IN VITRO STUDIES OF ^{99m}Tc-PERTECHNETATE BINDING BY HUMAN SERUM AND TISSUES

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Of clinical interest because of its widespread use in nuclear medicine, $^{99m}TcO_4^-$ was found to bind to human serum proteins and to a variety of human tissues. At 37°C and pH 7.4, approximately 80% (equilibrium dialysis) or 65% (ultrafiltration) of $^{99m}TcO_4^-$ was found to be bound to serum proteins. Binding was increased in the cold and by an acid shift in pH. It was decreased in debilitated patients and by a variety of pharmaceuticals. For equimolar concentrations, relative effectiveness in blocking serum $^{99m}TcO_4^-$ binding in vitro was: sodium salicylate > $KClO_4$ > acetylsalicyclic acid > propylthiouracil > prednisone > KI. By a variety of techniques, albumin was shown to be the primary if not the only serum $^{99m}TcO_4^-$ binding protein. Serum binding is weak, as additionally evidenced by complete release on electrophoresis or with prolonged dialysis in an open system.

After equilibration with insoluble tissue fractions, $^{99m}TcO_4^-$ binding per gram was: abnormal synovium = normal synovium > liver > red blood cell membrane > brain. After equilibrium dialysis with minced whole tissues, "equilibration" binding per gram of wet weight was: liver > serum > thyroid > kidney > muscle > skin > abnormal synovium > brain > normal synovium. Kinetic studies of these tissues suggested that $^{99m}TcO_4^-$ binding was completely reversible only in serum and the synovial specimens with retarded efflux particularly from the liver and thyroid specimens.

In recent years ^{99m}Tc has become the most frequently used radiopharmaceutical in the nuclear medicine clinic. Because its physical characteristics make it an excellent imaging agent, it has been tried in some chemical form for scanning virtually every organ. In its simple pertechnetate form ($^{99m}TcO_4^-$) it is excluded by normal brain tissue (but not by necrotic or neoplastic brain) and is concentrated by the thyroid gland, the salivary glands, the gastric mucosa, and a variety of other tissues. It also concentrates in areas of inflammation. These characteristics of $^{99m}TcO_4^-$ have led to its use as an agent for scanning the brain, thyroid, salivary glands, and joints.

Despite this wide clinical application, little basic information about the biological characteristics of pertechnetate has been reported after the original studies of Andros, et al (*1*). Their work and studies by Beasley, et al (*2*) and our laboratory (*3*) indicate that pertechnetate is distributed into a much larger volume of distribution than is iodide, an ion whose physiology is similar in many respects. When radioiodide and radiopertechnetate were administered simultaneously (*3*), early serum pertechnetate concentrations were higher than those of iodide despite the ultimate greater volume of distribution.

These observations suggest that pertechnetate, much more than iodide, is bound in some fashion to serum and also to tissues. Preliminary studies by ourselves and others (*4,5*) clearly demonstrated that serum protein binding occurs. The present work was undertaken to characterize this binding of pertechnetate to human serum proteins and to evaluate the possible importance of its binding to human tissues. Such information may be of value in clarifying the mechanisms for scan positivity.

METHODS

Source of sera and tissues. Serum samples were obtained from healthy, normal volunteers ("healthy" sera), from individual chronically ill patients at the Buffalo V. A. Hospital ("sick" sera), and from a pool of serum obtained from in-patients at the Buffalo V. A. Hospital collected by the Clinical Laboratory ("sick" pool). All normal tissue samples and

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the atrophic thyroid sample were obtained at autopsy. Two samples of abnormal synovium from patients with rheumatoid arthritis were surgically removed during synovectomy. A third sample of abnormal synovium was obtained at autopsy from a patient with severe gout.

Nonequilibrium dialysis. When intercomparison of the binding characteristics of serum proteins was to be carried out without reference to absolute binding levels, dialysis in an open system against running tap water was used. Serum was enriched with $^{99m}TcO_4^-$ and placed in a sack of Visking membrane. A sample of the same enriched serum specimen was retained to serve as an undialyzed standard. All samples were studied in duplicate. The closed dialysis bags containing the various specimens to be compared were suspended from the top of a large beaker by strands of silk thread. The beaker was filled with tap water and placed under a gentle stream of tap water for the entire period of the dialysis. At the end of the period of dialysis, the bags were placed in test tubes suitable for scintillation counting with attention to maintaining a constant geometry. All samples including the standards were then counted in a well scintillation detector. The percent of the radioactivity remaining at the end of dialysis was expressed as the net counts in the dialysis bag/net counts in the standard. The ordinary period of dialysis by this technique was 4 hr.

Equilibrium dialysis. To arrive at equilibrium binding values, certain samples were subjected to equilibrium dialysis for 20 hr with shaking at 37°C. In these studies, a 2-ml sample was placed inside a Visking membrane which was closed and washed. This bag was then weighted to insure submersion and placed in a 50-ml Lusteroid tube. Ten milliliters of phosphate buffer, pH 7.4, 0.077 molar, was added. One milliliter of the same material placed within the dialysis bag was pipetted in duplicate to serve later as a counting standard. After incubation with shaking at 37°C for 20 hr, the dialysis bag was removed from the Lusteroid tube. Its contents were quickly aspirated by hypodermic syringe, and 1 ml was pipetted for counting. One milliliter of the buffer in which the serum had been incubated was also pipetted in duplicate. The percent of the pertechnetate that was free was calculated by each of the following techniques, which were found to give comparable results:

$$\text{(A) percent free} = \frac{\text{counts in buffer}}{\text{counts from inside dialysis membrane}} \times 100,$$

$$\text{(B) percent free} = \frac{\text{counts in buffer}}{\text{counts in standard} - (5\times \text{ counts in buffer})} \times 100.$$

For special studies, the temperature of the dialysis or the pH of the buffer were altered.

