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Review article

The development of new radiopharmaceuticals*

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Abstract. The development of new radiopharmaceuticals is the basis of the continuing growth of nuclear medicine. Chemical interactions of electron clouds in their three-dimensional conformations bring together, in the process of molecular recognition, the reaction of antibody and antigen, receptor and ligand, enzyme and substrate, hormone and response site. This convergence enables the computer design of molecules such as ligands to fit computer-displayed conformational models showing active centres, positive and negative charges and other interactions. Indeed, given a particular molecule, a complementary binding structure can be devised. The hybridoma approach to monoclonal antibody production is being superceded by the bacterial bioengineer. The gene for the hypervariable region from the spleen cells of immunized mouse can be coupled with the myeloma gene. The polymerase chain reaction can duplicate the DNA a million times over in 20 min and the result transfected into a bacterial plasmid to produce the antibody. These scientific problems are soluble in principle and are being solved. However, so much damage to this developing biological field is being done by regulatory authorities that one must ask who should or can regulate the regulators. These problems have to be overcome in order to provide the new radiopharmaceuticals that are the food and wine of nuclear medicine.

Key words: Radiopharmaceuticals – Molecular recognition – Genetic engineering – Heart – Monoclonal antibody

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The new radiopharmaceutical is the life-blood of nuclear medicine. If you look back over the past 6 years the gamma cameras have hardly changed and yet nuclear medicine has progressed in an almost unrecognizable way. The question is whether the development of new radiopharmaceuticals is, in fact, a game of chance with some individual or some company winning the game and drawing an ace, or whether it is a scientific discipline cleverly worked out with each stage identified to give a designer molecule.

In this context, I wish to consider the development of new radiopharmaceuticals under several headings: the physics approach; the chemical approach; the physiological approach; the pharmacophore and the active analogue approach; computer-aided radiopharmaceutical design; receptor-binding ligands; genetic engineering of antibodies and labelling sites; the polymerase chain reaction; the clinical approach (Table 1).

The physics approach

As an example of the physics approach, Brookhaven decided to produce some thallium Tl 201. They put it into a few guinea-pigs and found that it went to the heart (Bradley-Moore et al. 1975). It was then rationalized that this was because its ionic radius was similar to potassium. The development of a new radiopharmaceutical depended on its interest to physicists, the range of gamma rays suitable for the imaging equipment and

Table 1. The development of new radiopharmaceuticals

The physics approach
The chemical approach
The pharmacophore and the active analogue
Computer-aided radiopharmaceutical design
Receptor-binding ligands
Genetic engineering of 'antibodies' and labelling sites
The polymerase chain reaction
The clinical approach

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the combinations of parent and daughter suitable for local generators. Nuclear medicine has greatly benefitted from the physics approach, as you have heard from Professor Tubiana (1989), with ^{131}I and ^{123}I, and the generator systems for technetium Tc 99m, Gold 195 m, gallium Ga 68 and so on. The physics approach will come to the fore later in this presentation.

Recent developments

I will review briefly, through the joint European Nuclear Medicine congresses of the past 6 years, some of the new chemistry that has happened. In 1984 in Finland there were I-123MIBG (Horne et al. 1984, 1985), the I-123 fatty acids (Notohamiprodjo et al. 1984), and the I-123 monoclonal antibodies (Granowska et al. 1984a, b).

In 1985 in London, ^{99m}Tc-HMPAO dominated. Ell et al. (1985a, b) showed beautiful images of the distribution of ^{99m}Tc-HMPAO in the brain. There the first introduction of a ^{99m}Tc cardiac agent occurred (Khalil et al. 1985) and the new ^{99m}Tc Technegas (Burch et al. 1985). The ^{99m}Tc anti-melanoma antibodies (Buraggi et al. 1985; Siccardi et al. 1986), the new generation of ^{99m}Tc-HIDA compounds (Evans et al. 1985), and the first of the ^{123}I-labelled hormones – insulin (Ng Tang Fui et al. 1985) – also came onto the scene.

In 1986 in Goslar, Rigo et al. (1986) described the first of the ^{99m}Tc-MIBI studies. Figure 1 shows a typical study with technetium MIBI, which shows the distribution of uptake in normal cardiac muscle and the areas of deficient uptake so beautifully and so much better than thallium 201. There were new antibodies against white cells (Danpure et al. 1986; Seybold 1988) and against myosin (Sochor et al. 1986; Khaw et al. 1987), a new use of ^{99m}Tc-DMSA (Clarke et al. 1986); the first use of ^{123}I-labelled body proteins, the low density lipoproteins from Sinzinger et al. (1986), and ^{123}I-MIBG was investigated in cardiac denervation studies by Wellman et al. (1986). Figure 2 shows the uptake of ^{111}In-anti-myosin in a recent myocardial infarct.

In 1987 in Budapest, ^{99m}Tc-MAG3 was the highlight (Jafri et al. 1987, 1988; Al-Nahhas et al. 1988). Figure 3 shows how much the background has been reduced by the use of a proximal tubular secreted technetium agent MAG3, as compared to ^{99m}Tc-DTPA. Technetium HMPAO white-cell labelling was introduced (Peters et al. 1987; Lavender et al. 1987). A normal ^{99m}Tc-HMPAO white cell scan shows a lack of lung uptake and the high spleen uptake. In Fig. 4 the defect in the liver colloid scan shows focal uptake on the white cell scan indicating the sites of active infection. The technique has been simplified by Solanki et al. (1988). There were a number of other compounds, including ^{111}In-labelled monoclonal antibodies (Granowska et al. 1987, 1989a). Figure 5 shows the uptake of indium-labelled anti-CEA in the primary rectal cancer in the pelvis.

In 1988 in Milan, the highlight was technetium-labelled anti-CEA from Baum et al. (1988, 1989). There were also labelled brain receptor agents for single photon tomography (Kung et al. 1989) and yttrium monoclonal antibody therapy (Hnatowich et al. 1985, 1988).

In 1989 in Strasbourg there was the detection of amyloid, using ^{123}I-serum-amyloid protein (Hawkins et al. 1989), technetium MIBI for parathyroid imaging introduced by Coakley et al. (1988; Fig. 6), and ^{99m}Tc-labelled monoclonal antibodies for many purposes (Baum et al. 1989; Granowska et al. 1989b, 1990a, b). There were also rhenium substitution for technetium (Maxon et al. 1989; Hanelin et al. 1989), strontium 89 (Blake et al. 1987), and samarium 153 (Boniface et al. 1989) for the therapy of bone metastases.

