 1981. Subsequently many other MAB have been developed which can recognise several ag (other than CEA) on colorectal tumours. The MAB designated as 791 T/36 (table 2) has been used to image both primary and metastatic CRC. After a prior intra-dermal skin test (1 µg) to rule out Type-1 hypersensitivity reaction, <sup>131</sup>I-labelled MAB is administered IV and images are obtained using a gamma camera linked to a computer.

The positivity rate is 70-75 % for localising primary tumours, and 85% for detecting liver metastases (table 5). The uptake per gram of tumour is 2.6 times that of normal colonic tissue, and the ratio of tumour : normal tissue levels of  $^{131}\text{I}$ -labelled MAB is > 3 times the ratio of tumour : normal tissue levels  $^{125}\text{I}$ -labelled normal IgG; indicating that the binding of MAB (791 T/36) is antigenically related rather than a non-specific binding like a normal Ig.

**False-negative:** The reasons for failure to localise tumours at times are due to; (a) variations in ag expression of the tumour; (b) problems in scanning (small rectal tumour masked by isotope in urinary bladder); (c) isotope emission variations giving limited image resolution (supra); and (d) poor image enhancement. This results from overlapping of images from the blood and tissue pool. This can be obviated by the following means: (1) *Rapid blood clearance*; Fragments of MAB which are rapidly cleared from the circulation may be used.  $\text{F(ab}^1)_2$  fragments have been used to image CRC and malignant melanoma. Alternatively, the initial injection of  $^{131}\text{I}$ -labelled MAB is followed by administration of LESA (liposome-entrapped second antibody) directed against the first antibody (MAB). The LESA-MAB complex is quickly engulfed by the reticuloendothelial cells. (2) *Separate labelling*; This is done by using normal Ig of a similar type to the MAB but labelled with a different radio-nuclide. The distribution of the normal Ig should correspond exactly with that of the active ab apart from the specific uptake of the latter in tumour deposits.  $^{111}\text{In}$  and  $^{67}\text{Ga}$  have similar characteristics, making paired labelling technique possible. Alternatively, the blood pool is labelled with  $^{99\text{m}}\text{Tc-RBC}$ . This latter image is then digitally subtracted from the  $^{131}\text{I}$ -labelled MAB image, to give only the enhanced tumour image.<sup>2</sup>

### Tomoscintigraphy

Tomoscintigraphy (TSG), a.k.a. emission tomography, gives a three-dimensional image of the distribution of the isotope. It therefore detects smaller tumour sites ( $10\text{ cm}^3$  vs.  $50\text{ cm}^3$ ) and has a higher detection rate (94% vs. 43%), compared to conventional rectilinear ISG (the latter figures), when imaging CRC and medullary thyroid cancers with  $^{131}\text{I}$ -labelled anti-CEA ab.<sup>2</sup>

### Radioimmunotherapy

This employs the same principle as RIL, to deliver a tumoricidal dose of radiation to the tumour site. Systemic administration of labelled MAB for treating melanoma or hepatoma has not met with much success because the fraction of the total administered dose reaching the tumour is low. However it has been possible to instil  $^{131}\text{I}$ -labelled MAB against human milk fat globule membrane ( $^{131}\text{I}$ -labelled anti-HMFG 2 MAB) directly into the pleural and pericardial cavities to treat these malignant effusions from ovarian cancer with 5000-7000 cGy (centi Gray) (1 cGy = 1rad) of radiation.<sup>2</sup>

### Miscellaneous tests

Tests to detect IgE in serum of atopic allergic patients use radio-labelled IgE, and use; (a) radioimmunodiffusion test (RID) (sensitivity  $<1000\text{ ng ml}^{-1}$ ); (b) competitive radioimmunosorbent test (RIST) (sensitivity= $50\text{ng ml}^{-1}$ ); (c) non-competitive RIST (more sensitive); and (d) double ab RID. Even more useful is the radioallergosorbent test (RAST), which measures specific anti-IgE in serum by using radio-labelled anti-IgE.

## Thyroid scan

Radioisotopes (table 6) are used in thyroid disorders for; (a) evaluating the thyroid function; (b) scintiscanning; and (c) therapy. The first has been enumerated in table 1B and shall not be elaborated any further.

### Scintiscanning

1. It defines areas of physiologically active, inactive or hyperactive tissue within the gland, its degree and uniformity.
2. It is highly valuable in the diagnosis of an autonomous toxic nodule (either solitary or the dominant nodule of a toxic nodular goitre).
3. It is often (but not always) helpful in evaluating the 'clinically solitary nodule' (infra) and occasionally helpful in thyroid carcinoma.
4. It helps to determine the location of the thyroid gland (normal, ectopic or aberrant location) and the presence or absence of any continuity between them. This helps to determine the treatment modality.
5. It locates stuma ovarii (ovarian teratoma with functioning thyroid tissue) or functioning metastases from carcinoma.

