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Measurement of inflammation

I. Application of technetium clearance to rheumatoid arthritis and animal models

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Radioactive isotope scanning has been used pictorially as a diagnostic aid in many diseases and has more recently been applied to measure clearance from the brain (McAlister, du Boulay, Houlder, and Cato, 1967). Technetium as sodium pertechnetate is a gamma emitter with a short half-life of 6 hours. McCarty, Polcyn, Collins, and Gottschalk, (1970) suggested that it was 90 per cent. bound to plasma protein. After intravenous injection, counts over a joint rise rapidly to a peak within 30 minutes. Dick, Neufeld, Prentice, Woodburn, Nuki, and Buchanan (1970) showed that the peak count over inflamed knees was greater than over normal knees, and the time taken to reach this peak was shorter. Small joints have also been studied by Collins, Deodhar, Nuki, Whaley, Buchanan, and Dick (1971) who showed that steroid therapy could reduce the peak count.

In this study we have re-examined protein binding of Technetium (99mTc) and its passage into joint fluid and have explored the application of this method to those experimental models of inflammation in rats and rabbits that are in current use.

Methods

(1) BINDING

A. Electrophoresis
This was carried out using the Shandon tank.

B. Equilibrium dialysis
Serum was incubated with 99mTc at different concentrations and dialysed against distilled water at 4° and 20°C. for 24 hours.

C. Ion binding resin
Serum was incubated at 37°C. with 99mTc and ion exchange resin for 60 minutes, then centrifuged and the radioactivity of the supernatant was measured. This cycle was repeated many times.

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D. Charcoal separation
To six series of eight tubes, 0·5 ml. of normal serum and dilutions of 99mTc from ½ to 1/128 were added (diluted with phosphate buffer pH 7·6). 99mTc was added to all tubes, 1 μc./ml. serum in the first series, 0·5 μc./ml. in the second and then 0·25, 0·12, 0·06, and 0·03 μc./ml., all in a volume of 0·5 ml. To all tubes, 0·5 ml. of charcoal suspension (1·25 mg./ml.) was then added; the tubes were immediately centrifuged and the supernatant sampled and counted. Two control tubes were used in each series, one containing 99mTc in serum but to which no charcoal was added, representing 100 per cent. binding, and one containing 99mTc but no serum to which charcoal was added. 87 per cent. of the isotope was removed under these conditions and this was used as a standard blank. The counts for this second control tube were subtracted from all others and the results were then expressed as a percentage of the 100 per cent. control tube.

The results in this experiment were checked in six patients having knee scans. Blood was taken 20 minutes after injection of 200 μc. of 99mTc. To the undiluted serum, the same charcoal mixture as used in the above experiment was added in the same proportion.

(2) TURPENTINE PLEURISY
The method of Spector (1956) was used. 0·1 ml. turpentine was injected intrapleurally. Sixty Wistar rats weighing between 200 and 250 g. were used. 99mTc was injected into the tail vein 30 minutes before harvesting the pleural fluid. Harvesting was carried out at 30 minutes and 3 hours after intrapleural injection. All bloodstained exudates were discarded. The animals were bled *via* the carotid artery. 1 ml. of serum and 1 ml. of exudate were counted in a liquid scintillation counter.

(3) RABBIT ARTHRITIS
Four rabbits were sensitized with complete Freund's adjuvant (CFA) and bovine serum albumin (BSA) intradermally on two occasions, spaced 3 weeks apart. After a further 3 weeks the left knee was challenged with BSA. The right knee was injected with an equal volume of isotonic normal saline (Dumonde and Glynn, 1962), Both knees were scanned every other day for 14 days, then

weekly. 50 μc. of 99mTc were injected into the ear vein. A box was designed which fixed the counting point and shielded it from the bladder and opposite knee. Counting was carried out for 20 seconds out of 30 seconds; the right knee was counted once and the left three times in a 2-minute cycle. Results were expressed as a ratio of counts for left and right knees.

(4) HUMAN STUDY

Six patients with large knee effusions were selected, four with definite or classical rheumatoid arthritis by the American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959), and two with ankylosing spondylitis. The target knee was scanned for 30 minutes after intravenous injection of 200 μc. of 99mTc. A needle was inserted into the joint and 5 ml. synovial fluid were aspirated at 10, 20, and 30 minutes. Samples of venous blood were taken at the same times. At the end of the experiment as much fluid as possible was aspirated from the knee whilst counting continued over the joint.

