

## 85. Some Methodical Aspects of Red Cell Labelling

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The differences in labelling procedures and also in the interpretation of red cell survival curves make direct comparisons rather difficult. I.A.E.A. took the initiative in the standardization of labelling methods and organized an international panel with a discussion of hitherto acquired experience.

For routine use, the methods of random labelling are quite satisfactory. Besides the differential agglutination technique, the sodium chromate and DFP methods are widely used. Each of them has certain advantages and limitations.

1. The required amount of labelled chromate is small, non-toxic, the substance is readily available and substantially less expensive than DFP.
2.  $^{51}\text{Cr}$  emits gamma rays and enables therefore external scanning. Its longer half life is for survival studies also advantageous. The radiation dose for the patient is negligible.
3.  $^{51}\text{Cr}$ -labelled erythrocytes lose some activity by elution. The loss of activity has often two components. The early elution in the first 24 hours is not fully explained and makes the interpretation of survival curves difficult. The range of  $^{51}\text{Cr}$  elution rates differs widely in normal individuals and especially in disease /1/. The elution may be influenced also by the method of labelling /2/. The rate of elution can only be estimated by comparing simultaneous Ashby and  $^{51}\text{Cr}$  curves, the use of some universal correction factors is impossible.

On the other hand, the elution of DFP is usually completed in 24 hours and the binding of DFP is irreversible. In addition, DFP offers a possibility for simultaneous study of survival of leukocytes and thrombocytes.

In recent years, some methodical details of erythrocyte labelling have been studied in several laboratories in Czechoslovakia. The amount of chromium should not exceed  $1\mu\text{g}/\text{ml}$  of red cells to avoid their possible damage. It should be added dropwise for

lowering the risk of a damage to the cells exposed to a high concentration of chromium ions.

Ascorbic acid for the reduction to trivalent chromium should not be used because its interference with the metabolism of erythrocytes /3/. A single washing with the ten-fold volume of saline is usually fully satisfactory for the removal of free sodium chromate.

A considerable improving of the DFP method consists in the elaboration of an appropriate method of labelling in vitro. Red cells have a limited capacity for binding of DFP and  $0.12$  to  $0.14\mu\text{g}$  of DFP per milliliter of packed red cells may be considered as a saturation dose /4,5/. For that reason it is necessary to label at least  $100\text{ ml}$  of packed cells.

The solution of DFP in propylene glycol is diluted shortly before use by physiological saline to the concentration of about  $5\mu\text{g}/\text{ml}$ . The activity of samples may be measured either in a liquid GM counter or after previous drying. The washed red cells are re-suspended about to the original volume. Two  $1\text{ ml}$  aliquots are pipetted into disposable boats of aluminium foil and dried at  $70^\circ\text{C}$ . The samples are then sealed in cellophane and wrapped around a suitable GM tube /6/.

DFP method gives a picture of the true survival of the erythrocyte without any necessary corrections. It is a method of choice for estimating a true survival time curve, especially when a quantitative estimate of hemolysis is desired. A good agreement between the life span obtained by the glycine- $^{14}\text{C}$  and DF  $^{32}\text{P}$  methods supports this conclusion.

### References

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