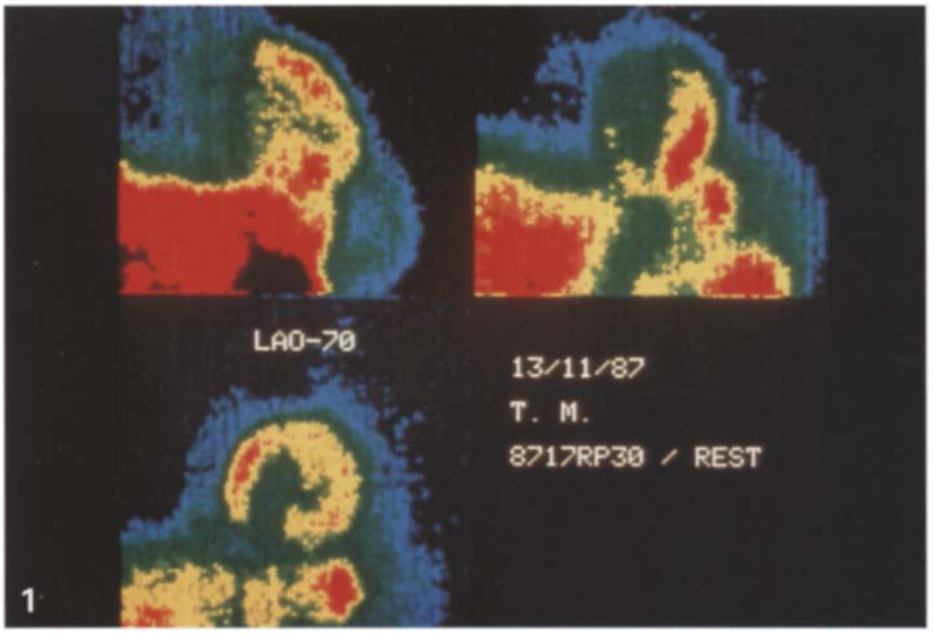
This brief survey has moved from the past to the present, identifying some of the recently developed new radiopharmaceuticals.

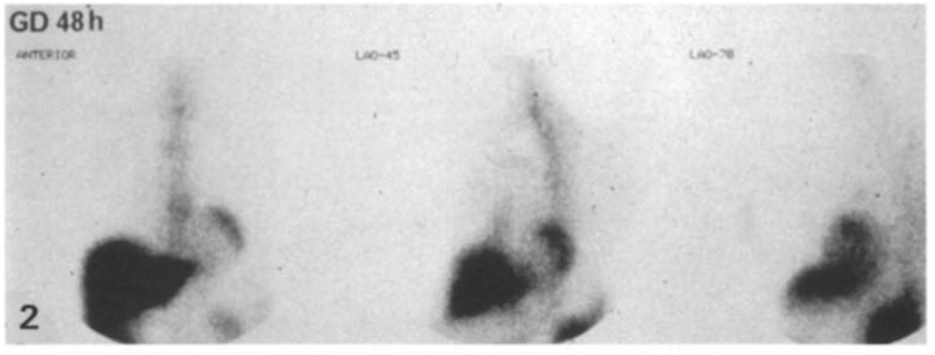
The chemical approach

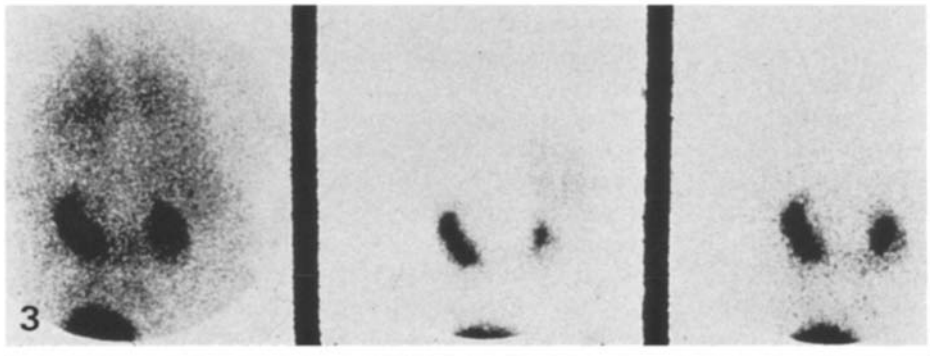
I will take the heart as an example of the chemical approach. It is concerned with structure-activity relationships and chemical specificity to enable the development of designer molecules. The chemists are interested in a number of features of each chemical compound: its electrostatic charge; whether it is an electron donor or receiver; the lipophilicity; the redox potential; the pH; the chirality, which is whether it is bent to the left or to the right; the species of technetium; chelation and bond-stretching. The theory was that if the technetium could not be reduced once the compound got into the heart, it would stay in the heart, whereas if it was reducible, then it would be a poor cardiac agent (Deutsch et al. 1981). Deutsch's theory dominated the chemists approach the seeking a technetium cardiac agent (Gerundini and Maffioli 1989).

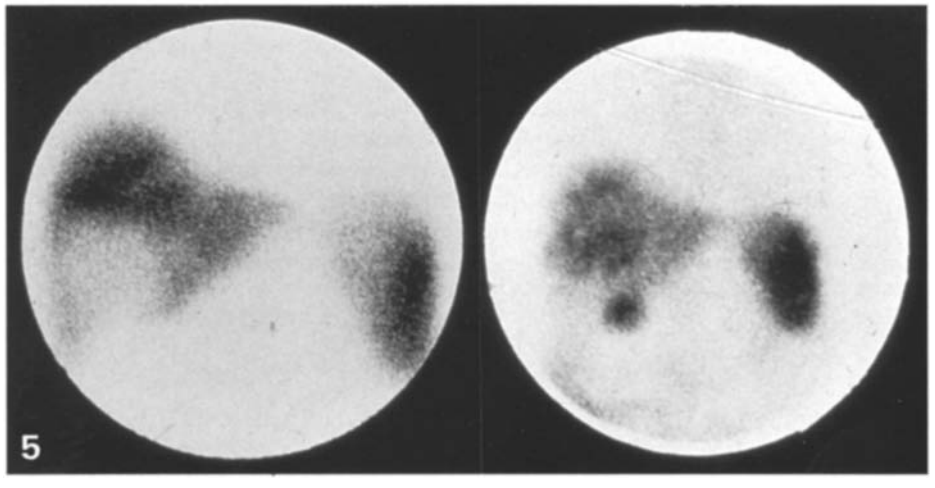
The Dupont solution to cardiac imaging was to take this to its logical conclusion and use technetium in its ground state, which could then not be reduced. They developed compounds for this: the iso-nitriles such as methoxy isobutyl isonitrile, MIBI (Baillet et al. 1989). However, its success was not due to the fact that it contained technetium in its ground state; it was a lucky accident, the play of the cards, that there was a protein in the cytosol that bound MIBI and gave it its particular properties as an excellent fixed cardiac agent (Mousa et al. 1987).