### Isotopes

**$^{131}\text{I}$ :** This isotope ( $T_{1/2}=8$  days) and the others are taken up by the thyroid just like the stable isotope ( $^{127}\text{I}$ ) and made to go through the same sequence of events (trapping, oxidation, iodination, coupling) in the biosynthesis of thyroid hormones. Therefore it forms the basis of all the thyroid function tests listed in table 1B. In this capacity it represents one of the earliest applications of radioisotopes in medicine. Its radioactive emission is used to scan the gland and treat thyrotoxicosis and cancer.  $^{131}\text{I}$  has a high energy of  $\gamma$  emission which a lower efficiency of detection by the gamma camera, as compared to  $^{123}\text{I}$ . For  $^{131}\text{I}$  uptake (RAIU) test  $5\text{ }\mu\text{Ci}$  is given orally and activity is counted at 4, 12, 24, 48 hours. For thyrotoxic ablation the dose is 140-160  $\mu\text{Ci}$  per gram of estimated gland weight, given orally. More is described under 'Therapy'.

**$^{123}\text{I}$ :** This is ideal but costly and not easily available. Its low  $T_{1/2}$  (13 hours) and lower energy of  $\gamma$  emission makes it more suitable for gamma camera performance, safer for children and pregnant women, and for performing serial measurements of thyroid function.

<sup>132</sup>I: Its short T<sub>1/2</sub> (2.3 hours) precludes its use in scanning but has been used in Werner's T<sub>3</sub> suppression test to predict whether a thyrotoxic patient will have a relapse after 6 months of antithyroid drugs. <sup>132</sup>I is administered IV and activity counted at 20 minutes. Failure to suppress <sup>132</sup>I uptake by T<sub>3</sub> administration indicates persistent thyroid-stimulating-immunoglobulin (TSI) control (TSH-independent), with likelihood of relapse.

<sup>99m</sup>Tc: This has been described in the previous issue. Since it is trapped by the thyroid but not organified, it cannot be used for thyroid function tests, and the scan information may not be the same as that with <sup>131</sup>I. But because of its numerous advantages (q.v.) it is ideal when thyroid morphology (but not function) is to be assessed.

### Special scans

These are aimed at; (a) detecting carcinoma; (b) improving the diagnostic accuracy of the same and; (c) detecting metastases from thyroid cancer. The first and third aims are fulfilled by TSG/ISG and total body scan / skeletal scan, respectively. The scans to improve the diagnostic accuracy of cancer are based on the premises that; (1) a cancer shows lack of affinity for radio-iodine (with rare exceptions); and (2) it has a high affinity for methionine because of its cellularity. <sup>99m</sup>Tc photoscanning and <sup>241</sup>Americium(Am) fluorescent scan are based on the first premise and <sup>75</sup>Selenomethionine scan is based on the second.

**Tomoscintigraphy:** TSG/ISG using <sup>131</sup>I-labelled anti-CEA ab to image medullary thyroid carcinoma has been described earlier.

**<sup>99m</sup>Tc photoscanning:** *Conventional rectilinear scanning* may miss a lesion < 1 cm because of the intimate relationship of the nodule to the surrounding normal parenchyma, and the scan may not accurately reflect the iodide uptake within the nodule because of the overlying normal parenchyma. *<sup>99m</sup>Tc photoscanning* techniques permitting oblique views overcome these disadvantages of rectilinear scanning. Thus a nodule which appears hypo-functioning in the A-P view (due to overlying tissues) may be confirmed to be non-functioning in the oblique view. These techniques permit better appraisal of the intrinsic radioactivity of a nodule and provide a more accurate index of the radio-nuclide content of a nodule. The positive correlation between lack of iodide uptake within a nodule and thyroid cancer is 10-20%. However, *colloid nodules, cysts and follicular adenomas* may also exhibit lack of iodide uptake and cannot be differentiated from cancer by scan alone.

**<sup>241</sup>Am fluorescent scan:** When parallel (collimated)  $\gamma$  photons from <sup>241</sup>Am are focussed on the thyroid gland, the I<sub>2</sub> atoms get sufficiently agitated to release 28.5 keV of x-ray which can be detected by fluorescence imaging. The quantum of x-ray emitted depends on the amount of I<sub>2</sub> atoms in the field. By the fluorescent scan, comparison of the I<sub>2</sub> content in a 'cold' solitary nodule (as seen in a conventional scan) and the corresponding area on the opposite side may be used to distinguish between benign and malignant lesions.

**<sup>75</sup>Se-methionine scan:** This may be used to distinguish between a benign 'cold' nodule and a malignant 'cold' nodule (as demonstrated in a conventional scan). However, the theoretical premise that an actively dividing highly cellular carcinoma will take up more <sup>75</sup>Se-labelled-methionine and show as a 'hot' spot is often belied by many well-differentiated carcinomas which are not sufficiently mitotically active to take up <sup>75</sup>Se-methionine.

