estimates made for individual and population doses was not recognized in the official studies.

However, the best way for a reader of *The Journal of Nuclear Medicine* to determine what my report says is to send for a copy of either the four-page summary or the full 300-page report. Copies can be obtained from the Three Mile Island Public Health Fund, 1622 Locust St, Philadelphia, PA 19103.

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## Electrophoretic Analysis of Technetium-99m MDP Complexes

**TO THE EDITOR:** In a recent journal article (*1*), Najafi and Hutchinson addressed a very important question: "what is the explanation for the occasional liver uptake in bone scintigraphy which is not readily explained by findings on paper chromatography?" The approach by the authors to answer this question and their subsequent conclusions are the subject of this correspondence.

As the authors stated, it is difficult to do preparative work with electrophoresis to study the biological behavior of each complex individually. Their goal of using electrophoresis to find conditions for the formation of a single technetium-99m (Sn) methylene diphosphonate ($^{99m}$Tc(Sn)MDP) complex appears naive to us, and several points should be considered in interpretating their data.

Electrophoresis separates on the basis of charge. The charge on the $^{99m}$Tc (Sn) MDP complex is a function of the pH of the solvent. Unfortunately, acetate (pKa = 4.75) is not a buffer at pH 7, so it is difficult to know the pH during electrophoresis. The authors' titration of MDP shows that the $pKa_3$ is ~7. At 0.02M, MDP likely was acting as its own buffer during electrophoresis. However, MDP would only be an effective buffer over the range of pH 6–8, with the buffering capacity greatest at pH 7 and weakest at the extreme of the range.

The authors followed the electrophoretic movements of radioactive complexes and showed that $^{99m}$Tc (Sn) MDP is the major complex. Addition of almost equimolar amounts of competing cations, phosphate, and methylphosphonate, will disrupt this complex. Their peaks C and D are likely the +2 and +3 complexes of $^{99m}$Tc (Sn) MDP. Assuming the pH of the preparation is the pH of electrophoresis, the presence of equal amounts of C and D at pH 6 would indicate a $pKa_3$ of ~6 for the $^{99m}$Tc (Sn) MDP. It is also reasonable to expect similar images (Figs. 7 and 8) using radiopharmaceuticals containing only C or D because they are different ionic species of the same chelate and would probably be identical in blood.

One of the main reasons that MDP has wide-spread use for bone imaging is that it is much less likely to be hydrolized than pyrophosphate. If the authors' hydrolysis scheme can be documented, a reference would be most helpful. Their hydrolysis of MDP shows the formation of methylphos*phonate* and their reference for synthesis is for methylphos*phonate*, but the text refers only to methylphos*phate*. This is quite confusing. It is reasonable to assume that adding almost equimolar amounts of a competing cation would disrupt the $^{99m}$Tc (Sn) MDP complex but the authors have not shown that hydrolysis happens in their kit (solution) or in commercial kits (lyophilized).

Although pH probably plays an important role in bone imaging with Tc-labeled diphosphonates, the authors neglected the role of the stannous ion and the effects of aging on stannous ion. The authors give no information on the pH of the commercial kit preparations. It should be noted that the Squibb kit contains ascorbic acid as a stabilizer while the Mallinckrodt kit does not.

The authors do state that high performance liquid chromatographic analysis would have been a much more informative system for the characterization of these complexes.

We, then, would urge readers to be skeptical in their conclusions of this report. To state that the reason for the occasional liver uptake seen in bone scintigraphy is due to the presence of methylphos*phate* or methylphos*phon*ate in MDP kits, we feel, is not warranted from the data reported.

### References


1. Najafi A, Hutchinson N: Electrophoretic analysis of different technetium-99m (SnC12) methylene diphosphonate complexes. *J Nucl Med* 26:524–530, 1985



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**REPLY:** We thank Drs. Shafer, and Elson for their comments concerning our recent article (*1*) in this Journal.

In this article we have *tried* to address and explore the reasons of occasional liver uptake in bone scintigraphy not readily explained by findings on paper chromatography. Indeed, at no place in this article did we attempt to show that this issue is solved nor that our effort to solve this problem has ceased. We have shown, however, in this article that the presence of methylphosphonate (which was stated methylphosphate incorrectly) in MDP kits will give rise to an increase in concentration of peak A according to our electrophoretic analysis which ultimately gives rise to accumulation of activity in the liver of a rabbit. Trace amounts of peak A were found in most of our technetium-99m ($SnCl_2$) methylene diphosphonate preparations including those that were prepared by using Mallinckrodt or Squibb MDP kits. In addition high performance liquid chromatography (HPLC) analysis on a 5-mo-old solution of methylene diphosphonate pH = 7 revealed the presence of methylphosphonate. We agree that the carbon-phosphorus bond is

very strong and therefore not easily hydrolyzable. We are puzzled by this ourselves.

HPLC analysis of $^{99m}Tc$ ($NaBH_4$) MDP at no carrier added level has shown only one peak. The results of our HPLC analysis using a DEAE ion exchange column* on $^{99m}Tc$ ($SnCl_2$) MDP suggested entrapment, followed by a slow release of the radioactivity from the column. Therefore, although it may appear naive, we had no other choice but to use electrophoresis for analysis and to try to find different conditions that only one peak produced. Of course the number of experiments for finding such different conditions were limited; however there are always more experiments to be performed. In addition in a *true* oxygen free environment, $SnCl_2$ should be stable indefinitely, and therefore Sn should not age.