The Mallinkrodt approach was to investigate a compound called Sal3TAME, chosen by the chemists because such ligands allow considerable adjustment of molecular parameters. This means that the chemist can play with the molecule, which is what a chemist likes to do, so that one can alter its properties such as the redox potential, lipophilicity and so on. The way that it was decided that this compound had cardiac uptake was through the concept of a window of lipophilicity. If the









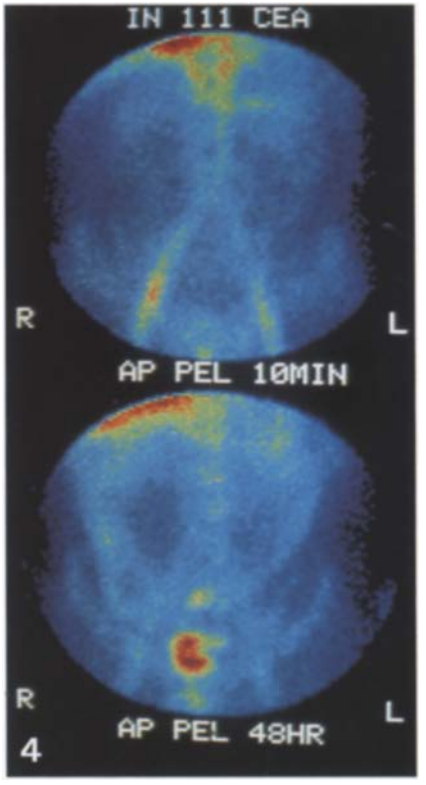


Fig. 1. ^{99m}Tc-MIBI image of the heart at rest at 1 h. Anterior, LA045, LA070. Loss of uptake in the septum and the inferior wall due to previous myocardial infarction is evident. Liver uptake is noted but does not interfere with the cardiac image in the sitting position

Fig. 2. ^{111}In-antimyosin image of the heart at 48 h. Anterior, LA045, LA070. Focal uptake in the free wall of the left ventricle at the site of recent myocardial infarction. Liver, marrow, and splenic uptake is also noted

Fig. 3. Horseshoe kidney, posterior views at 15 min: left 400 MBq ^{99m}Tc-DTPA, centre 100 MBq ^{99m}Tc-MAG3, right 100 MBq ^{123}I-orthoiodohippurate. The high background with DTPA contrasts with the low background of MAG3

Fig. 4. Liver abscess: left, ^{99m}Tc-colloid scan showing a large focal defect; right, ^{99m}Tc-HMPAO leucocyte scan showing focal areas of increased uptake at sites related to the focal defect. The normal high splenic and low lung activity is evident

Fig. 5. ^{111}In-anti-CEA monoclonal antibody radioimmunoscintigraphy anterior views of the pelvis at 10 min and 48 h. Note the specific uptake in the rectal adenocarcinoma at 48 h and in adjacent (uninvolved) lymph nodes. Vascular, marrow, large-bowel and high-liver uptake is noted

compound took 6–9 min to move to a chosen point on the chromatogram, it had the right sort of lipophilicity to get into the heart (Nosco et al. 1989). This very chemical approach indicated the likelihood of maxium heart and minimal liver uptake. It was also shown that an electron donor in the five-position gave much longer cardiac retention, whereas in the six position with an electron remover, there would be less cardiac retention. Using this chemical approach, the compound was then labelled with ^{99m}Tc and in the dog the uptake of this compound in the heart was better than that of MIBI from Dupont (Nosco et al. 1989).

The Amersham approach was to go back to the idea of Deutsch et al. (1981): that preventing the reduction of technetium would give cardiac retention. What excites a chemist is to have a bond longer than anybody else has. In one particular compound of DMPE, synthesized at Amersham, the technetium V – nitrogen bond is all of 0.2 Å longer than anyone has ever discovered before (Dilworth et al. 1989). The trouble is that this elongated bond allows easy reduction, so this particular compound was of no use. DMPE was used as a basis of a series of over 150 different derivatives to obtain a molecule that went into the heart and stayed in the heart (Kelly et al. 1989). This occurred with the ether derivatives known as PL37 and P53 that have been successfully used in man by Lahiri et al. (1989). Thus, there is now a new Amersham agent for the heart. This, then, is the chemical approach to the development of new radiopharmaceuticals, which uses chemical criteria to decide the direction of the research.

The physiological approach

Next, the physiological approach says that the study of man is man, but can a simple physiological model system be found to mimic this? The usual approach is to go from the guinea-pig to the minipig to man, but the Noah's Ark phenomenon is well known: that so many compounds have been successful in animals but have failed the last hurdle of man. In the antibody world a sort of murine immunological Folies Bergère is used. Not another nude mouse study please, for it is an inappropriate model for man! In vitro cell binding of antibody does not necessarily mean that there will be in vivo binding because of the phenomenon of biological barriers preventing access. Nevertheless, simple physiological model systems provide an alternative to the chemical approach. One very good example is given by the development of HMPAO.

How does a compound enter a tissue? There are a number of ways: by diffusion, by lipophilicity, by active transport, by carrier, by receptor or antigen binding. There are a number of ways in which it can stay in: by jamming enzymes such as selenocholesterol in the adrenal, by enzyme catalysis, by protein binding and by metabolism and depositing the radionuclide at the site, such as metals in the proximal tubules of the kidney. The HMPAO story is worth looking at from this point of view because Neirinckx at Amersham created a physiological model, which was very simple and allowed him to select, from thousands of different HMPAO-like compounds, those most likely to go to the brain and stay there. He had great fun in persuading us that this was all related to glutathione binding (Neirinckx et al. 1987), which it is not. But it was a good cover story and he believed in it and so did many others. In fact, as Costa et al. (1989) have demonstrated, it is a protein in the cytoplasm that is binding this HMPAO and has nothing to do with glutathione, as El-Shirbiny et al. (1989) and Powell et al. (1989) have shown.

Another way of binding compounds in the brain are through specific receptors. A whole range of ^{123}I compounds are now being developed to bind cerebral receptors and are waiting for clinical applications (Kung et al. 1989). Whereas the chemical approach appears limited to defining chemical criteria for cell entry, the physiological approach appraises both entry and residence requirements.