Ultrafiltration. To avoid possible alterations in binding caused by introduction of possibly competing buffer ions, a series of studies were also performed by an ultrafiltration technique (Schleicher and Schuell apparatus). Ultrafiltration was ordinarily performed in the cold room at 4°C although a series of comparative studies at 37°C were performed. It was found that the percent of the radioactivity entering the ultrafiltrate was variable during the first ½–1 hr of ultrafiltration but that the concentration was constant thereafter. For this reason, the radioactivity appearing in the ultrafiltrate during the first hour is not reported here but instead the average of the concentration appearing between 60 and 90 and between 90 and 120 min is presented.

In all cases an aliquot of the serum to be ultrafiltered was retained as a counting standard. The percent of the radioactivity presented here as "free" is

$$\text{percent free} = \frac{\text{radioactivity in ultrafiltrate}}{\text{radioactivity in equal volume of serum}} \times 100.$$

Studies of dried homogenized tissues. Human tissues were thoroughly homogenized, washed twice with distilled water, and freeze dried. After drying, they were pulverized. Thirty-milligram aliquots of the solid, dry powder were then measured into a small centrifuge tube. One milliliter of phosphate buffer, pH 7.4, 0.077 molar, enriched with a tracer amount of $^{99m}TcO_4^-$, was added to the tube. These tubes were then incubated with shaking at 37°C for 12–18 hr. At the end of incubation, they were centrifuged and the supernatent removed. The pellets of tissue powder were washed once with nonradioactive buffer and recentrifuged. The radioactivity in the pellets and in an aliquot of the original $^{99m}TcO_4^-$-enriched buffer were counted. After correction of the counts to equalize the weights of tissue and buffer, results were expressed as a tissue:medium (T:M) ratio.

Dialysis of intact tissues. Human tissues were frozen until use. They were thawed rapidly with attention to avoid drying. They were cut into pieces as nearly uniform as possible and portions weighing 0.5 gm were placed in small dialysis bags which were then closed. These bags were kept moist until use. An empty dialysis bag was used as a blank. All of the dialysis bags were weighted and suspended by silk thread from the top of a 1,000-ml beaker so that they were immersed in 300 ml of 0.9% saline which contained approximately 30 μCi of $^{99m}TcO_4^-$. This beaker containing the bags was then incubated with shaking at 37°C. The bags were added to the beaker at staggered times to allow removal and counting of each bag precisely 15, 30, 60, and 120

min after incubation was begun. In each case the bag to be counted was quickly removed, placed in a well counter tube, and counted for 1 min. It was then replaced in the incubating solution. Times of incubation were adjusted for the time out of solution. Counts in the empty dialysis membrane were subtracted from those of the dialysis membranes enclosing tissues to obtain net tissue counts. Aliquots of the incubating solution were also counted and a tissue:medium ratio was calculated after correction for specimen weight.

At the end of this phase of the experiment, 10 mCi of additional $^{99m}TcO_4^-$ was added to the beaker and the tissues were allowed to incubate with shaking at 37°C for 17 additional hours. At the end of this time, the bags were removed from solution, gently dried between tissues, and counted. These counts were compared with counts in the enriched buffer solution in which the 17-hr incubation had been done for determination of T:M ratio. Each of these bags was then placed in a 50-ml Lusteroid tube containing 10 ml of nonradioactive pooled human serum. These tubes were incubated with shaking at 37°C. One tenth-milliliter samples in duplicate of the serum on the outside of the dialysis bag were obtained during incubation at the following times: 1 min, 15 min, 30 min, 60 min, 2 hr, and 4 hr. At the end of 4 hr, the dialysis bags containing the tissues were counted once more to ascertain the amount of radioactivity remaining attached to the tissues. Again, counts in the empty dialysis bag were subtracted as a blank.

All counts were corrected for radioactive decay before other calculations.

Equilibrium studies with Biogel columns. Columns of Biogel P 60 were equilibrated with solutions of $^{99m}TcO_4^-$ in a variety of tracer concentrations. After equilibration had been demonstrated by constant levels of radioactivity in the column efflux, various amounts of human serum albumin were added to the column. Binding of $^{99m}TcO_4^-$ was inferred from a peak of increased radioactivity in the column efflux corresponding to the protein peak measured by absorption at 280 mμ.

Serum electrophoresis. Serum previously enriched with $^{99m}TcO_4^-$ was subjected to agar gel electrophoresis in Tris-maleate (pH 8.6) buffer at 90 volts and also in barbiturate buffer (pH 8.6) at 100 volts and at 45 volts. In additional attempts to identify the specific serum protein responsible for pertechnetate binding, nonradioactive serum previously subjected to agar gel electrophoresis was then briefly exposed to $^{99m}TcO_4^-$ followed by quick rinsing. The location of the radioactivity on the slides was then identified by radioautography or scintillation counting of strips of the electrophoretic medium.

