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$^{99m}$Tc-H-D RBC as the spleen scanning agent is prepared by using reducing agent, $^{99m}$TeO$_4^-$ and heating (49.5°C, 15 minutes). The object of the present study is to evaluate the osmotic fragility of the membrane of $^{99m}$Tc-H-D RBC to evaluate the mechanism of the spleen sequestration of $^{99m}$Tc-H-D RBC. For this purpose the C.P.C. (Coil Planet Centrifuge) method was used. This new method provided us with more sensitive and quantitative information than the ordinary Parpart method on the osmotic fragility of the membrane of RBC.

The results were as follows: 1) The osmotic fragility of the membrane of $^{99m}$Tc-H-D RBC is remarkably elevated by the heating (15 or 30 minutes), whereas the difference between 15 and 30 minutes is not clearly seen. Clinically the agent heated 15 minutes is most suitable for spleen specific scan but the one heated 30 minutes isn’t suitable for the imaging of liver and spleen. 2) The addition of reducing agent and/or $^{99m}$TeO$_4^-$ also showed slight elevation of the osmotic fragility of the membrane. It is concluded that osmotic fragility is not the single factor of splenic sequestration of $^{99m}$Tc-H-D RBC.

Application of $^{99m}$Tc-Labelled Heat-Denatured Erythrocytes to Splenic Clearance Study

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Splenic clearance rate of denaturated erythrocytes has been measured usually with $^{51}$Cr-labelled cells to investigate splenic function. In this report, we examined if or not $^{99m}$Tc-labelled erythrocytes can be used for this purpose in association with spleen scintigraphy.

Erythrocytes were at first treated with stannous pyrophosphate in saline solution for 5 minutes at the room temperature and then incubated with pertechnetate. The labelled erythrocyte suspended in the saline were incubated at 49.0±0.5°C for 15 minutes. At the end of each step, erythrocytes were washed sufficiently with the saline.

On observation with scanning electron microscope, SEM, erythrocytes appeared just slightly spherocytic after the tinning procedure. Their osmotic resistance was slightly reduced as examined by coil planet centrifuge method.

After the heat-denaturation process, $^{99m}$Tc-labelled erythrocytes were more uniformly spherocytic than $^{51}$Cr-labelled ones which beared numerous protrusions and might be called echino-acantho-spherocyte. Significant difference was not observed in the 50% hemolysing saline concentration between $^{99m}$Tc-heat-denatureted cells and $^{51}$Cr-cells which had been incubeted at the same temperature for 45 minutes, by “Parpart’s method”, while the range of hemolysis was significantly more narrow in the former than in the latter. The disappearance curve of the former in the circulation was plone to simulate single exponential fashion. When the individual variation of the damage degree was corrected using osmotic fragility, average difference in clearance rate between these cells was only 4.3% of an average of normal clearance rate in 32 measurement in 30 cases.

From these results, it was concluded that tinning process brought about a slight damage on the cell membrane, which enhanced the labelling efficiency and made the denaturation effect more
uniform, and that \(^{99}\text{Tc}\)-heat-denatured cells could be used for splenic clearance study in stead of \(^{51}\text{Cr}\)-ones.

Double tracing study using \(^{99}\text{Tc}\)-heat-cells and \(^{51}\text{Cr}\)-labelled NEN (N-ethylmaleimid)-treated cells was carried out in 30 subjects. The result was satisfactory so far as the double tracing technique was concerned.

Spleen Scintigraphy with \(^{99}\text{Tc}\)-Labelled red Blood Cells

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Recently a new kit for \(^{99}\text{Tc}\)-labelled red blood cells (RBCs) has been available and utilizing this kit, spleen scintigraphy was done. Method is as follows: 1 withdraw from patient 2 ml of heparinized blood. 2 Add 0.3 \(\mu\)g of stannous pyrophosphate solution, prepared just before use and incubate for 5 minutes at room temperature, mixing gently. 3 Centrifuge and discard all the plasma layer. 4 Add 0.5–1.5 mCi (about 0.5 ml) of \(^{99}\text{Tc}\)-pertechnetate and incubate for 5 minutes at room temperature mixing gently. 5 Wash and centrifuge the suspension and adjust its volume to 2 ml with physiologic saline. 6 Place the suspension in water bath for 15 minutes at 49.5 ±0.5°C, or add 1.5 mg of Bromomercuryhydroxypropane (BMHP) to the suspension. 7 Inject and 30 minutes—3 hours later take spleen scintigraphy.

Comparing above standard method of 84.9% labelling yields, smaller amount of stannous pyrophosphate (0.12 \(\mu\)g) showed higher labelling yields of RBCs 90.5%. Concerning to time for heat damaged RBCs, 20–30 minutes is suitable because of cardiac image from incomplete damage and of hepatic image from exess damage to RBCs. It is better to use BMHP for routine spleen scintigraphy because this method is much less troublesome and it takes much shorter wasting time.

Spleen scintigraphy with \(^{99}\text{Tc}\)-RBCs kit (TCK-11) from CIS is rather simple and gives smaller amounts of radiation to medical stuffs for preparation and spleen scintigrams are good, even in the case of splenomegaly. \(^{99}\text{Tc}\)-spleen scintigraphy gives lesser radiation to patients and much less interference to other isotopic tests or examinations than \(^{51}\text{Cr}\)-spleen scintigraphy.