The active analogue

Let us consider next the pharmacophore and active analogue approach (Marshall 1984) and the computed-aided radiopharmaceutical design. The concept of a pharmacophore is to look at features common to a set of drugs or compounds binding to and acting on the same receptor, drugs which are responsible for recognition and transduction of the appropriate response. The traditional chemical appraisal is to compare their two-dimensional chemical structures. Take, for example, gamma amino butyric acid (gaba) 1, gamma amino butyric acid 2, and picrotoxin, a drug which acts on the gaba-binding neuroreceptor (Glen 1984); see Fig. 7. With this sort of widely accepted chemical approach, the likelihood of receptor binding is based on the fact that the arrangements of molecules look similar.

The active analogue approach states that one needs to vary the shape of the ligand to test the shape of the receptor. In this way, hopefully the common variables that lead to recognition and binding can be separated from the variables that lead to activation. This is required because one does not want to destroy the system or activate the system while it is actually being imaged. The pharmacophore approach emphasizes the minimal volume requirements of the particular compounds in order to fit the particular shape of the receptor-binding site. This leads to the consideration of the process of molecular recognition.

Molecular recognition

Molecular recognition is the basis of biological interaction: substrate with its enzyme, a hormone with its re-

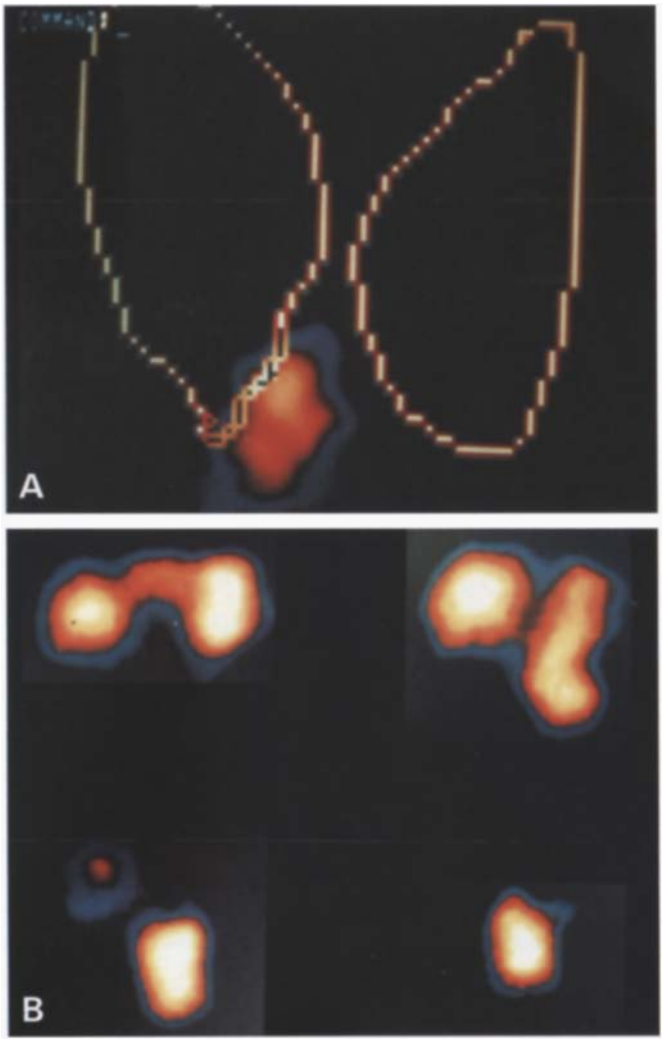


Fig. 6A, B. Parathyroid adenoma. **A** ^{99m}Tc-MIBI/^{99m}Tc-pertechnetate scan showing site of adenoma inferior and medial to the right lobe of the thyroid. **B** Transverse tomography of above with descending sections: *top* anterior; *bottom* posterior. *Left top*, mid-thyroid section; *right top*, posteriorly placed parathyroid adenoma on the right with the thyroid anterior; *left and right bottom*, parathyroid adenoma. The posterior inferior location of the parathyroid adenoma is highlighted by tomography for which ^{99m}Tc-MIBI provides the high count rate

ceptor, the drug with its metabolic site and the antigen with its antibody. It may be demonstrated that three dimensionally an enzyme acting on its substrate and an antibody acting with its antigen are very similar. Indeed, the interaction between all these groups pairs is by the same sort of mechanism (Lerner and Tramontano 1988).

What is the mechanism? Here one has to enter into the quantum mechanics of molecular design. To you and me, a molecule is given by a chemical formula or a stick and ball model (Fig. 7). However, to a receptor or an enzyme or an antibody, a molecule is a blob of electron density with atomic nuclei only as a positive centre to hold a variable cloud of negative charge together, and so our simple chemistry has become a different sort of chemistry with a chemical ectoplasm, a persona around the molecule that is what actually matters. Therefore, when one compares molecules, one can no longer compare the shapes of chemical formulae as described above, but one really has to compare molecules through the similarity of their electron density clouds (Fig. 8).

What is quantum mechanics? The chemical and physical properties of molecules are strongly dependent upon their electronic structure. The techniques of quantum mechanics can measure and calculate the distribution of electrons, the geometrical relationships, the energies of the molecules, the electrostatic potential and their thermodynamic properties. The Schroedinger equation is used, which relates the energy, E, for a molecule in a specified nuclear geometry and the wave form: $H\psi = E\psi$. From the wave function, ψ, the electron density map, H, and electrostatic fields can be calculated (Richards 1989).

