TECHNICAL DIFFICULTIES IN ^{99m}Tc-LABELING OF ERYTHROCYTES

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Even though labeling the red cell with ^{99m}Tc is difficult (*1*), the favorable radiation characteristics of ^{99m}Tc make it most desirable for scintographic studies. Transit-time studies would be greatly facilitated if one could successfully label an intravascular component such as the mature red cell. Moreover, if the red-cell membrane could be modified by physical or chemical agents and still retain its label, selective splenic or hepatic scans could be performed.

Fisher, Wolf and Leon (*2*) have described a method of "labeling irreversibly and with high yield" normal human erythrocytes. They further state that heat-damaged red cells give excellent splenic scans. We have attempted to repeat their studies and have uniformly met with failure. Our method has essentially been that of Fisher *et al* which is as follows:

Twenty milliliters of blood were withdrawn from a hematologically normal volunteer into a syringe containing 5 ml of acid citrate dextrose solution (Squibb ACD Modified List 1031). The erythrocytes and plasma are separated by centrifugation at 1,500 rpm for 5 min. The red cells are washed two times in isotonic saline and then resuspended to the original hematocrit. A 200 mCi Abbott generator #8305, Lot T-037, with a yield of 140 mCi in 30 cc of eluate was used as the labeling source. One millicurie of ^{99m}Tc was added to the saline suspended red cells in a ratio of 1 mCi to 10 cc of packed cells. After 20 min of incubation at 37°C with occasional stirring, the red cells were repetitively washed with saline. Each saline supernatant was appropriately diluted and counted against a 1-mCi standard of ^{99m}Tc. It has been noted on multiple experiments that repetitive washing reduces the "bound" radioactivity dramatically and by the seventh wash little more than 1% or 2% of the added activity remains.

To further document this finding, a sample of ^{99m}Tc "labeled" red cells was mixed with a sample of blood that had been labeled in the routine manner with ^{51}Cr (*3*). It can be seen (Fig. 1) that after the initial washing of ^{51}Cr-labeled red cells, very little radioactivity at the 320-keV level is lost. However, there is progressive elution of technetium from the red cells, and after the seventh washing background levels are being approached (^{51}Cr and ^{99m}Tc activity were determined by the Technical Measurement Corp. Gammascope, Model 101). It is obvious from this study that neither high-percentage binding nor irreversibility was obtained by this method. When

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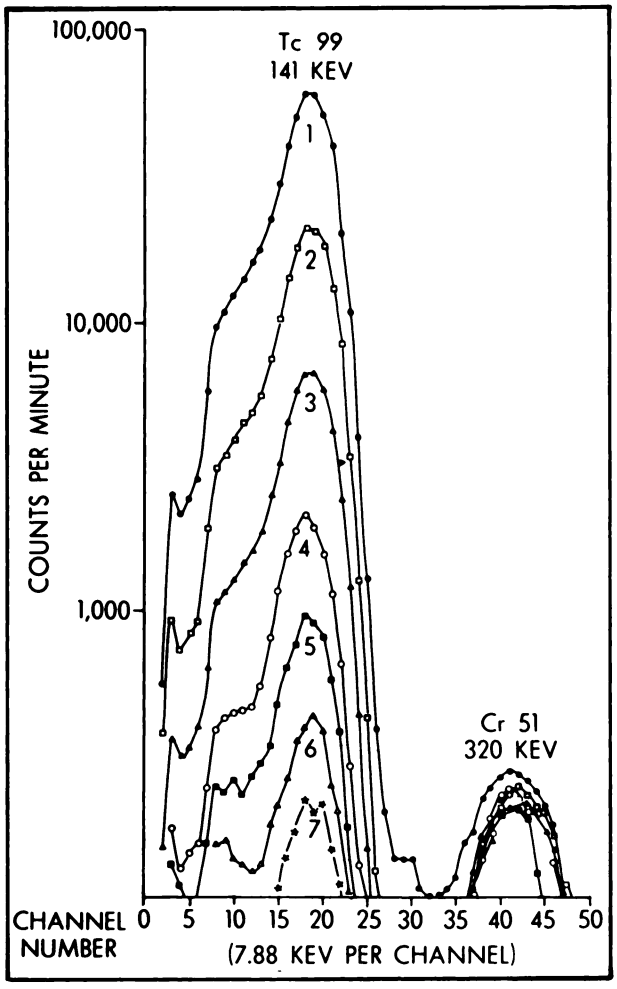

FIG. 1. Seven repetitive isotonic saline washes of ^{99m}Tc and ^{51}Cr-labeled red cells.

the repetitive saline washings were delayed until heating cells to 49.5°C as described by Fisher *et al* (*2*), the same results are observed.

Increasing volumes of ^{99m}Tc eluate were added to constant volume of saline-washed red cells in an attempt to overcome the poor labeling efficiency. It was observed that when an equal volume of saline-washed packed cells and ^{99m}Tc eluate were mixed an irreversible red-cell agglutination occurred (Fig. 2). The etiology of the agglutination phenomenon was hypothesized to be due to radiation-induced decomposition of water with peroxide formation (*4*) or trace-metal contamination. The former possibility has been tested with negative results, and the latter is being investigated. Trace-metal contamination in the form of aluminum is considered most probable because of the known construction of the generator columns which used phosphomolybdate adsorbed on aluminum oxide (Al_2O_3). Studies have confirmed the RBC agglutinating effect of aluminum at concentrations of 5 μg/ml. A marked variation has been noted in the aluminum content of the three commercially available technetium generators we have tested by atomic emission and absorption spectroscopy. The Atomic Energy Commission specification regarding generators states that the aluminum content of the eluate shall not exceed 500 μg/10 mCi of ^{99m}Tc (*5*). None of the generators exceeded this specification.

Further studies are in progress to more efficiently label red cells and to evaluate the *in vitro* and *in vivo* effect of red cells exposed to the eluate of a technetium generator.

We also have speculated that the variability in the preparation of technetium-sulfur colloid and technetium-albumin may be related to variations in aluminum content of the generator eluent. Complete description of the source of technetium may in the future result in reproducible procedures in more than one laboratory.

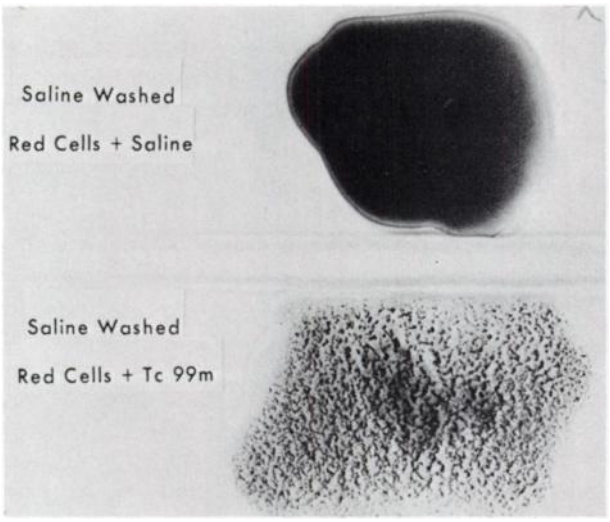

FIG. 2. Effect of ^{99m}Tc on saline washed red cells.

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