Results

(1) BINDING

With a mixture of serum and 99mTc we were unable to obtain a band of radioactivity with electrophoretic studies. Work with dialysis gave inconsistent and unreproducible results. We found that after repeated washings of the bag, high counts (up to 20 per cent. of the total) could still be obtained, suggesting that 99mTc adhered to the bag. The resin study showed that the count of the isotope that remained in the serum became lower and lower on repeated washings. (Full decay corrections were carried out). Results of protein-binding experiments using the charcoal separation technique are shown in Fig. 1. Mean protein binding of the isotope in six sera from patients having joint scans was 90 per cent.

(2) TURPENTINE PLEURISY

The results are expressed as a ratio of exudate to serum (counts per 10 seconds per 1 ml.). Means were at $\frac{1}{2}$ hour 87 per cent. and at 3 hours 82 per cent. The mean volume of exudate was 0·8 and 1·9 ml. respectively. Binding to exudate remained as in serum (90 per cent.).

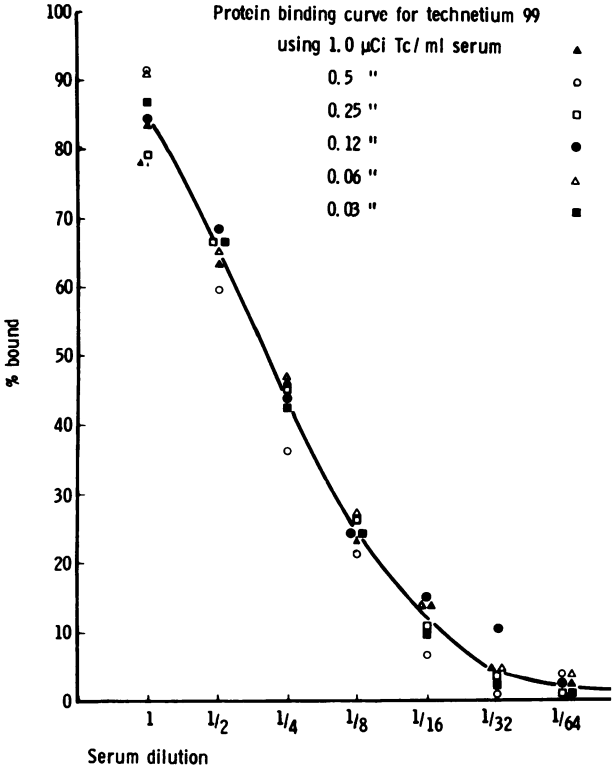

FIG. 1 *Protein binding curve for 99mTc*

(3) RABBIT ARTHRITIS

The rabbits developed acute arthritis of the left knee 2 days after challenge; this was confirmed histologically in a parallel study. We obtained large differences between the two knees from Days 1 to 5 using 99mTc and scanning both knees. In the chronic phase a difference of up to 14 per cent. and at least 3·5 per cent. between the two knees was obtained (Fig. 2). This is consistent with the histological

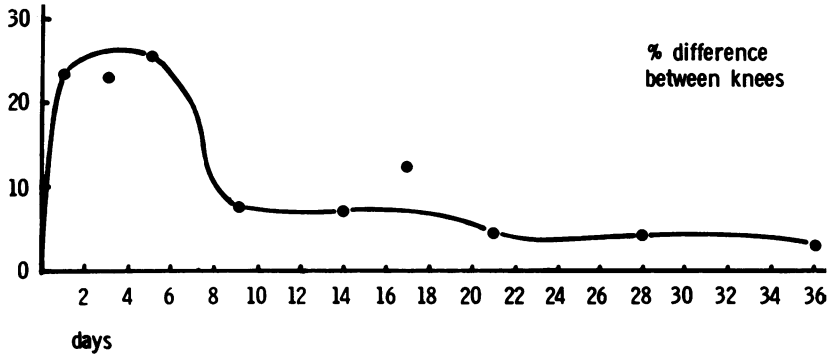

FIG. 2 *Progress of development of rabbit arthritis using 99mTc as a measure*

findings in this model described by Dumonde and Glynn (1962).