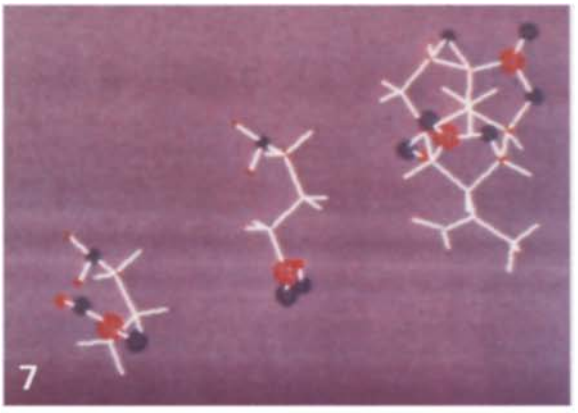
Computer-aided design

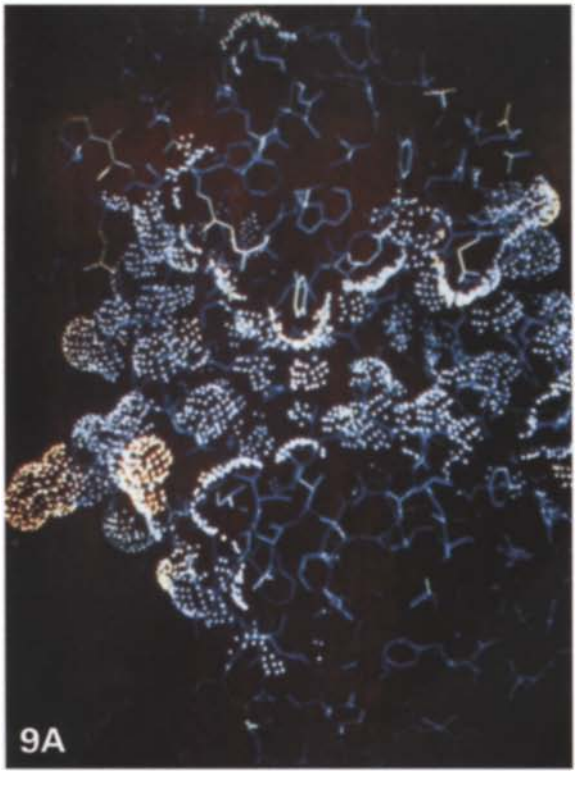
This approach requires computer-aided design (Blundell 1984). An example is a molecule of the enzyme Renin. The active centre is seen as a sort of cleft between cloudy mountains. Blue indicates hydrogen proton attracting areas and red the hydrogen-proton-repelling areas in the active centre of the enzyme (Fig. 9A).

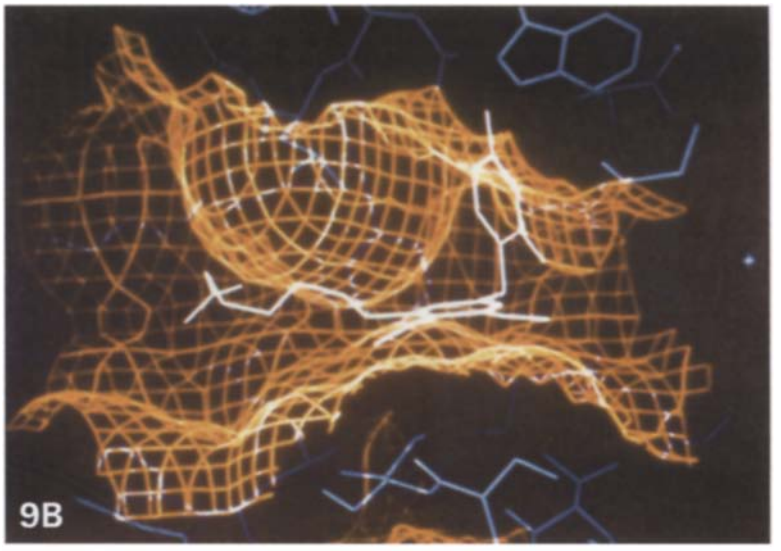
Thus, it can be appreciated that recognition mechanisms depend on the three-dimensional arrangement of electron density in the substrate or ligand molecule, the three-dimensional conformation of the receptor, and their surface interactions. Consider the active site of the enzyme hydrofolic reductase, which is responsible for part of the integrity of cell walls and against which trimethoprin, the antibiotic and methotrexate, the anticancer agent, act (Glen 1984; Fig. 9B). See how the ligand fits into position and where there are possible spaces or other combining sites. This is how new anticancer molecules are designed now.

Receptor-binding ligands

Furthermore, having obtained a design of a molecule in three dimensions, one can ask the computer to create its receptor, the antithesis of that molecule. The computer produces a molecular net in the shape of the complimentary structure that would act as a receptor for the particular molecule. This receptor characterization allows the synthesis of analogue molecules to bind to the receptor. For example, one can take insulin and create its receptor in three dimensions and then test a three-dimensional set of insulin-like molecules to see if they will fit into the conformation of that receptor, not as chemical formulae, but as patterns of interactions of electron clouds (Fig. 10).







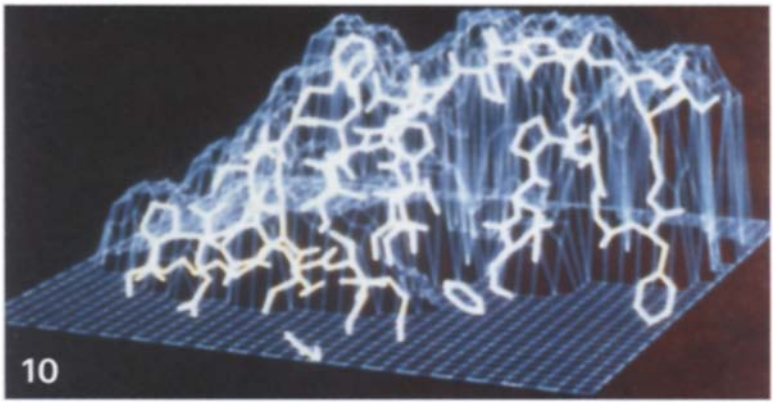


Fig. 7. Chemical stick and ball models: *left* gaba 1; *centre* gaba 2; *right* picrotoxin. They indicate the traditional analogue approach (published with permission of IBC)

Fig. 8. Chemical ectoplasm, the electron cloud around a small molecule

Fig. 9. A Computer-aided electron cloud representation of the active centre of the enzyme renin: blue hydrogen proton-attracting areas and red hydrogen proton-repelling areas (published with permission of IBC). **B** Computer-aided electron cloud representation of the active centre of the enzyme dihydrofolate reductase with its ligand (published with permission of IBC)

Fig. 10. Computer-aided synthesis of the complementary receptor in blue to the complex ligand (insulin-like growth factor) in white (published with permission of IBC)