TABLE 1. EFFECT OF TEMPERATURE ON BINDING OF $^{99m}TcO_4^-$

Serum	Session	Technique	Percent free 4°C	Percent free 37°C
Healthy	A	Ultrafiltration	7.1	43.8
Healthy	B	Ultrafiltration	7.7	33.5
Healthy	C	Ultrafiltration	6.6	29.7
Healthy	D	Ultrafiltration	6.9	27.3
Healthy	D	Equilibrium dialysis	2.9	19.0
Healthy	E	Equilibrium dialysis	3.6	23.0
Sick	E	Equilibrium dialysis	5.7	29.6

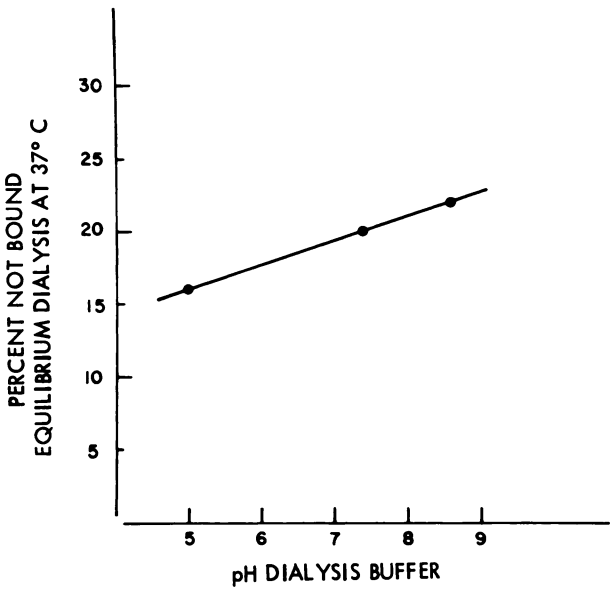

FIG. 1. Effect of pH of dialysis buffer on binding of $^{99m}TcO_4^-$ to human serum.

Gel chromatography. Column chromatography of serum previously enriched with $^{99m}TcO_4^-$, either endogenously or exogenously, was performed at 4°C in a cold room using a Biogel P 200 column 30 cm in height. The columns were eluted with 0.9% saline solution. Four-milliliter fractions were collected and assayed for UV absorbance at 280λ and later for content of radioactivity.

RESULTS

Serum binding of $^{99m}TcO_4^-$. Serum obtained from normal healthy persons bound approximately 80% of $^{99m}TcO_4^-$ in an equilibrium dialysis situation when the study was done at 37°C (Table 1). In studies in which the ultrafiltration technique was used, somewhat larger percentages were found to be present in the ultrafiltrate.

A variety of factors were found to affect serum pertechnetate binding. It is sharply temperature-dependent (Table 1). On the average the percent

TABLE 2. SIMULTANEOUS STUDIES OF $^{99m}TcO_4^-$ BINDING BY SERA FROM HEALTHY NORMAL SUBJECTS WITH BINDING BY SERA FROM HOSPITALIZED PATIENTS

Session	Source of "sick" serum	Technique		Percent free: Healthy	Percent free: Sick
E	Convalescent after nearly fatal illness	Equilibrium dialysis	4°C	3.6	5.7
			37°C	23.0	29.6
F	Clinical laboratory pool	Ultrafiltration	4°C	7.2	13.3
G	Clinical laboratory pool	Ultrafiltration	4°C	7.4	12.9
H	Patient with chronic lung disease	Ultrafiltration	4°C	7.0	6.9
	Patient with metastatic carcinoma				9.1

found in the "free" compartment was greater by a factor of 5.3 at 37°C than at 4°C. This relationship held equally for ultrafiltration and for equilibrium dialysis.

It is also related to the pH of the NaH_2PO_4-Na_2HPO_4 buffer used in equilibrium dialysis. Figure 1 shows that binding is more avid at pH 5 than pH 7.4 and less avid at pH 8.6.

Casual study of specimens obtained from hospitalized patients or from a "serum pool" obtained from the Clinical Laboratory suggested that patients with clinical illness might bind pertechnetate less well than do normal controls. To confirm this observation, a series of paired studies were done in which specimens obtained at the same time from a healthy normal volunteer and from a sick patient or pool of patient serum were studied simultaneously. These data are presented in Table 2. Because a variety of drugs will inhibit pertechnetate-serum binding, the individual patients chosen for this comparative study were receiving no medication. One of the individual patients reported in Table 2 had serum binding identical to his control. In the other five paired studies, the "free" fraction was greater for the "sick" than for the healthy serum.

When tagged serum was dialyzed against running water, measurements were made of the radioactivity remaining bound to the serum rather than to the "free" fraction. In these studies, considerably less radioactivity was found to be bound to the serum after 4 hr dialysis than after equilibrium dialysis or ultrafiltration. This observation suggests a readily reversible type of binding. To test this hypothesis, a series of tagged serum samples were dialyzed simultaneously against running water, and duplicate bags were removed at varying time intervals after initiation of the dialysis. In one of these experiments (Fig. 2), ^{125}I-iodide and ^{131}I-thyroxine were added to the serum for comparison with $^{99m}TcO_4^-$. The $^{99m}TcO_4^-$ disappearance curve can easily be separated into a fast and a slow component,

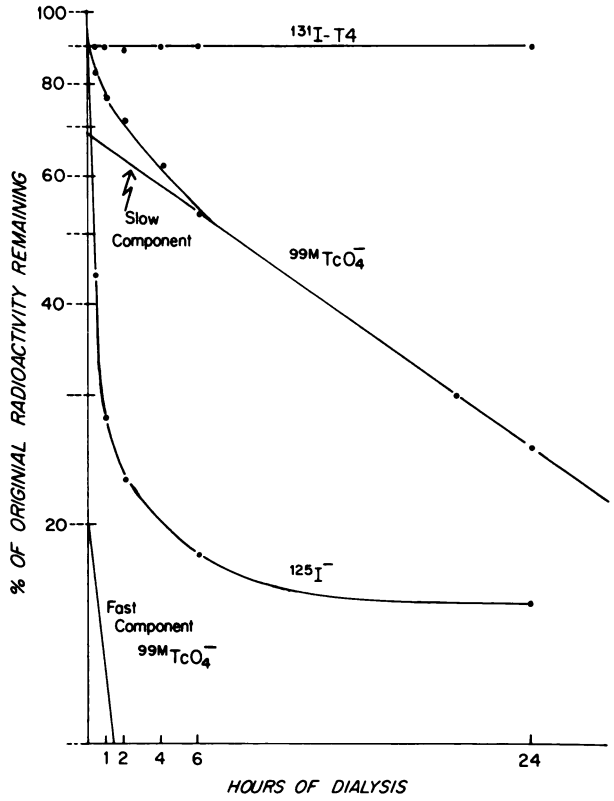

FIG. 2. Removal of $^{99m}TcO_4^-$ from human serum during continuous dialysis against running tap water. Late phase of disappearance curve was "stripped" to demonstrate fast component. Simultaneous disappearance of thyroxine (^{131}I-T_4) and iodide ($^{125}I^-$) are shown for comparison.