**<sup>131</sup>I total body scan:** It is performed in thyroid cancer as a screening procedure for metastases, to detect their site(s) and to assess the functional status of such deposits. (1) *Screening:* TBS is performed after total thyroidectomy. Pre-operative TBS is useless because secondary deposits do not take up <sup>131</sup>I in the presence of functioning thyroid tissue. Routine screening is not necessary for well-differentiated tumours. (2) *Site(s):* If a solitary secondary is present local radiotherapy to the site (instead of systemic <sup>131</sup>I therapy) will be beneficial. All hepatic, pulmonary and skeletal metastases appear as 'hot' spots. Post-operative neck scan is essential; (a) some functioning thyroid tissue commonly remains even after total thyroidectomy; (b) determination of its size and location permits later differentiation from local recurrence, (c) if significant amount is present, it should be ablated prior to systemic therapy with <sup>131</sup>I. (3) *Status:* Post-operatively, the metastases may demonstrate sufficient function to take up <sup>131</sup>I. This is further enhanced by TSH administration 5 days before. Very rarely, a large mass of secondary may produce mild hyperthyroidism (functional autonomy). Scan is of predictive value; if the secondary shows good <sup>131</sup>I uptake, therapy with same is likely to be beneficial.

**Procedure:** It is performed several weeks after surgery. Ten units of TSH are administered 5 days before the day of scanning. Three days later 2 mCi of <sup>131</sup>I is given orally. This ablates any residual thyroid tissue in the neck and demonstrates any functioning metastases. On the day of TBS urinary <sup>131</sup>I is also measured. When functioning metastases are demonstrated, they are treated with therapeutic doses of <sup>131</sup>I.

**<sup>99m</sup>Tc PYP skeletal scan:** Invasive follicular cancer metastases to the bones (sternum, skull, humerus, spine, ilium, knee bones etc) appear as 'hot' spots in <sup>99m</sup>Tc stannous pyrophosphate(PYP) scan. More will be discussed under 'Bone scan' in the next issue.

### <sup>131</sup>I therapy

In cancer, <sup>131</sup>I ablates the whole tissue; in hyperthyroidism it reduces the functioning mass below a critical level.

**Metastases:** Anaplastic metastases do not respond to <sup>131</sup>I ablation. Only follicular carcinoma or follicular elements in papillary carcinoma accumulate sufficient quantities of <sup>131</sup>I. Solitary metastases may be locally irradiated but multiple deposits require <sup>131</sup>I systemically. All normal thyroid tissue must have been ablated by surgery and/or radio-ablation and the patient must be hypo-thyroid when the treatment is given. Following therapeutic dose (50-200 m Ci) of <sup>131</sup>I, T<sub>4</sub> (0.2-0.3 mg day<sup>-1</sup>) is started (for thyroxin replacement and for TSH suppression). One year later TSH is stimulated large doses of TRH (so as to maximise the stimulation of any residual functional tissue) and the <sup>131</sup>I scan is repeated. Re-ossification of the sites of deposits is looked for, as well as uptake of <sup>131</sup>I, if any. If present, a further therapeutic dose of <sup>131</sup>I is administered.

**Thyrotoxicosis:** (1) Radiation and  $^{131}\text{I}$  are avoided in; (a) *children* (interference with growth, subsequent development of papillary carcinoma); (b) *pregnancy* (intra-uterine growth retardation, chromosomal defects, mutations in the foetus); (c) *< 45 years of age* (delayed hypothyroidism) and; (d) *retrosternal goitre* with obstructive symptoms (goitre may enlarge and worsen the thoracic inlet obstruction). (2) Toxic nodular goitres do not respond well to  $^{131}\text{I}$ . (3) In elderly cardiac patients (surgery contraindicated),  $^{131}\text{I}$  is given first and anti-thyroid drugs are started 48 hours later and continued until  $^{131}\text{I}$  has had effect. (4) In case of failure hyperthyroidism to respond to  $^{131}\text{I}$ , radio-ablation of thyroid gland with same (and  $\text{T}_4$  replacement) is required.

The dose is 140-160  $\mu\text{Ci}$  per gram of estimated gland weight. For toxic nodule (hyper-functioning adenoma) this is usually 10 m Ci and for toxic multinodular goitre it works out to 20-30 m Ci. Due to a large proportion of sub-lethal cell damage, there is progressive *delayed hypothyroidism* whose incidence has been variously reported as 40 to 80% after 10 years. Some have advocated a dose of 80  $\mu\text{Ci}$  of  $^{131}\text{I}$  per gram of thyroid, but this just delays the onset of hypothyroidism. *Radiation thyroiditis* is an occasional complication which occurs within 7-10 days and is associated with excessive release of hormone into the blood.

### **Solitary nodule**

The problem of clinically solitary nodule (CSN) revolves around 3 questions. Is it truly solitary? Fifty percent prove to be otherwise on exploration. What is its activity status? Is it benign or malignant (the most important consideration)? See table 7.