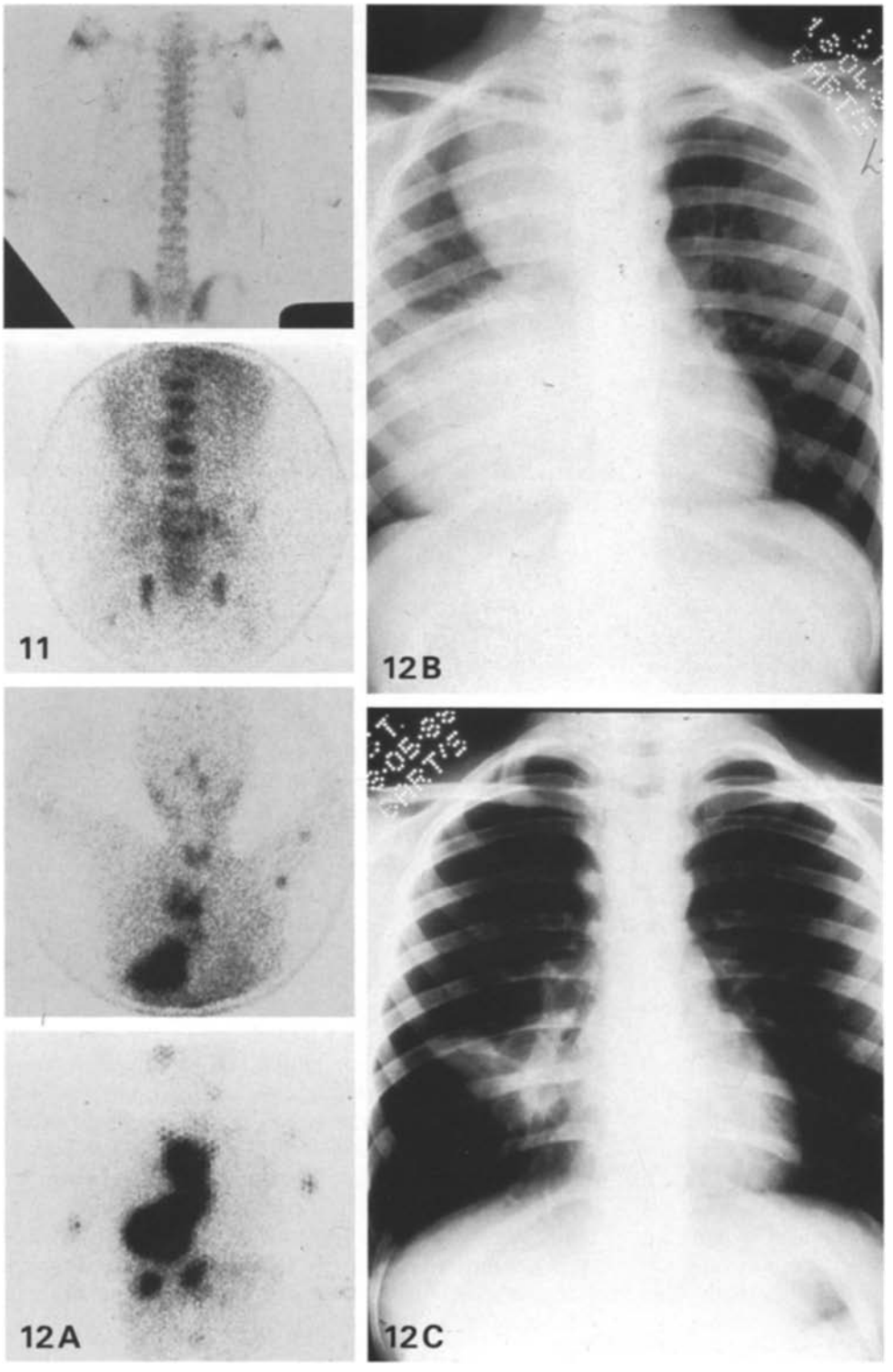


Fig. 11. Neuroblastoma in a child. *Top:* ^{99m}Tc-methylene diphosphorate bone scan is almost normal; *bottom:* ^{123}I-MIBG scan shows focally increased uptake in multiple vertebrate due to marrow involvement with neuroblastoma

Fig. 12A–C. Neuroblastoma in a child. **A** Intense mediastinal and left chest uptake of ^{123}I-MIBG; **B** chest X-ray before ^{131}I-MIBG therapy; **C** Chest X-ray after ^{131}I-MIBG therapy (3 GBq) showing the reduction of the tumour mass

In summary, one can synthesize the ligands and one can then synthesize the receptor. One can test the structure activity relationships, the rotational barriers, the conformation volume, and the interactions. This gives a new three-dimensional approach to the chemistry of receptor interactions between molecules large and small. This is summarized in a text by Richards (1989).

On more familiar territory, the various receptor-binding ligands in practice include ^{123}I-MIBG, Tc 99m (V) DMSA, F18 Oestradiol and a new set of I-123-labelled hormones, of which somatostatin is the most exciting. The power of these agents as radiopharmaceuticals for imaging and therapy is illustrated by ^{123}I-MIBG in neuroblastoma. Figure 11 shows on the left at the top a normal bone scan, and yet the patient is full of neuroblastoma, demonstrated by the bone marrow up-

take of MIBG. Figure 12A shows a massive tumour in the mediastinum and lung, both with MIBG and on the chest X-ray. Figure 12B is before MIBG therapy and Fig. 12C is after MIBG therapy in childhood neuroblastoma, showing that it is able to provide sometimes complete, but many times partial remission in this disease.

Octreotide is a somatostatin analogue and its active centre contains the sequence phenylanaline, tryptophan, lysine. Changing the phenylanaline to tyrosine gives I-123 tyrosine 3 octreotide, which is also able to bind somatostatin receptor sites. Pancreatic cancer and its metastases may be demonstrated (Krenning et al. 1989). It has also been used for carcinoid and small cell lung cancer. Indeed there is a whole range of tumours with somatostatin receptors. Those of particular excitement for the endocrinologists are the insulinomas, gastrinomas, vipomas, glomus tumours and medullary carcinoma of the thyroid.

These new receptor agents and there are many others coming from tumours, such as bombesin and all sorts of possible analogues, give a combination of optimism and pessimism, for, provided that the receptor is present, the abnormality or the tumour will be detected and radioreceptor therapy could follow. However, if it does not have the receptor, the image will be negative. The concepts of false-positives and false-negatives are really not applicable to this receptor type of situation.

Genetic engineering

The next development to consider is the genetic engineering of antibodies and labelling sites. Any company that is not into genetic engineering and still calls itself a major radiopharmaceutical company is now lost. Monoclonal antibodies are produced by the hybridoma technique, and this important, but tedious undertaking is about to become outdated. The old-fashioned view of a gamma globulin was that the antigen was the lock and the antibody the key. Three-dimensionally, in fact, the antibody is female with the hypervariable centres forming a cleft, whereas the antigen is male, and its epitopes stick out from the surface. On some occasions one is looking for promiscuous antibodies with high sensitivity and on other occasions rather virginal antibodies with high specificity.

Now monoclonal antibodies may be labelled with technetium and the Schwarz and Steinstraesser technique (1987), developed at Behring is the way to go. 2-Mercapto ethanol is added in a 1000:1 molar ratio to the antibody to open its sulphur-sulphur bonds, and the product is then frozen. To label this antibody 'kit', a normal bone-scanning agent such as methylene diphosphonate is added to provide the tin and to provide a weak chelator of technetium to prevent non-specific binding to the protein (Mather and Ellison 1990). ^{99m}Tc is added and binds through SH bonds to give a technetium-labelled antibody that is totally stable in vivo in the sense that there is no uptake of free pertechnetate by the thyroid over 24 h (Baum et al. 1989; Granowska et al. 1989b, 1990a). This new approach enables the application of the philosophy that we have spoken of for many years (Britton et al. 1985; Britton and Granowska 1987): "the shorter the half-life, the greater the administered activity, the higher the count rate, the less the noise from the signal, the earlier the detection and the smaller the lesion detected."