each of which follows a simple logarithmic pattern. Radiothyroxine concentration remained virtually unchanged throughout the 24 hr of dialysis after an initial small drop. The radioiodide disappeared very rapidly in keeping with its relatively unbound state. A second study carried out over 48 hr confirmed the $^{99m}TcO_4^-$ disappearance pattern. In this study the slow component of the distribution curve was unchanged even after 48 hr.

Using this method of dialysis against running water, the effects of propylthiouracil, sodium per-

chlorate, triiodothyronine, and iodide added to serum in concentrations approximating extreme levels which might be achieved clinically were studied. In each case a single serum sample from a given subject was dialyzed in five pairs of 2-ml samples placed in dialysis membranes and dialyzed simultaneously against running water. In each case, one pair of samples were unaltered and to each of the other pairs propylthiouracil 1 mg, sodium perchlorate 4 mg, triiodothyronine 0.2 μg, or iodide 25 μg were added to the 2 ml of serum. The results of these studies on nine samples are given in Table 3. In these concentrations, with this system, neither triiodothyronine nor iodide significantly altered the $^{99m}TcO_4^-$ binding. However, propylthiouracil and sodium perchlorate both caused a significant reduction in the percent which remained attached to the serum after 4-hr dialysis.

Further studies of the effects of pharmaceuticals on serum-pertechnetate binding were made using the technique of ultrafiltration at 4°C. In these studies, simultaneous ultrafiltrations were performed with varying amounts of added pharmaceutical. Figure 3 gives the results. In general the inhibition of pertechnetate binding was directly related to the amount of pharmaceutical with leveling off at very high concentrations. It will be seen that, mole for mole, salicylate and perchlorate were the most effective of the substances tested while iodide and prednisone were relatively ineffective. While these studies were all in vitro, a single in vivo study was done in which 1.2 gm of aspirin was given to a normal individual after a control serum had been obtained. One and one-half hours later a second serum sample was obtained which was submitted for chemical determination of salicylate content. When this pair of sera were studied by ultrafiltration, the increase in ultrafiltratable $^{99m}TcO_4^-$ from the control to the post-aspirin serum was exactly parallel to the slope of the sodium salicylate curve.

Various studies were undertaken to identify the serum protein responsible for $^{99m}TcO_4^-$ binding. When human serum albumin was added to phosphate buffer to produce a final concentration of 2.5 or 3 gm/100 cc, the extent of binding of this solution in equilibrium dialysis and open dialysis systems was similar to that of normal human serum samples (Table 4). The binding of pertechnetate by human serum albumin was quantitatively studied by the ultrafiltration technique (Fig. 4). Progressively increasing concentrations of human serum albumin bound greater amounts of $^{99m}TcO_4^-$ although this was not a linear function. At the usual physiological levels, the results were in the range usually observed in normal sera. Similar studies using 7S human gamma globulin, also given in Table 4 and Fig. 4, show that pertechnetate binds to a much smaller degree to this protein. It should be pointed out that the usual concentrations of gamma globulin are smaller than those of albumin in normal human serum, and such data do not indicate whether gamma globulin also binds when albumin is present.

When a $^{99m}TcO_4^-$-tagged sample of whole serum or of human serum albumin was gel filtered on Biogel P 200 in phosphate buffer (pH 7.4, 0.05 *M*), a very small peak of radioactivity ($<1\%$) coincided with the albumin peak (excluded volume) in the column efflux. The majority of the radioactivity was retarded by the column and eventually eluted in a single broad peak. This suggests that a very small

TABLE 3. PERCENT OF ORIGINAL $^{99m}TcO_4^-$ RADIOACTIVITY REMAINING AFTER 4-HR DIALYSIS AGAINST RUNNING WATER

		Percent of radioactivity bound after dialysis for 4 hr			
Subject	Control serum	Propylthiouracil 50 mg% added	$NaClO_4$ 200 mg% added	Triiodothyronine 10 μg% added	Iodide 500 μg% added
OD	27.3	10.9	19.1	28.4	29.3
BR	27.9	8.2	18.7	29.6	24.5
HA	28.5	6.1	13.7	25.7	23.5
LI	43.4	22.5	25.6	46.5	43.1
BW	43.6	22.9	24.5	44.7	45.5
TO	37.8	24.9	24.1	36.2	36.0
JW	46.8	24.2	25.6	41.7	41.8
TB	32.9	13.6	16.6	34.7	34.8
MC	20.9	9.9	13.8	23.3	21.8
Mean	34.3	15.9	20.2	34.5	33.3
s.d.	9.0	7.6	4.9	8.4	9.0
t (difference from subject's own control)		13.35	8.42	—0.17	1.01
P (difference from subject's own control)		0.001	0.001	n.s.	n.s.

