Development of a New Cholescintigraphic Agent
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It is reported that lipophilic character of a chelate may be responsible for the rapid excretion through the bile. On this basis, a lipophilic $^{99m}$Tc-penicillamine ethyl ester ($^{99m}$Tc-Pen ethyl ester) was chosen for this study.

The $^{99m}$Tc-Pen ethyl ester was prepared using the SnCl$_2$ method and analyzed by thin layer chromatography and paper electrophoresis. Various complexes were detected, whenever a slight change of the labeling condition, such as pH, concentration of SnCl$_2$, reaction time, was carried out. Among these complexes, a high recovery of $^{99m}$Tc in the bile of rats was observed in a complex prepared by addition of a freshly prepared solution of $2 \times 10^{-2}$ M of SnCl$_2$ to a mixture of $^{99m}$TcO$_4^-$, $10^{-2}$ M of Pen ethyl ester and phosphate buffer (pH 7.7). This complex was more than 96% extractable into ethyl acetate and excreted through the bile more rapidly than the unesterified complex ($^{99m}$Tc-Pen Complex I). This result clearly indicates that the lipophilicity of a chelate closely relates with a rapid hepatobiliary clearance.

When this complex was intravenously administered in mice, the radioactivity retained in kidney, liver and blood were considerably higher than observed in $^{99m}$Tc-Pen Complex I, though these two complexes were much the same in the concentration in gallbladder. This phenomenon can show the lower stability in vivo of $^{99m}$Tc-Pen ethyl ester.

In order to protect this complex from the decomposition in vivo by stabilizing it chemically, histidine was added to it. The addition of histidine showed a better organ distribution.

Evaluation of Resin-Sn$^{2+}$ and Its Application on $^{99m}$Tc-DOPA Labeling
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In our pursuit for technetium labeling mechanism, DOPA (3-(3,4-dihydroxyphenyl)-L-alanine), a clinically interesting biogenic amine, was considered as an interesting ligand to be studied, since in its presence, hydrolysis is predicted to compete with (monomer) complex reaction formation.

The labeling method itself is a very simple and rapid procedure because only a mixing of DOPA ($10^{-2}$M), buffer solution (Na acetate, pH 5.6) and $^{99m}$TcO$_4^-$ eluate (2 ml) in a syringe followed by the addition of Resin-Sn$^{2+}$ ($10^{-3}$M, 2 mg) with an up-side-down movement is involved. The mixture is ready to be injected after a filtration through a Millipore filter.

Sephadex column chromatography (G-15) was used for the analysis and from the elution time the monomer and the polymer complexes were determined, which showed a great similarity to the data reported on penicillamine-$^{99m}$Tc complexes.

A very severe pH range, concentration of Resin-Sn$^{2+}$ or DOPA was needed for the optimal condition of a monomer complex preparation, as compared with the labeling of penicillamine. The use of Sn$^{2+}$ in solution even at very low concentration produced a rather high percentage of polymer complex. The use of Sn$^{2+}$ adsorbed on cation exchange resin (Resin-Sn$^{2+}$), prevented the hydrolyzed complexes and increased the monomer complex yield.

A selective mechanism is postulated. Once the


\[ 99m\text{TcO}_4^- \text{ is reduced to } \text{TcO}_3^{2-}, \text{ the labeling reaction proceeds through a competitive reaction between the formation of the complex and hydrolysis of the reduced } 99m\text{Tc species, depending upon the ligand coordinating atoms or the bonding strength with technetium.} \]

An O-O coordinating ligand, such as DOPA, ascorbic acid, where the hydrolysis easily took its course, the use of Resin-Sn\(^{2+}\) appeared as an effective tool to be applicable. So even with this very week chelate, a monomer complex was able to be labeled. In animals studies, this complex showed higher stability than the polymer complex.

**In Vitro Studies on Tc (IV) and Its Human Serum Albumin Binding**

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The hydrolysis and the human serum albumin (HSA) binding of tetravalent TcCl\(_6^{2-}\) were investigated in vitro by the use of \(^{99}\text{Tc}. UV absorption spectra showed that TcCl\(_6^{2-}\) was stable for 20 min or so at pH 3 and decomposed immediately at pH 7. But the valency of Tc was assumed to be kept IV. When Tc (IV) was reacted with HSA, Tc was considered to bind to HSA in a monomeric Tc (IV) state such as TcCl\(_6^{2-}\) at both pH regions. The number of binding sites was 2 and the association constants were approximately \(1.6 \times 10^4 \text{ M}^{-1}\) and \(2.5 \times 10^4 \text{ M}^{-1}\) at pH 3 and 7, respectively. The values of association constants showed that these bindings were attributed to coordinating bonds. The fact that the association constants of the monomer Tc(IV)-HSA interaction were not much higher than these of other metals and organic compounds suggested that the monomer Tc(IV)-HSA complex was not always stable in vivo. While, it was observed that the polymer Tc(IV) appeared if more than \(1 \times 10^{-5} \text{ M}\) of Tc(IV) concentration was used at pH 7. At pH 7 TcCl\(_6^{2-}\) was hydrolyzed to the various species of Tc (IV), which had various reactivities to HSA. It was assumed that some of the hydrolyzed Tc(IV) species had high affinity for biopolymers such as HSA.

**Simple Method for Preparing \(^{99}\text{Tc}-sulfur Colloid with Metallic Sn.**

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Various kinds of \(^{99}\text{Tc}-colloid have been used for imaging the liver so far, and \(^{99}\text{Tc-Sn-phytate is widely used due to the simplicity of preparation at the present time. However, this is not satisfactory, since kidneys are always visualized as well. Therefore we investigated a new simple method of preparation of liver imaging agent considering the method with sulfur colloid, which has been known to visualize the liver but kidneys.**

Procedures

Five ml of \(^{99}\text{TcO}_4^-\) solution and 1 \(\mu\)l of 1 N HCl are added. The solution is eluted through the column containing Sn and Ag metal plates, mixed with 25 \(\mu\)l of sulfur solution consist of Na\(_2\)S and sulfur 5 mg/ml each, stirred, and pH is adjusted below 3. The efficiency of labelling was between 96\% and 97\%. A beautiful image of liver and spleen, but kidneys was obtained using the agent prepared as mentioned above.