**'Hot' nodule:** Isotope scan is useful if there is hyperthyroidism. Demonstration of a 'hot' nodule (autonomous toxic nodule, hyper-functioning adenoma) determines the mode of treatment (surgery /  $^{131}\text{I}$  therapy). The site of over-activity is a useful guide to the surgeon if excision is planned. On the other hand, such a lesion is never malignant; therefore conservative treatment may be justified (if anaesthetic risk is unusually high). Finally,  $^{131}\text{I}$  is the treatment of choice in patients > 45 years old. The suppressed surrounding thyroid tissue does not take up  $^{131}\text{I}$ ; so delayed hypothyroidism is unlikely. Due to all these considerations, thyroid scan is essential in autonomous toxic nodule.

**Malignancy:** Scan cannot diagnose malignancy with certainty. Various methods to improve the diagnostic accuracy of malignancy (TSG/ISG,  $^{99\text{m}}\text{Tc}$  photo-scanning techniques,  $^{241}\text{Am}$  and  $^{75}\text{Se}$ -methionine scans) have been described. Each has its own shortcomings.

**Drawbacks:** (1) Rectilinear scan misses lesions < 1 cm in size (due to intimate relation of nodule with surrounding normal tissue) and also deep-seated lesions (due to masking effect of contiguous overlapping normal tissue). Photo-scanning techniques partly overcome these problems. (2) A 'cold' nodule is benign in 75-90% of cases (viz. cyst, adenoma, focal thyroiditis) and malignant in 10-25% of cases. A large number of innocent swellings will thus be over-treated if operation is based purely on scan findings. (3) Five percent of 'warm' nodules (same uptake as surrounding normal tissue) are malignant (some well-differentiated carcinomas have normal or even increased uptake). If surgery is routinely avoided in all 'warm' nodules, these carcinomas will be missed. Given these drawbacks of scan coupled with the fact that scan findings do not influence the treatment of CSN (except autonomous toxic nodule), some workers have proposed that its routine use in all cases should be discontinued.<sup>3</sup>

### **Ectopic/aberrant thyroid**

The role of scan is best understood if it is studied in conjunction with a working classification of ectopic/aberrant thyroids (table 8). For ectopic thyroid, scan helps to; (a) establish the diagnosis (differential diagnosis of midline neck swellings); and (b) often demonstrates this to be the only functioning thyroid tissue. Thus treatment can be tailored accordingly. For mediastinal (aberrant) thyroid, scan establishes both the above-mentioned points besides demonstrating continuity (or otherwise) with the cervical gland. This helps in determining the operative approach. Not all aberrant thyroid tissue metabolises iodine, and lack of function on scan does not exclude the possibility of thyroid tissue within a mass.

## **Hepatic scan**

Radioisotope scan of the liver (table 9) demonstrates; (a) the liver size and shape; (b) focal lesions (tumours, cysts, abscesses etc); and (c) intra-hepatic lesions or extrinsic compression (viz. subphrenic abscess). Lesions greater than 2-3 cm are readily detected. The normal liver presents an even distribution of activity.

### **Colloid scan**

Radioactive colloid particles (viz.  $^{99\text{m}}\text{Tc}$  sulphur colloid) of 1  $\mu$  diameter are phagocytosed by the cells of the reticuloendothelial system (RES) i.e. Kupffer cells lining the hepatic sinusoids, macrophages in the splenic red pulp and mononuclear-phagocytic cells of the bone marrow. Normally the hepatic uptake predominates because of its greater bulk and concentration of Kupffer cells. The splenic image is smaller than the liver and the vertebral bodies are not seen in between the two. The uptake is uniform in both organs.

Focal liver lesions produce filling defects (negative scans; 'holes'; 'cold spots') and generalised diseases produce diffuse reduction in liver uptake. Any parenchymal liver disease may decrease activity of the hepatic RES cells and cause apparent defects in the liver image. Additionally, any disease causing significant destruction of the Kupffer cells, e.g. extensive metastases, causes decreased liver phagocytosis of the labelled colloid and increased phagocytosis by the spleen and marrow of the vertebral bodies. Then there is decreased tracer density in the liver, more prominent visualisation of the spleen (which is not necessarily enlarged) and visible vertebral bodies between the hepatic and splenic images.

### **Rose Bengal scan**

Unlike  $^{99m}\text{Tc}$  sulphur colloid (or any other colloid),  $^{131}\text{I}$ -Rose Bengal (RB) is taken up by the hepatocytes and excreted by them into the biliary system. Therefore the former is for hepato-splenic scanning and the latter is for hepato-biliary scanning. Since the hepatocytes and Kupffer cells are intimately related to each other in the hepatic lobular architecture and both are uniformly distributed in the liver, destruction of one is bound to affect the other, giving scan findings which are substantially similar in both the scans. Excretion of RB into the biliary system and its concentration in the gallbladder causes problems with delineation of the liver edge. RB has been used to differentiate between intra-hepatic cholestasis and complete extra-hepatic obstruction. In the latter condition there is failure of the isotope to enter the duodenum while in the former situation some radioactivity is seen in the small bowel lumen. However this differentiation not well defined and is of limited clinical utility.