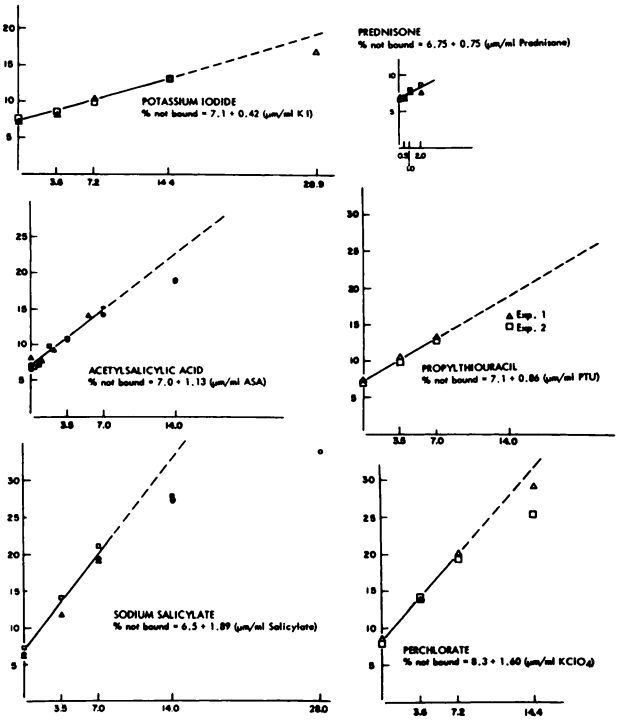

FIG. 3. Effect of various pharmaceuticals on binding of $^{99m}TcO_4^-$ to human serum. Horizontal axis of each curve is μm of pharmaceutical per ml of serum. Vertical axis is percent of serum radioactivity which is ultrafilterable.

amount of $^{99m}TcO_4^-$ is tightly and possibly irreversibly bound.

When human serum albumin was added to Biogel P 60 columns previously equilibrated with $^{99m}TcO_4^-$ in buffer, peaks were observed in radioactivity corresponding to the protein elution peak. The radioactivity in this area amounted to 20 ± 4% of the total radioactivity. To attempt to clarify the apparent discrepancy between these and the dialysis experiments, the binding of $^{99m}TcO_4^-$ to Biogel was studied. Increasing weights of dry Biogel were added to a standard solution of pertechnetate in buffer. The beads were allowed to swell, were centrifuged, and the radioactivity of the supernatant was measured. At the highest Biogel concentration, practically all the buffer was taken up by the gel. Under these conditions this batch method approached the column situation, and it was found that 25% of the radioactivity was bound to the gel. This explains, at least in large part, why the percent radioactivity bound to serum protein (albumin) appeared less in the case of gel filtration as compared with dialysis. Other ion competition was also in evidence as it was found that the percent bound increased when the gel filtration was carried out without added ions. However, smearing prevented accurate determination of the radioactivity bound in the absence of other added ions.

Attempts were made to confirm the nature of $^{99m}TcO_4^-$ serum binding proteins by electrophoresis of serum enriched with the radioisotope. When electrophoresis was done by standard agar gel techniques using 90 volts and 140 mA, the $^{99m}TcO_4^-$ was found to separate completely from all of the serum components and to migrate toward the cathode. After 15 min of electrophoresis, the radioactivity could be detected near the cathode end of the slide whereas the serum had barely begun to separate. By 30 min virtually all of the radioactivity had disappeared into the buffer. Similar results were found after electrophoresis at 100 volts and 90 mA and even when the study was done at 45 volts and 11

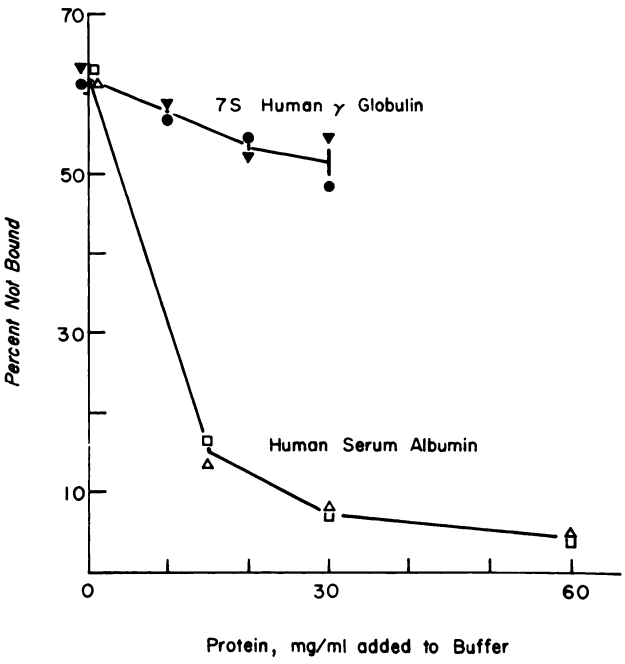

FIG. 4. Ultrafiltration of $^{99m}TcO_4^-$ added to various concentrations of human serum albumin or of 7S human gamma globulin.

TABLE 4. COMPARISON OF BINDING OF $^{99m}TcO_4^-$ TO NORMAL SERUM, TO HUMAN SERUM, ALBUMIN, AND TO HUMAN 7S GAMMA GLOBULIN

Binding agent	Technique	Percent free
Normal serum	Equilibrium dialysis 37°C	18.6
Human serum albumin 2.5 gm%	Equilibrium dialysis 37°C	28.6
Human 7S gamma globulin 1.5 gm%	Equilibrium dialysis 37°C	95.0
Normal serum	Ultrafiltration 4°C	7.1
Human serum albumin 3.0 gm%	Ultrafiltration 4°C	7.4
Human 7S gamma globulin 1.5 gm%	Ultrafiltration 4°C	53.4

TABLE 5. LYOPHILIZED, PULVERIZED TISSUE RESIDUA INCUBATED WITH $^{99m}TcO_4^-$ IN PHOSPHATE BUFFER (0.07 *M*, pH 7.4) FOR 18 HR WITH SHAKING. MEANS OF DUPLICATE OBSERVATIONS