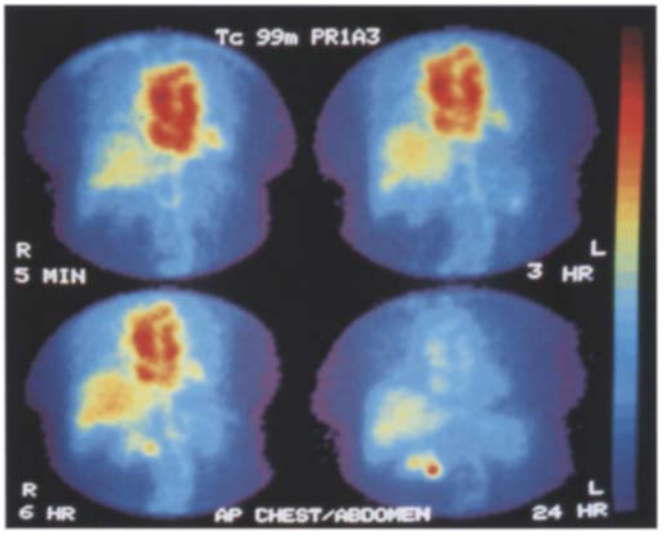


Fig. 13. Radioimmunoscintigraphy with ^{99m}Tc-PR1A3 monoclonal antibody against a columnar epithelial antigen at 5 min, 3, 6 and 22 h as illustrated: anterior views of chest and abdomen in a patient with transverse colon adenocarcinoma. The tumour uptake is evident at 6 and 22 h. The cardiac blood pool activity fades with time and the final liver uptake is less than that in the tumour at 24 h (courtesy of Dr. Granowska)

Figure 13 shows a series from Granowska et al. (1990a) of ^{99m}Tc-labelled antibody PR1A3 against colon cancer at 5 min, at 3 h, at 6 h and at 24 h. At 24 h, the uptake in the transverse colon tumour is much greater than that in the liver and it is easily visible at 6 hours. Analysis of the surgical specimen gave a tumour to mucosa ratio of 63:1. This is a successful technique. As ^{99m}Tc is available to any routine nuclear medicine department and as this method of labelling antibodies is robust, a request for a labelled antibody study can be accepted, fulfilled and the result given out within 24 h.

What about the hybridoma? It may now be replaced by the bacterial bioengineer. In principle and now in practice it is only necessary to isolate the hypervariable genes from the spelen cells of the immunized mouse, add the myeloma gene, transfect the coupled gene and grow it in a bacterial plasmid and the bacteria produces the specified antibody for use (Riechmann et al. 1988; Rodwell 1989). Not only that, however, the gene can be manipulated and this wonderful new machine, the polymerase chain reaction (PCR) can give cell-culture-

free synthesis of genomic DNA (Saiki et al. 1988). This particular PCR enzyme from Thermus aquaticus (Taq) allows one to take any single DNA molecule, attach an oligonucleotide partner primer at each end and then in 20 min it will give a million copies of DNA. This is revolutionizing the whole approach to genetic engineering. One then just takes this DNA and introduces it into some vector like a bacteria and it will brew your antibody for you.

One can go further. One can look at all this chemistry of adding radiolabels to antibodies for example by making special chelating compounds and substituting it with genetically engineered products. There is the Kemptide sequence which is leucine, arginine, arginine, alanine, serine, leucine, glycine, and this binds phosphorus naturally (Kemp et al. 1976). One could in principle genetically engineer the Kemptide sequence on to the antibody and have an antibody that will bind phosphorus P32, presented by an active protein kinase (Foxwell et al. 1988). P32 may be the best antibody-linked therapy agent with its 14-day half-life and 2 MeV energy to treat tumours (Boye et al. 1984). Alternatively, one can look at the metallothionein protein and genetically engineer this natural chelating protein to bind heavy beta-emitting metals for therapy or indeed ^{99m}Tc (Epps et al. 1989). If it is thought that a group of cysteine molecules is the way to bind technetium, these could be genetically engineered onto a $F(ab')_2$ fragment to give an antibody that will bind technetium without any biotechnical problems.

In breast cancer, it has been shown that a monoclonal antibody against the human milk fat globule HMFG2 binds a particular amino acid sequence in the glycoprotein on the cell surface, which consists of aspartine, threonine, arginine whereas a more cancer-specific antibody SM3 binds the proline, aspartine, threonine, arginine, and proline. It has been discovered that in malignant tissue the two prolines have been exposed because the mucin glycoprotein is partly deglycosylated. Therefore, an antibody that only binds to the larger sequence allows it to be a little bit more cancer specific than the widely used HMFG2 (Burchell et al. 1987). Figure 14 from Granowska et al. (1990 b) shows technetium SM3 uptake in an ovarian tumour at 5 min, 6 h and 24 h. The SM3 antibody was labelled with ^{99m}Tc using the Schwarz technique, modified as described above (Mather and Ellison 1990).

Given this or another particular amino acid sequence, one can obtain the corresponding RNA and thus the DNA sequence. The PCR reaction could then be used to create synthetic antigens. Or else, if the DNA sequence is known, as in some oncogenes (Hamlyn and Sikora 1983), one could reverse the process and obtain the amino acid sequence. One could then synthesise its three-dimensional structure by computer, work out the bond flexibilities, determine the receptor conformation and create one's own molecules that fit that particular receptor to test out analogue molecules for a new radiopharmaceutical. Indeed, there is an explosion of computer-aided chemistry, which is the basis of the radiopharmaceuticals of the future.

Regulate the regulators

The problem is man. The unjustified risk-related fear and unreasonable concern that is now being expressed by regulatory authorities in relation to biological products that have quality assurance and quality control already of a much higher standard than a blood transfusion is about to stop the introduction of genetically produced compounds and other biological products that have been described above.

One aspect is their attack, for it can put in those terms, on monoclonal antibodies where a committee for proprietary medicinal products have made Guidelines (1988) (which have the force of regulations in the EEC) applied to the marketing of antibodies. This body has decided that antibodies are dangerous. And what is the basis of that decision which is going to affect all of us? I have compared the regulatory guidelines that they have created with an antibiotic (Britton 1990).