### **Gallium scan**

$^{67}\text{Ga}$  Gallium (Ga) citrate is concentrated more in neoplastic and inflammatory cells than in hepatocytes. Thus a hepatoma or liver abscess appears as a 'cold spot' in the other scans and as a 'hot spot' in the  $^{67}\text{Ga}$  scan. In liver abscess  $^{67}\text{Ga}$  is picked up by the rim of inflammatory tissue around the abscess (not by the abscess contents), thus outlining the periphery of the lesion. This is also characteristic of a large neoplasm with central necrosis.  $^{67}\text{Ga}$  is useful in diagnosing malignancy with cirrhosis because the tumour shows increased uptake and the fibrous bands decreased uptake. By the other scans, decreased uptake by both fibrous bands and neoplasm may cause a malignancy to be missed (false-negative). Moreover, cirrhosis without malignancy may show irregular uptake and filling defect(s) in the other scans, due to regenerating nodules and distortion of lobular architecture, thus giving a false-positive diagnosis of neoplasm, which can be refuted by a  $^{67}\text{Ga}$  scan.

**Accuracy:** Isotope and CT scans are equally sensitive (95%; vs. 75% for USG). However lesions <2 cm are often falsely negative in isotope scan. The overall diagnostic accuracy is 74%, which can be improved to 93% by supplementing with USG and CT scans.

### **Scanning in hepatic disorders**

**Cirrhosis:** Isotope scan is more informative than CT scan or USG in this condition. The scan findings with  $^{99m}\text{Tc}$  sulphur colloid may be; (a) diffuse reduction in uptake; (b) irregular uptake; (c) 'cold spots' (giving a false-positive diagnosis of malignancy). When there is portal hypertension and splenomegaly the most characteristic features are patchy and decreased uptake in a shrunken liver and increased uptake in a large spleen and in the bone marrow. Malignancy in cirrhosis may be missed by a colloid scan, as described earlier.

**Malignancy:** Any malignancy, primary or secondary, appears as 'cold spot(s)' in  $^{99m}\text{Tc}$  colloid or  $^{131}\text{I}$ -RB scans and as 'hot spot(s)' in  $^{67}\text{Ga}$  scan. The last is the best for delineation of hepatoma in cirrhosis. When there is extensive destruction of Kupffer cells by multiple metastases, there are multiple filling defects in the liver and a proportionately greater uptake of labelled colloid (but not others) by the RES cells of the spleen and vertebral bone marrow. This typical finding is seen with colloid scan in any condition producing extensive destruction of RES cells of the liver.

For diagnosis of primary tumours CT scan is the most accurate, and the most reliable combination is CT scan and scintigraphy. The false-positive results with  $^{99m}\text{Tc}$  sulphur colloid (the agent of choice) is 26% (vs. 12% and 17% with CT scan and USG respectively).

**Abscess (pyogenic, amoebic):**  $^{99m}\text{Tc}$  sulphur colloid gives the most reliable results. Abscesses appear as 'cold' areas in colloid or  $^{131}\text{I}$ -RB scan. More than one may be present. A proper scan includes anterior, posterior, oblique and lateral views, to determine the exact location and extent of a 'cold' area within the liver substance. Lesions < 2 cm rarely appear on a scan. These scans do not distinguish between abscess, hepatoma or secondary deposit(s), but they are useful in confirming the diagnosis and particularly in identifying left lobe abscess, which is difficult to diagnose by other means.  $^{67}\text{Ga}$  scan shows 'hot' areas outlining the uptake by the inflammatory tissue itself. It is considered less useful than the colloid image and is thus not widely employed. It has been suggested that addition of  $^{67}\text{Ga}$  study of a liver abscess may provide additional information in assessing the size and resolution in response to treatment.

$^{111}\text{In}$  Indium-WBC (autologous) has been used to define their progression into areas of inflammation or abscess. But normal liver also accumulates such a label. So it may not be of much use in defining pyogenic abscess. *Labelled metronidazole* has been suggested as a possible agent for imaging amoebic abscess, by virtue of selective accumulation of the same in the abscess contents. This may provide a method of specifically identifying amoebic abscess and differentiate it from other intra-hepatic space-occupying lesions.

**Miscellaneous:** *Haemobilia* demonstrates a filling defect (cavity containing necrotic tissue, clots and bile) by  $^{99m}\text{Tc}$  colloid scan. Such a scan may be of use in diagnosing *liver wounds*, though scintiscan rarely yields information not provided by other modalities.  $^{131}\text{I}$ -RB (but not others) may be helpful in differentiating *intra-hepatic cholestasis* from complete extra-hepatic obstruction, as described earlier.