Human tssue	cpm/30 mg: Tissue pellet	cpm/30 mg: Supernatant	Concentration ratio
Liver	12,196	1,124	10.8
Brain	4,431	1,272	3.5
Abnormal synovium (alcohol preserved)	17,781	1,100	16.2
Normal synovium (alcohol preserved)	17,895	1,075	16.6
Normal synovium (in saline until frozen)	32,936	924	35.6
Red blood cell membrane	5,224	1,202	4.3

mA. In later studies nonradioactive serum was separated by electrophoresis and the slide was then exposed to a solution of $^{99m}TcO_4^-$ either by dipping or by spraying one of the radioisotopes. Subsequent measurement of the distribution of the radioactivity by well counting of aliquots of the slide or by autoradiography was technically difficult because of high background radioactivity on the slide. However, a band of radioactivity corresponding to the location of the albumin band was clearly seen. These studies are not sufficiently clear-cut to permit any conclusions about binding by other proteins in addition to albumin.

$^{99m}TcO_4^-$ binding to insoluble tissue fractions. When the insoluble lyophilized fractions from liver, brain, normal and abnormal synovium, and erythrocyte membrane were incubated with $^{99m}TcO_4^-$, all of these tissues were found to concentrate the tracer (Table 5). Concentration by the brain tissue and erythrocyte membrane was least. In contrast, the insoluble liver fraction concentrated the $^{99m}TcO_4^-$ well, but the specimens of synovial tissue were the most avid of those tested. As the abnormal synovium available for study had been preserved in alcohol, the normal synovium for comparison was divided when it was received, a portion being preserved in alcohol and a portion in saline. Alcohol preservation markedly decreased the avidity of this synovial specimen for $^{99m}TcO_4^-$. No difference in specific binding was seen between the normal and the abnormal synovial samples on a unit wet weight basis.

$^{99m}TcO_4^-$ binding to minced tissue fractions. To include the activity of soluble cell fractions as well as the insoluble fractions studied above, small weighed sections of intact tissues were dialyzed against buffer. Results of these studies are given in Tables 6 and 7. Table 6 shows the uptake phase of this study in which tissue-containing dialysis bags were bathed in a solution containing $^{99m}TcO_4^-$. Three separate experimental sessions are presented, and each figure entered into the table is the mean of duplicate samples. As mentioned above, all of these represent tissue:medium ratio after correction for uptake by the dialysis bag itself, size of specimen, and radioactive decay. In Table 7 similar results are given for a followup experiment in which the dialysis bag, now containing radioactive tissues, was dialyzed against nonradioactive serum. In this case the radioactivity leached out of the tissues by the bathing serum was measured at varying time intervals. The values expressed in the table are those calculated to be remaining in tissues. In the right hand column are presented the actual radioactivity measurements found in the tissues at the end of the 4-hr leaching dialysis. In the tables the means of duplicate samples are presented with identification of the experimental session in which this particular pair of samples were

TABLE 6. TISSUE:DIALYSATE RATIOS (500 MG SLICES OF TISSUES IN DIALYSIS MEMBRANE BATHED IN 0.9% NaCl SOLUTION CONTAINING A TRACER OF $^{99m}TcO_4^-$. 37°C WITH SHAKING). MEANS OF DUPLICATE STUDIES

Tissue	Exp. No.	Dialysis time: 15 min	30 min	60 min	120 min	18 hr
Normal thyroid	2	0.33	0.50	0.65	0.86	2.57
Normal thyroid	3	0.36	0.51	0.72	0.97	1.02
Normal thyroid	3	0.30	0.44	0.66	0.92	1.07
Myxedema thyroid	3	0.31	0.48	0.67	0.92	0.65
Liver	1	0.27	0.44	0.59	0.91	2.45
Liver	2	0.38	0.61	0.75	1.03	4.27
Normal synovium	1	0.21	0.26	0.32	0.38	0.42
Normal synovium	2	0.30	0.51	0.64	0.76	0.89
Normal synovium	3	0.36	0.51	0.72	0.96	0.80
Abnormal synovium	1	0.38	0.46	0.56	0.72	0.80
Abnormal synovium	2	0.40	0.56	0.69	0.84	1.17
Brain	1	0.11	0.20	0.25	0.39	0.89
Brain	3	0.09	0.14	0.24	0.37	0.54
Muscle	1	0.20	0.41	0.54	0.70	1.01
Muscle	2	0.44	0.52	0.72	0.93	1.50
Skin	2	0.18	0.30	0.37	0.48	0.98
Skin	3	0.20	0.29	0.44	0.63	0.78
Kidney	2	0.54	0.57	0.75	1.00	1.38
Kidney	3	0.34	0.51	0.76	1.04	0.92
Serum (cadaver)	1	0.62	0.92	1.23	1.41	1.50
Serum (normal)	2	1.35	1.70	2.20	2.75	2.40

TABLE 7. ELUTION PHASE—CALCULATED TISSUE:DIALYSATE RATIOS (BASED ON DIALYSATE USED FOR EQUILIBRATION)