In order to introduce a new antibiotic into medicine one has to go through a number of steps, including its scientific validation. What is the scientific validation for these regulatory guidelines (1988)? There are no references. There is no discussion of the scientific basis of the regulatory guidelines. There is no experimental work and there is no explanation for the reasons for each of the statements in the regulatory guidelines. There is no assessment of efficacy, essential for an antibiotic. Do these regulations help or not? It is easy to separate a virus particle from a gamma globulin, for example, and

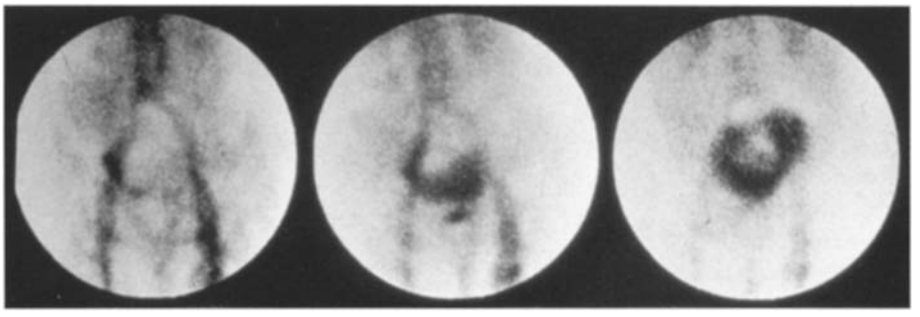


Fig. 14. Radioimmunoscintigraphy with ^{99m}Tc-SM3 monoclonal antibody against a stripped mucin core glycoprotein antigen at: *left* 10 min; *centre* 6 h; *right* 22 h; anterior views in a patient with ovarian cystadenocarcinoma. Specific tumour uptake increases with time around a focal defect due to the cyst (courtesy of Dr. Granowska)

these particular regulatory guidelines demand that tests be made for 14 different murine viruses at each stage of the production procedure. The death rate from natural murine viruses is about ten per decade worldwide (Minor 1990). The death rate from cancer is somewhat more!

What about toxicity? This must be tested extensively for an antibiotic. The toxic effects of these regulations have already been able to stop nuclear medicine specialists in Holland from using antibodies that they have been using for 6 years; they have been able to stop the development of antifibrin antibodies from the animal to the human stage; and they have stopped the cancer research unit in Nottingham from using an antibody they developed and were using for immunoconjugate therapy for cancer. Further side-effects are evident in the flurry of activity in the medical insurance world, which is trying to tell us that we can no longer be insured if we use antibodies because they might be dangerous.

What are the cost-benefit evaluations of these regulatory guidelines? The risks from these mouse viruses and from the other things they insist commercial companies should go through are quite trivial, but the costs are enormous. One company, Unipath, expects half of its costs in developing a new radiolabelled antibody to be for regulatory requirements. Another company, Amersham International, no longer feels that it is worth its while to wade through such regulatory swamps and has withdrawn from the monoclonal antibody field until reason prevails. Consider a conversation with a patient with malignant disease: "Isn't there a new drug treatment for me with antibodies which I saw on television?" "Yes, but we are not allowed to use it." "What is the problem?" "The antibodies are made from mouse cells and there is a one in ten-million chance that you might get a mouse virus disease." "What is it like?" "Like mild flu usually". "So for this I cannot have a new treatment for cancer that might prolong my life."

These are the side-effects of the new regulatory guidelines from the EEC by a group of people, as far as one can see, that have no scientific or clinical support for their destructive actions in legislating monoclonal antibodies and how they are prepared and, indeed, have no insight into the management of cancer in man. They contravene the European Communities Council's own directive (1987) where it is stated: "Whereas high-technology medicinal products requiring lengthy periods of costly research will continue to be developed in Europe only if they benefit from a *favourable regulatory environment*, particularly identical conditions governing their placing on the marked throughout the Community;" and "Whereas the need for the adoption of new technical rules applying to high-technology medicinal products or for the amendment of existing rules must be examined during a preliminary concertation between the Member States and the Commission with the competent Committees *so as not to endanger the advance of pharmaceutical research* while at the same time ensuring optimum protection of public health within the Community" (my italics).

I submit that these guidelines, which in effect will have the authority of regulations, do not meet the European Communities Council's Directive of creating a *favourable regulatory environment* and do endanger the advance of pharmaceutical research. On the one hand, they will have a knock-back effect on scientific laboratories trying to develop antibodies for human use against cancer and other diseases; on the other, they will have a knock-forward effect discouraging commercial companies from entering this field.

In conclusion, in the development of new radiopharmaceuticals the scientific problems are exciting and soluble, but the problem with man is man. I think it is up to this European Association and many other scientific societies to try and regulate the regulators, because in this world a distorted perception of risk is grossly damaging the real benefits.

The clinical questions

What then are the clinical questions? We want new radiopharmaceuticals to tell us whether a tissue is dead or alive, as in the brain after head injury or stroke or in the damaged heart before a by-pass operation. We want to know whether a tissue is alive and recoverable, again in the brain and the heart and the kidney with poor function due to an outflow obstruction. We want to know if a tissue is alive and dangerous, such as the jeopardized myocardium. We want to know whether it is cancer or not. We want to know whether a system is functioning normally. We want to be able to detect disease early, particularly cancer and atheroma and to diagnose infections and also autoimmune diseases. We

Table 2. The clinical questions

Dead or alive?	–	Brain/stroke
	–	Heart/bypass operation
Alive and recoverable?	–	Brain
	–	Heart muscle
	–	Kidney
Alive and dangerous?	–	Jeopardized myocardium
	–	Cancer or not
Is it functioning normally?		
Demonstrate inflammation?	–	Infection
	–	Autoimmune disease
Detected early	–	Cancer
	–	Atheroma
Treated with targetted radionuclides?		
Mark biological ageing?	–	Free radicals
	–	Degenerative processes

want to look at their responses to treatments. We want to give therapy with radionuclide-labelled compounds because they are potentially so selective that they can be targetted to the right site. Looking ahead, we want some markers of chronological and biological ageing. We want some markers of free radicals and we want some markers of degenerative processes (Table 2).

In conclusion, I hope I have convinced you that the development of new radiopharmaceuticals is related to the chemical persona of the molecule and how it interacts with the persona of another molecule. I hope that I have indicated that the way to develop a new radiopharmaceutical is to start with the clinical question and then to look at the physiological basis of how a compound is entering the cell and binding in the cell; to look at the chemical and molecular basis of the three-dimensional interaction; and to look at the possibilities of genetic engineering. Then you will have the new radiopharmaceuticals. These are the food and wine of nuclear medicine.

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