We agree that the pH of the solvent will effect the charge on the $^{99m}Tc$ (Sn) MDP complex. However since we used 0.1 N acetate buffer (solution) pH = 7.0 as the electrophoresis solvent and standardized each test such that only 1.0 $\mu$l of the labeled MDP (0.02*M*) solution was added to the electrophoretic paper saturated with 0.1 N acetate buffer at pH = 7, we expect the pH remains at 7 or very close to it. In fact by using colored solution, we estimated the area of the acetate saturated paper that 1.0 $\mu$l of MDP would effect to be 0.4 $cm^2$. We measured the acetate content of this area to be 20 $\mu$l. If we assume that the 1.0 $\mu$l of MDP and 20 $\mu$L of acetate will mix on the paper, the variation of pH would be between 6.7–7.1 with the addition of MDP at pHs between 4.5–7.5.

Our titration of MDP confirmed the results found by others that MDP has $pKa_3 \sim 7$. The titration curve, however showed that over the range of pH 6–8, MDP is present with a -2 or -3 charge as competing anions (not cations), and therefore at pH above 6 very little but nevertheless some $MDP^{-3}$ is present. We thought $MDP^{-3}$ may have higher formation constant than $MDP^{-2}$ for complexing with either Sn or Tc and although at pH = 6.5 the mass amount of $MDP^{-3}$ is less than $MDP^{-2}$, it is enough to bind to all the technetium present.

We do know that the Squibb kit contains ascorbic acid. We think that ascorbic acid may also disrupt the MDP complex formation. The additional peak E demonstrated in these kits is probably associated with this.

Finally since completion of this study, we have prepared our "in house" MDP kit at pH = 7.0 and lyophilized them for 3 days (at 25°C and 45 mtorr). We have not noticed any liver uptake in the bone scans in our clinical laboratory using these kits and electrophoretic analysis has shown absence of peaks A and B.

## FOOTNOTE

†Water Assoc., Milford, MA.

### References


1. Najafi A, Hutchinson N: Electrophoretic analysis of different technetium-99m ($SnCl_2$) methylene diphosphonate complexes. *J Nucl Med* 26:524–530, 1985



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## Measurement of Glomerular Filtration Rate Using Technetium-99m DTPA

Recently Gates (*1*, *2*) proposed a simple technique for measuring glomerular filtration rate (GFR) without blood samples. In patients who were undergoing technetium-99m diethylenetriaminepentaacetic acid ([$^{99m}Tc$]DTPA) renography total GFR values were computed from the data stored in a computer during the early period of the study by reference to a calibration curve. The calibration curve was derived from data on patients who had undergone [$^{99m}Tc$]DTPA renography and had GFR measured by one of the established techniques, e.g., the one-injection–two blood sample technique of Brochner-Mortensen (*3*). Unlike a similar method proposed by Lee et al. (*4*) Gates' technique required no additional images to those acquired for routine renal studies and the only additional requirement was the counting of a [$^{99m}Tc$]DTPA standard made up from the vial used for injection.

Gates' method of deriving GFR is based on his finding that there is a strong correlation (r = 0.97, n = 51 Sy.x = 7 ml/min) between the percentage of [$^{99m}Tc$]DTPA in the kidneys during the early phase of the renogram and GFR. To calculate the percentage renal uptake it is necessary to allow for gamma-ray attenuation by tissues overlying the kidneys which in turn, requires a knowledge of kidney depth. Gates suggests that kidney depth may be predicted with sufficient accuracy using a relationship, proposed by Tonnesen et al. (*5*) between patients weight/height (w/h) ratio and kidney depth. This latter relationship was derived from ultrasound measurements.

We have attempted to confirm Gates' finding but obtained poor correlation between percentage [$^{99m}Tc$]DTPA uptake and GFR both in adults (r = 0.37, n = 32, Sy.x = 23 ml/min.) and in children (r = 0.53, n = 32, Sy.x = 18 ml/min.). This lack of correlation is not due to labeling deficiency as the average labeling efficiency of the [$^{99m}Tc$]DTPA was greater than 98%. The lack of correlation may be due to the inaccuracy in predicting kidney depth from the regression equation of Tonnesen et al. Tonnesen et al. did not quote a standard error on their regression equations relating w/h ratio to kidney depth but a correlation coefficient of r = 0.865 suggests that it is substantial. In our institution a study comparing kidney depth measurements obtained by the w/h ratio of Tonnesen et al. and depth measurements obtained by skin-surface marker to center of [$^{99m}Tc$]DMSA activity in each kidney yielded a standard error of 2 cm. With our camera-collimator system (GE 400T) this difference would yield a 33% error in renal uptake for a commonly found kidney depth of 6 cm.

In conclusion, therefore, we suggest that GFR measurements obtained by the method of Gates are unreliable unless an accurate means of measuring kidney depth is utilized.

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