Tissue	Exp.	1 min	15 min	30 min	60 min	120 min	4 hr	Observed ratio in bag at end of study
Normal thyroid	2	2.55	2.35	2.21	2.08	1.96	1.90	1.83
Normal thyroid	3	0.98	0.82	0.71	0.58	0.48	0.40	0.37
Normal thyroid	3	1.04	0.88	0.77	0.64	0.50	0.44	0.27
Myxedema thyroid	3	0.61	0.45	0.35	0.21	0.09	0.02	0.05
Liver	1	2.44	2.38	2.35	2.25	2.28	2.26	2.18
Liver	2	4.27	4.26	4.26	4.25	4.25	4.25	3.49
Normal synovium	1	0.35	0.26	0.20	0.13	0.06	0.03	0.03
Normal synovium	2	0.83	0.55	0.41	0.21	0.05	—0.05	0.05
Normal synovium	3	0.76	0.58	0.49	0.36	0.23	0.18	0.16
Abnormal synovium	1	0.73	0.53	0.40	0.25	0.13	0.07	0.07
Abnormal synovium	2	1.10	0.82	0.64	0.41	0.21	0.16	0.16
Brain	1	0.78	0.66	0.60	0.53	0.43	0.29	0.29
Brain	3	0.50	0.44	0.40	0.34	0.26	0.19	0.16
Muscle	1	0.96	0.81	0.73	0.63	0.56	0.48	0.47
Muscle	2	1.45	1.19	1.06	0.87	0.72	0.63	0.56
Skin	2	0.94	0.82	0.77	0.66	0.52	0.40	0.35
Skin	3	0.74	0.60	0.58	0.47	0.36	0.26	0.17
Kidney	2	1.32	1.00	0.84	0.66	0.40	0.26	0.24
Kidney	3	0.88	0.68	0.59	0.46	0.32	0.26	0.18
Serum (cadaver)	1	1.43	1.00	0.75	0.22	0.10	0.02	0.02
Serum (normal)	2	2.33	1.77	1.43	0.70	0.36	0.23	0.16

studied. Results of averaging all available values for a given tissue are shown graphically in Figs. 5 and 6. Human serum protein binds $^{99m}TcO_4^-$ very rapidly and binding levels after 18 hr of incubation are no higher than after 2 hr. The serum specimens also were very quick to release $^{99m}TcO_4^-$ when dialyzed against a solution of nonradioactive serum. The normal synovium specimens concentrated and released the $^{99m}TcO_4^-$ in a manner very similar to that of serum although at a lower level. This was also true of the abnormal synovium specimens studied, except that some increase in radioactivity concentration was noted between 2 and 18 hr. Samples of brain tissue showed a relatively low level of concentration, particularly during the first 2 hr. After 18 hr of incubation the brain concentration ratio remained below 1.0 but residual concentration failed to drop to ratios as low as those of serum even after 4 hr of leaching dialysis. Samples of skin, muscle, and kidney tissues show a relatively slow uptake of the tracer with a peak concentration at 18 hr considerably greater than that after 2 hr of incubation. These tissues released $^{99m}TcO_4^-$ fairly rapidly during the leaching dialysis against nonradioacitve serum but showed some relative retention when compared with the specimens of serum or synovium. Both the normal thyroid tissues and a specimen removed from a patient with severe myxedema showed an early uptake of $^{99m}TcO_4^-$ comparable to that of the other tissues although at a higher level than any other tissues except serum. The normal thyroid tissues continued to concentrate the radioactivity leading to a relatively high concentration ratio after 18-hr incubation. The specimen of myxedema thyroid showed no further increase in radioactivity after 2 hr. During the second, "leaching" experiment, the specimen of myxedema thyroid released virtually all of its radioactivity. On the other hand the specimens of normal thyroid retained a large fraction of their radioactivity even after 4 hr of incubation against nonradioactive serum. This finding suggests some tightly and possibly "irreversible" binding to the thyroid tissue.

The studies of liver tissues, shown only in the tables, showed a pattern of uptake during the first 2 hr of incubation comparable to that of the other tissues studied with the concentration ratios higher than any of the other tissues except serum. Subsequently, however, the liver tissue continued to concentrate radioactivity in a fashion which was slowly if at all reversible. After 18-hr incubation, the liver:medium concentration ratio was even higher than the serum:medium ratio. Subsequently, during the leaching experiments the hepatic tissue released virtually none of its radioactivity into the bathing nonradioactive serum. These results strongly suggest that the mechanism of binding to the liver differs from that of other tissues or that a barrier to release has been formed. To evaluate the possible clinical significance of this observation, scanning over the liver area was performed in patients 6 hr after intravenous administration of $^{99m}TcO_4^-$ for brain scan-

ning. No significant increase in concentration of radioactivity was seen in the hepatic area. This discrepancy supports the postulated barrier mechanism which may in part be due to postmortem changes within the liver.

DISCUSSION

The usual distribution of $^{99m}TcO_4^-$ in the various organs and tissues of the body has been clinically described in connection with brain scanning, scanning of the salivary glands, physiologic studies of the thyroid, thyroid scanning, joint scanning, stomach scanning, and scanning for abnormal areas within the intestine. In most of these areas transport of $^{99m}TcO_4^-$ appears to be comparable to that of iodide. However, it has been observed that the total-body distribution of $^{99m}TcO_4^-$ is quite different from that of iodide. Serum radioactivity concentrations after intravenous injection tend to be higher during the early hours with a greater ultimate volume of distribution. Serum binding of the ion, documented here, can explain this early relative increase in serum radioactivity concentration and the high tissue binding affinity for pertechnetate ion shown in this study can account for the ultimate distribution into a relatively large volume.

