GEL CHROMATOGRAPHY IN THE ANALYSIS OF ^{99m}Tc RADIOPHARMACEUTICALS

In a recent article entitled "A possible artifact in gel chromatography of some ^{99m}Tc-chelates" (*1*), Valk, Dilts, and McRae expressed doubts about the reliability of Sephadex columns in the analysis of radiopharmaceutical products. They found that when samples of ^{99m}Tc-Sn gluconate were eluted with saline from a Sephadex G-25 column, there was considerable variation from sample to sample in the fraction of activity retained by the column. However, in spite of this irregular chromatographic behavior, all eluted fractions exhibited the same biological activity in the rat as the unfiltered preparation. They drew the conclusion that "the results of analytical separations must be correlated with studies of the biological behavior of the separated fractions to establish the validity of the analytical technique," i.e., gel chromatography.

During the past year a collaborative research program has been carried on between the Division of Nuclear Medicine, Downstate Medical Center, and the Department of Applied Science, Brookhaven National Laboratory, for the study of the chemistry of reduced pertechnetate in the presence of weak complexing agents including sodium gluconate. The work is based on the phenomenon which Valk, et al have called "possible artifacts" in their article. They reasoned (correctly, we believe) that some exchange of technetium may have occurred between weakly complexed technetium and the Sephadex gel. The latter is a cross-linked polysaccharide, and McRae, et al had reported that pertechnetate which is reduced with Sn(II) will complex with carbohydrates (*2*). The basis of our research (briefly outlined in this letter) is set forth on the next page.

In the vast majority of gel chromatographic separations, the largest particles—colloids and macromolecules—do not permeate the gel matrix to any extent and thus traverse the column most rapidly and are eluted first. Small particles and molecules permeate the gel matrix to the greatest extent and therefore traverse the column slowly and hence are eluted later. Generally in gel chromatography the assumption is made that there is no specific interaction between the sample solute and the gel. We think, however, that in the present case there is a definite interaction between technetium and the Sephadex. The research is organized on the basis of the following hypothesis: a reduced technetium species (valence as yet unknown) forms weak complexes with compounds containing hydroxyl groups (polyols and substituted polyols), and that Sephadex gels which possess such groups can have an affinity for reduced technetium, thus retaining it on the column. If the hypothesis has validity, one should be able to study (at least qualitatively) the relative strengths of weak complexes of this technetium species by placing an appropriate solution on a Sephadex column and eluting the column with various concentrations of different water-soluble polyols. The resulting elution pattern will then depend on the concentration of the eluant and its complexing ability for the reduced species relative to that of the Sephadex. If the eluant is a very dilute solution of a weak complex former, one may observe varying amounts of technetium retained on the column or a considerable amount of tailing. A more concentrated solution of the same eluant could yield a satisfactory separation of the technetium from the column. Thus, we have found that when $^{99m}TcO_4^-$ is reduced with either $SnCl_2$ or $NaBH_4$ in the presence of sodium or calcium gluconate and an aliquot of the reaction mixture is eluted from a Sephadex column with 0.2 *M* (or even 0.1 *M*) sodium gluconate (at pH 8), practically all of the technetium activity appears in the fraction where the complex would normally appear. However, when elutions of a similar aliquot of the same reaction mixture from a similar column were carried out with saline solution, either no activity or a small fraction of the total activity was found in the eluate, depending on the initial concentration of gluconate in the reaction mixture, the volume of the aliquot taken, and other factors. This is what we believe did happen in the work of Valk, Dilts, and McRae since they used 1 ml of a gluconate solution with concentration range from 1–10% (W/V). This also explains their finding that the chelate fraction eluted with saline from one column contained more than 95% of the applied activity, but that an aliquot of this chelate fraction applied to a second column and eluted with saline lost 65% of *its* activity to the second column. We attribute this reported difference to dilution of the gluconate by the saline eluant in the second chromatographing.

If Sephadex gels are to be used for the analysis of relatively weak complexes of reduced pertechnetate, the eluant should not be a saline solution but rather a solution of the complexing compound at a sufficiently high concentration to ensure a satisfactory chromatographic separation from any residual pertechnetate.

The conclusion which we draw from our observations and those of Valk, Dilts, and McRae is that a deeper knowledge of the chemistry of reduced technetium in water is urgently needed. In this connection, separations on paper will probably produce

phenomena similar to those seen on Sephadex, since paper is also a polysaccharide, and the reduced pertechnetate species will, we believe, be held on cellulose as it is on Sephadex. This is not an artifact but rather a consequence of a specific and ascertainable interaction between the solid phase of the separating system and the desired solute in solution. Its appearance should not condemn the particular system of separation. An understanding of it will permit a more general and more intelligent use of the analytical tool of which it is an integral part.

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THE AUTHORS' REPLY

As stated in our previous communication (*1*), we also believe that there is interaction between reduced ^{99m}Tc and Sephadex. Exchange of ^{99m}Tc between a chelating agent and Sephadex during chromatography must depend on the relative affinities of chelating agent and Sephadex for ^{99m}Tc and on the concentration of chelating agent present. However, this relationship between concentration of the chelating agent gluconate and fraction of activity retained on the column is not as straightforward as suggested by Steigman, et al. They state that, when eluting with saline, the fraction of activity found in the eluate depends on "the initial concentration of gluconate in the reaction mixture, the volume of the aliquot taken, and other factors." It appears that the "other factors" are very significant. We did *not* use gluconate solutions of varying concentrations and in the description of methods we stated that 10% Ca gluconate was used in these experiments. The volume of the aliquot taken was always 1 ml. Despite these constant factors, retention of activity by the gel varied from less than 5%–70% on the first pass through the column. The explanation suggested by Steigman, et al is consistent with the results of one of our experiments but is not consistent with the results of the four other experiments reported by us.

We agree with Steigman, et al that dissociation of weak ^{99m}Tc-chelates during gel chromatography is most probably a result of dilution of the chelating agent by saline eluant. We have also observed the effect of dilution in separations performed on paper. When ^{99m}Tc-Sn-gluconate is analyzed by paper chromatography in 85% methanol, no clear peak is obtained. Instead, a band of variable height is seen beginning at the origin and extending a variable distance from the origin. If the paper is impregnated with 10% Ca gluconate and dried before the sample is applied, chromatography with methanol shows a single peak at the origin. These findings suggest that dilution on paper does cause breakdown of the chelate. Oxygen was not excluded from our system and the reduced technetium was probably reoxidized during chromatography and then migrated as TcO_4.

Interaction between reduced technetium and polysaccharides is also seen in situations where no dilution occurs. When ultrafiltration of ^{99m}Tc-Sn-gluconate was performed using a cellophane filter, 30–60% of the activity adhered to the filter and did not appear in the filtrate.

We agree with Steigman, et al that further knowledge of technetium chemistry is needed. The above phenomena must all result from specific chemical interactions and in this sense they are not artifacts. However, when an analytical system is used to determine the chemical components of a radiopharmaceutical, any component that forms during analysis and which was not present in the original sample is an artifact.

Gel chromatography is a valuable technique for studying the chemistry of technetium and for analysis of technetium compounds. However, when any technique is used for analysis of radiopharmaceuticals, results should be correlated with biological studies whenever possible in order to avoid misinterpretation of results. If such correlation had not been carried out in this case, the phenomenon under discussion would not have been detected.

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