### **Hepatic blood flow**

In comparison to BSP or indocyanine green clearance studies, radioisotopes (table 9) provide a simpler method of estimating hepatic blood flow (HBF), especially in cirrhosis with portal hypertension.  $^{99m}\text{Tc}$  injected IV is removed mainly by the liver. Its disappearance rate from the peripheral blood is determined by; (a) external monitoring; and; (b) analysis of radioactivity in serial blood samples. This is then used to calculate the HBF. There are inherent sources of *error* in the technique which become magnified in the presence of liver disease. In marked hepatic damage uptake of  $^{99m}\text{Tc}$  is decreased irrespective of changes in HBF, and extra-hepatic removal (spleen etc) is increased, leading to unreliable results. A portacaval shunt further complicates the picture.

# Splenic scan

## Colloid scan

$^{99m}\text{Tc}$  sulphur colloid scan has been described in detail under hepatic scan, including the normal and some abnormal splenic findings in liver disorders (portal hypertension, hypersplenism, liver metastases etc). Colloid scan may show a sub-capsular haematoma in *splenic trauma*. *Splenic cysts* appear as splenomegaly and filling defect, but show no evidence of hypersplenism, in the form of decreased survival of RBC and increased splenic sequestration (infra).

## Labelled-cell scan

**Isotopes:** They are; (a) autologous RBC labelled with sodium( $^{51}\text{Cr}$ )chromate ( $\text{Na}_2^{51}\text{CrO}_4$ ) or diisopropylfluoro ( $^{32}\text{P}$ )phosphate (DIPFP); and (b) autologous platelets labelled with  $\text{Na}_2^{51}\text{CrO}_4$  or  $^{111}\text{In}$  or  $^{113m}\text{In}$  (table 10). These scans are used to determine; (a) the life span (or  $T_{1/2}$ ) of the blood cells; (b) the role of spleen in inappropriately sequestering and destroying the cells (i.e. to diagnose if there is hypersplenism); (c) if splenectomy is likely to be beneficial; and (d) to assess if there is compensatory increased haematopoiesis in the marrow in the face of excessive splenic destruction.

**Procedure:** Patient's own RBC are heated to  $50^\circ\text{C}$  for 1 hour (to render them spheroid and more vulnerable to splenic sequestration), mixed with radioactive solution (to label the RBC) and injected back into the circulation. Serial counting of radioactivity indicates the rate of disappearance of the cells from the circulation and simultaneous daily scan over liver, spleen and praecordium (for reference) shows the concentration of the labelled cells in these organs and the rate of splenic sequestration. The procedure is similar with platelets.

**Life span /  $T_{1/2}$ :** Following injection of labelled RBC into the circulation the percentage decrease in radioactivity is noted over successive days and is plotted in the Y-axis of a graph as a function of the number of days in the X-axis. A linear fall of radioactivity to 50% in 30 days indicates the normal  $T_{1/2}$  of RBC and disappearance of radioactivity in 120 days indicates the normal life span. A measured  $T_{1/2} < 20\text{-}25$  days is considered as accelerated rate of destruction. Similarly the life span of *platelets* (normal=8-10 days) has been found to be significantly reduced in patients with splenomegaly and idiopathic thrombocytopenic purpura (ITP). However attempts to demonstrate a decreased longevity of circulating WBC (normal=6-12 days) have not been clinically helpful.

**Splenic sequestration:** That the spleen is the culprit can be assessed by splenic scan with a columnated detector, after the labelled cells have been injected. A selective rise in spleen : praecordial ratio of radioactivity suggests significant splenic sequestration. Normally the graphs of spleen : praecordial and liver : praecordial ratios are evenly matched. Using  $^{51}\text{Cr}$ -RBC it has been shown that the spleen is the major site of haemolysis in pyruvate-kinase deficiency. Similarly, in ITP, infusion of  $^{51}\text{Cr}$ -platelets causes a rise of radioactivity predominantly in the spleen. (In hypersplenism up to 90% of the platelet pool may be sequestered in the spleen, as against 20-40% in the normal spleen). However the mechanism of leucopenia in hypersplenism is not clear because there is no satisfactory method of determining the relative importance of spleen in the destruction of circulating WBC. *Hyposplenism* (splenic atrophy), as may occur in sickle-cell infarcts, celiac disease etc, is documented by splenic scan and by decreased clearance of  $^{51}\text{Cr}$ -RBC.

**Splenectomy:** Using the above two techniques a satisfactory result from splenectomy may be predicted when; (a) the  $T_{1/2}$  of  $^{51}\text{Cr}$ -RBC is  $< 50\%$  of normal (i.e.  $< 15$  days); and (b) when the spleen : liver ratio of radioactivity is  $> 2 : 1$ . This prediction approaches 80-90% accuracy in cases of haemolytic anaemia while in other conditions (myeloid metaplasia, malignant lymphoma, leukaemia etc) the predictive value is variable.