In studies of the kinetics of joint scanning, we have demonstrated that differentiation between normal and abnormal joints is most clearly delineated within the first 30 min after isotope injection (*6*). In the present study, both normal and abnormal synovia concentrate the radioactivity quickly with early concentration ratios greater than those for skin and

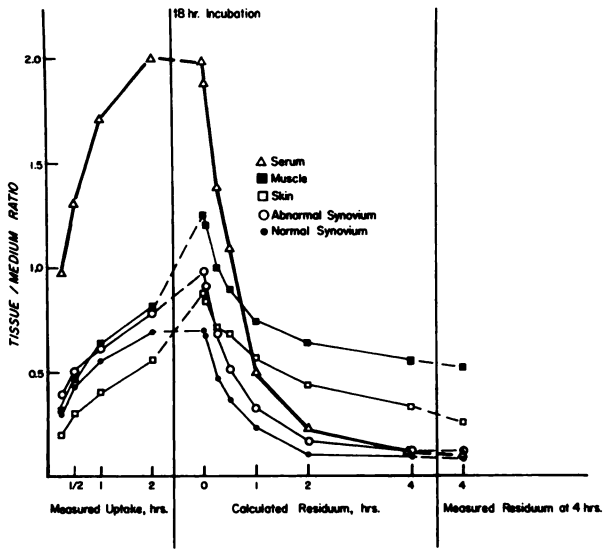

FIG. 5. Dialysis studies with minced human tissues. Vertical axis is [radioactivity in the tissue (corrected for blank)]:[radioactivity in buffer]. Horizontal axis is divided into three experimental periods: dialysis of tissue against buffer; dialysis against nonradioactive serum; and final tissue residual radioactivity.

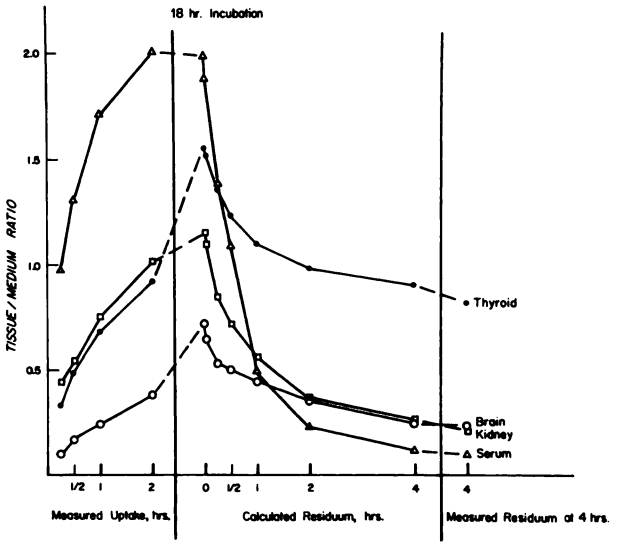

FIG. 6. Dialysis studies with minced human tissues. Vertical axis is [radioactivity in the tissue (corrected for blank)]:[radioactivity in buffer]. Horizontal axis is divided into three experimental periods: dialysis of tissue against buffer; dialysis against nonradioactive serum; and final tissue residual radioactivity.

comparable with those for muscle. The concentration ratio for abnormal synovium is not significantly greater than that for normal synovium but the greater amount of synovium present in both the acute and chronically inflamed joint may markedly increase this difference. This is supported by our failure to observe scan negativity in the presence of clinically enlarged synovium. Increased blood flow probably causes the difference in timing noted clinically.

It has been demonstrated in the rat that under certain conditions a non-pertechnetate technetium product is formed after uptake by the thyroid (*7,8*). This product is not dischargeable after inhibition of the thyroidal trapping mechanism. This observation is analogous to the in vitro situation seen in this study. In the present experiment the rate of formation of nonremovable materials in the thyroid tissue was intermediate between the very high rate observed in the liver and the much lower rate observed in other tissues. As the intact human thyroid has been observed to discharge virtually all of the previously concentrated $^{99m}TcO_4^-$ when the concentrating mechanism is inhibited (*9,10*), it seems likely that the irreversible mechanism observed in this experiment is characteristic of the in vitro situation using postmortem tissues. There is no evidence in these studies of an active "trap" in the thyroid because the tissue:medium concentration ratios do not reach unity until after 120 min. Binding of $^{99m}TcO_4^-$ by thyroglobulin remains a possibility which cannot be ruled out.

The observation that pertechnetate is relatively

excluded by brain tissue, even when the tissue is a postmortem specimen in which no blood-brain barrier mechanism should be functioning, is of interest. It seems likely that normal brain tissue has some mechanism for relative exclusion of this ion even in the absence of the usual physiologic mechanisms. This may be related to the relative hydrophobicity of brain tissue in which the lipid insoluble ion cannot readily dissolve. It would be of interest to do similar studies using brain tumor tissues.

Human serum albumin has been labeled with $^{99m}TcO_4^-$ for use in the nuclear medicine laboratory (e.g., *11*). To achieve true labeling, reduction of the pertechnetate ion has been found to be necessary. It is important to distinguish between this labeled albumin resulting from a deliberate radiochemical procedure and the loosely bound labeled albumin described here.

While our studies do not prove that albumin is the only serum protein which binds $^{99m}TcO_4^-$, they show it to be the major binding protein and sufficient to explain the observed phenomena. It is easily displaced by increase in temperature, increase in pH, or by the presence of a variety of competing molecules. This serum binding probably serves as a transport mechanism for delivery of the pertechnetate ion to its large tissue pool.

The exact binding affinity cannot be measured since carrier pertechnetate is not available so that the precise number of moles of pertechnetate per mole protein for different levels of free ligand concentration cannot be determined. However, all the evidence points to a low degree of kinetic stability which would imply a rapid diffusion of the ligand away from the albumin molecule. In the case of the extremely small amount of radioactivity tightly bound to albumin, a different mechanism must be involved, the significance of which is not clear at present. Where binding of the pertechnetate to whole cells or tissues were studied, conclusions in molecular terms are precluded because of the possibility of a diffusion or other barrier becoming established which cannot be distinguished from tight binding.

The possibility that binding characteristics may underlie scan positivity can be entertained. Although blood flow plays a role, the binding of pertechnetate to serum albumin and to tissues such as synovia must be important. Where a positive scan is due to a localized area of inflammation in soft tissues, extravasated serum albumin may bind the pertechnetate more than do the surrounding or normal tissues. Finally, exclusion of pertechnetate by the normal brain may well be due to the hydrophilic nature of the pertechnetate ion.

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