(4) SYNOVIAL FLUID STUDY

The results are shown in Fig. 3. Synovial fluid levels are expressed as a percentage of blood levels. Removal of up to 30 ml. of fluid did not affect the count rate over the knee.

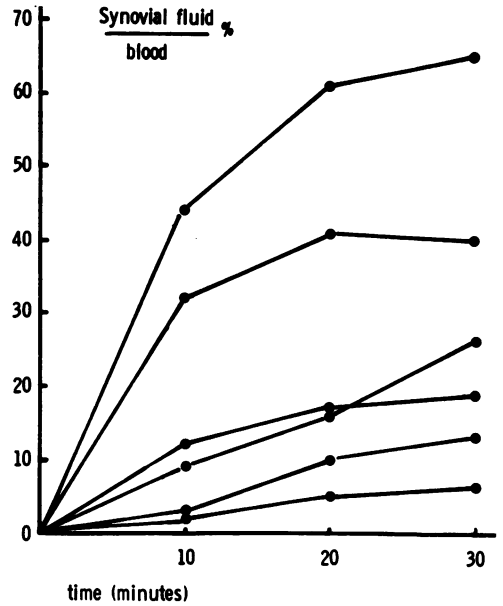

FIG. 3 *Passage of 99mTc into synovial fluid*

Discussion

Previous work suggested that 99mTc was 90 per cent. bound to plasma protein. Our initial dialysis experiments proved unreliable: one source of error was the radioactivity that remained in the bag membrane after repeated washings. Another possible source of error in the dialysis experiments was the need to wet the dialysis membrane before the experiment so that the contents of the membrane were diluted. The previously reported figure of 90 per cent. protein binding was confirmed by a charcoal precipitation experiment. Some ion binding resin work also carried out showed repeated removal of 99mTc from a mixture of serum and protein, suggesting that the binding was weak.

Little emphasis has been placed on the possible extension of the use of 99mTc to the assessment of animal inflammation particularly in response to therapy. Turpentine pleurisy is widely used in the evaluation of drugs in acute inflammation. It seems that in this model the vessels become maximally permeable very early. Results with 99mTc at ½ hour show that much more radioactivity appears in the exudate than if I^{131} labelled albumin is used (Di Rosa, Giroud, and Willoughby, 1971). This very early maximal vascular permeability of acute inflammation is quite different from the chronic rheumatoid knee effusion and our work again emphasized the difference between experimental models and human disease. In turpentine pleurisy, exudate from an initially normal vascular bed is studied, whereas in rheumatoid arthritis the whole vascular bed is dilated at the start of any experimental procedure.

The model of arthritis in rabbits was used because of its chronicity, its histological similarity to human rheumatoid disease, and its convenience for repeated scanning. Because results are expressed as a percentage of a normal joint, the isotope dosage does not need to be accurately controlled. The model suffers the disadvantage of being slow to induce and in most laboratories is applicable only to rabbits. 99mTc count appears to be a useful method for quantitation of the acute phase of this model but in the chronic phase, differences between inflamed and normal joints are probably too small to be affected by drugs.

99mTc passes slowly into synovial fluid and the size of an effusion does not affect count rate over the joint. It was of interest that synovial fluid 99mTc concentration was higher in the two patients with ankylosing spondylitis, though the numbers were too small to draw any firm conclusions. This could be explained by the observation that 99mTc tends to stay more within the vascular compartment in rheumatoid arthritis (Huskisson, Berry, Browett, and Balme, 1973).

Because isotope methods are objective and because of the ease of accurate counting, they offer an advantage over traditional crude methods which rely so much on the influence of patient and observer. They might usefully be applied to techniques of drug assessment, such as other models and skin tests. However the use of another isotope with more predictable distribution may have greater value.

Summary

99mTc was shown to be 90 per cent. protein bound in human blood at the concentrations used in these experiments. It passed into the exudate produced in response to intrapleural turpentine both at half and 3 hours after instillation. There was a large increase in counts over the affected joint phase of arthritis induced by BSA and CFA in rabbits. In the chronic stage of the model the difference was small. 99mTc passed slowly into synovial fluid and the volume of an effusion did not affect the count rate over the joint.

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