**Marrow function:** In ITP the bone marrow often shows compensatory increase in immature megakaryocytes. This may be detected, at least on principle, by the use of surface scanning after injection of  $^{113m}\text{In}$ -platelets. This technique may be a helpful diagnostic aid in predicting the value of splenectomy. (Extramedullary haematopoiesis at times leads to the formation of paravertebral masses visible on chest x-ray).

*The subsequent issues will contain applications of radioisotopes in the remaining organ-systems of the body.*

## References

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<b>Table 1A</b>	
<b>Radio-iodine preparations and their uses</b>	
<b>Radio-iodine labelled preparations</b>	<b>Clinical applications</b>
<sup>125</sup> I-labelled antigen (peptides, glycoproteins, OFA, viral antigens etc)	Radioimmunoassay (RIA) of hormones and serum levels of CEA, AFP, POA, PSA, HbsAg etc
<sup>123</sup> I-, <sup>131</sup> I-MAB (monoclonal ab against colorectal, medullary thyroid cancers (MTC), malignant melanoma etc)	Radioimmunolocalisation and tomoscintigraphy of the corresponding tumours (see text)
<sup>131</sup> I-PAS (polyclonal antiserum against CEA, AFP, insulin, HCG etc)	Tumour localisation: Colorectal, MTC (anti-CEA); germ cell tumours of testis, ovary, hepatoma (anti-AFP); insulinoma (anti-insulin); choriocarcinoma (anti-HCG)
<sup>131</sup> I-ant-HMFG2 (human milk fat globule membrane)	Radioimmunotherapy of malignant pleural and pericardial effusions from ovarian cancer
<sup>125</sup> I- $\alpha$ bungarotoxin (snake venom)	RIA of acetylcholine receptor ab; diagnosis of myasthenia gravis
<sup>131</sup> I-labelled snake venom	Experimental: To show that absorption of snake venom is reduced by 70% after tourniquet application

*Note: Radio-iodine can be used to label a wide variety of substances.*

<b>Table 1B</b>	
<b>Radio-iodine preparations and their uses</b>	
<b>Radio-iodine preparations</b>	<b>Clinical applications</b>
<sup>123</sup> I	RAIU test in children, pregnancy and serial assessments of thyroid function (because of low radioactive dose)
<sup>132</sup> I	T <sub>3</sub> suppression test after 6 months of antithyroid drugs, to predict relapse
<sup>131</sup> I	1) Radioactive T <sub>3</sub> uptake, radio-iodine uptake (RAIU), T <sub>3</sub> suppression, TSH stimulation tests; PB <sup>131</sup> I, FT <sub>4</sub> , FT <sub>3</sub> estimations; resin T <sub>3</sub> uptake; 2) Thyroid, whole body scan; 3) therapy of toxic nodule, toxic nodular goitre, thyroid and skeletal metastases ablation
<sup>131</sup> I-Rose Bengal	Liver, biliary scan
Na( <sup>125,131</sup> I)diatrizoate (labelled Hypaque)	Total renal function tests, radioisotope renogram
<sup>125</sup> I-fibrinogen	Leg deep vein thrombosis
<sup>125,131</sup> I-colloidal human serum albumin	Liver, splenic scan, liver blood flow studies
<sup>131</sup> I-cholesterol derivatives	Adrenal scan

<b>Table 2</b>	
<b>Types of monoclonal antibody (MAB)</b>	
<b>MAB raised against</b>	<b>MAB active against</b>
Anti-melanoma MAB (NU 4 B)	Melanoma cells, astrocytoma (many cell types), foetal astrocytes
Anti-osteogenic sarcoma MAB (791 T/36)	Estrogenic sarcoma, colorectal cancer (primary, metastatic), ovarian, breast cancer
Anti-neural MAB	Neuroblastoma
Anti-human milk fat globule membrane (HMFG 2)	Breast cancer metastases in marrow and in serous effusions, ovarian cancer metastases in serous effusions
Anti-breast cancer MAB	Breast cancer cells (prognostic indicator)
Anti-haemoglobin MAB	Occult bleeding per rectum in colorectal cancer (early detection)
Anti-CEA MAB	Colorectal, medullary thyroid cancer
F(ab <sup>1</sup> ) <sub>2</sub> fragments of MAB	Colorectal cancer, malignant melanoma

*Note: Any of these MAB may be radio-labelled and used to image the corresponding tumour or to treat it.*

<b>Table 3</b>			
<b>Comparison of 3 isotopes in ISG</b>			
	<sup>131</sup> Iodine	<sup>123</sup> Iodine	<sup>111</sup> Indium
Gamma emission	High energy	Low energy	Suitable range
Camera performance	Low efficiency of gamma camera detection	Efficient detection by gamma camera	Efficient detection
Image interference	Masking by thyroid uptake and urinary excretion	Masking by thyroid and bladder excretion	
Half life	8 days	13 hours	2.8 days
Labelling	Easily attached as radio-label	Easily attached	Difficult to attach to MAB; chelating agent required
Image	Positive image; radio-label attached to tumour	Positive image	Negative image; taken up by normal liver, metastases appear as filling defects
Suitability	Not ideal	Limited use in clinical work	Ideal for ISG and TSG

<b>Table 4</b>	
<b>Immunoscintigraphy (ISG) with labelled polyclonal antiserum (PAS)</b>	
<sup>131</sup> I-labelled antibody (in PAS)	Tumour localisation
Anti-CEA (carcinoembryonic ag)	Colorectal cancer-primary, secondary (84%), recurrent (77%); medullary thyroid cancer
Anti-AFP ( $\alpha$ -fetoprotein)	Germ cell tumours of testis, ovary; hepatoma
Anti-insulin	Insulinoma
Anti-HCG (human chorionic gonadotrophin)	choriocarcinoma

<b>Table 5</b>		
<b>Comparison of <sup>131</sup>I ISG and <sup>99m</sup>Tc scan in liver metastases</b>		
	<sup>131</sup> I-labelled MAB ISG	<sup>99m</sup> Tc sulphur colloid scan
Smallest metastasis detected	1 cm	2 cm
Accuracy	85%	63%
Nature of image	Positive image (tumour localised)	Negative image (filling defect)

<b>Table 6</b>		
<b>Isotopes used in thyroid studies and immunology</b>		
Thyroid	<sup>123</sup> I, <sup>132</sup> I	Function tests (certain occasions)
	<sup>131</sup> I	Function tests, scan therapy
	<sup>99m</sup> Tc-pertechnetate	Scan, photoscan techniques
	<sup>241</sup> Americium	Fluorescent scan
	<sup>75</sup> Se-methionine	Tumour scan
	<sup>99m</sup> Tc-PYP	Skeletal scan
Radioimmunology	<sup>125</sup> I-antigen	Radioimmunoassay
	<sup>123</sup> I-, <sup>131</sup> I-, <sup>111</sup> In-labelled ab	Tumour imaging
	<sup>99m</sup> Tc-RBC	Blood pool labelling
	<sup>67</sup> Ga citrate	Paired labelling (with <sup>111</sup> In)

<b>Table 7</b>	
<b>Scan findings in thyroid pathology</b>	
<b>Thyroid pathology</b>	<b><sup>131</sup>I or <sup>99m</sup>Tc scan</b>
Cyst	Non-functioning ('cold') nodule
Adenoma	Non-functioning ('cold') nodule
Carcinoma (follicular, papillary, undifferentiated)	Non-functioning ('cold') nodule
Well differentiated carcinoma (occasionally)	Normal functioning ('warm') nodule
Toxic nodule (hyper-functioning adenoma)	Hyper-functioning ('hot') nodule, complete non-uptake by surrounding thyroid due to suppression; TRH stimulation – uptake by suppressed tissue (confirms functional status)
Multinodular goitre	Heterogeneous uptake, thyromegaly, hyperfunctioning areas (+), contiguous retrosternal extension (+)
Adenomatous goitre	Hypo-/ non-functioning
de Quervain's thyroiditis	Hypo-/ non-functioning
Hashimoto's thyroiditis	Heterogeneous (patchy) uptake, some areas devoid of <sup>131</sup> I
Riedel's thyroiditis	No uptake

<b>Table 8</b>		
<b>Thyroid in abnormal location</b>		
	<b>Ectopic</b> (in path of descent; along course of thyroglossal tract)	<b>Aberrant</b> (not along path of descent)
Site	-Lingual -Cervical (midline thyroglossal)	Intra-thoracic: -Anterior mediastinum } substernal or -Superior mediastinum } plunging -Posterior mediastinum (rare)
Origin	Developmental	Acquired (first 2); developmental (last)
Relation to neck thyroid	May be only thyroid present	First 2 continuous with neck thyroid (common blood supply); last one separate from neck thyroid

*Note: Struma is not ectopic thyroid. It is teratoma of ovary with thyroid tissue in it.*

<b>Table 9</b>	
<b>Radioisotopes in liver studies</b>	
<b>Study</b>	<b>Isotope/ radio-pharmaceutical</b>
Colloidal scan	<sup>198</sup> Au colloidal gold
	<sup>125, 131</sup> I colloidal human serum albumin
	<sup>99m</sup> Tc sulphur colloid (most common)
Rose Bengal scan	<sup>131</sup> I Rose Bengal
Gallium liver scan	<sup>67</sup> Ga Gallium citrate
Miscellaneous scan	<sup>99</sup> Mo Ammonium molybdate
	<sup>111</sup> Indium-WBC (pyogenic abscess)
	Labelled metronidazole (amoebic)
Liver blood flow	Same as for colloid liver scan
	<sup>32</sup> P chromic phosphate

<b>Table 10</b>	
<b>Isotope preparations for splenic scan</b>	
Colloid scan	<sup>99m</sup> Tc sulphur colloid (same as for liver scan)
Labelled cell scan	
	-RBC
-Platelet	Sodium( <sup>51</sup> Cr)chromate
	<sup>111, 113m</sup> Indium (In)