The Measurement of the Survival of Human Erythrocytes by In Vivo Tagging with Cr$^{51}$

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THE DETERMINATION of the average survival time or life span of erythrocytes enhances markedly the appraisal of the etiology of anemias and other pathologic states. The most direct approach is to measure the survival of the erythrocytes within their natural environment. Therefore, techniques permitting this approach have involved the use of isotopes of iron (Fe$^{59}$), nitrogen (N$^{14}$) and carbon (C$^{14}$) and have been cumbersome due to the complexities of the analysis of these isotopes and the factor of reutilization of the isotope by erythrocytes. The differential agglutination technique of Ashby, although very useful, permits only the appraisal of donor cells within a second individual.

An in vitro method has been developed for tagging a random population of erythrocytes with radioactive chromium (Na$_2$Cr$^{51}$O$_4$) and measuring their in vivo survival following the auto- or homotransfusion of the tagged cells. Under these conditions a plot of the erythrocyte decay rate as measured by the decrement of radioactivity is curvilinear in the normal state. This represents a departure from the expected rectilinear function which is an expression of destruction of cells controlled by only a single factor, a constant determinant cell life span. This deviation from the rectilinear function has been considered to be due to either a random "leak" of chromium from intact cells or a random cell death from unknown factors possibly related to the process of in vitro tagging. The mechanical and physical trauma of the in vitro tagging procedure can be obviated by tagging the red cells in vivo. Erythrocytes have been tagged in vivo by the intravenous injection of 200–300 microcuries of Na$_2$Cr$^{51}$O$_4$. The procedures and results of this approach are given below.

METHODS

Three hundred microcuries of Na$_2$Cr$^{51}$O$_4$* in ten ml. of saline are injected into an antecubital vein. This results in the tagging of both erythrocytes and plasma proteins. The plasma protein radioactivity persists for a period which is dependent on the quantity injected. With 200 uc this activity persists for approximately 35 days; following the injection of 300 uc it can be detected up to 45 days.

The radioactivity in both a 4 ml. sample of hemolyzed blood (hemolyzed by freezing and thawing), and in a 4 ml. sample of plasma collected at the same time is measured in a well-type gamma sensitive scintillation NaI-Th crystal unit. The hematocrit reading of each venous blood sample is measured in Wintrobe tubes. From these data the radioactivity

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present within the erythrocytes contained in the 4 ml. sample of whole blood is calculated. The amount found 24 hours after the intravenous injection is designated as the "100 per cent" value. The radioactivity present in similar samples obtained on any subsequent day is related to this initial sample as per cent of initial activity. Corrections for physical decay of the isotope in the various samples may be obviated by saving all samples and counting on one day.

The subjects tested were either members of the medical staff or patients requiring nursing care for chronic diseases which did not affect the hematologic state. Peripheral blood studies of these cases were repeatedly normal.

The study was satisfactory up to 60 days. It was not feasible to continue beyond this time due to the low activity of the samples. The scintillation crystal unit has a background signal of 2.4 counts per second and one microcurie of Cr\(^{51}\) diluted to 4 ml. will record 1400 c/s.

**RESULTS**

The data obtained were treated as outlined to produce the curves for in vivo survival as seen in figure 1. The survival curves of the in vivo tagged cells are presented for comparison with similar studies of cells tagged by the in vitro method. The two methods produce similar resultant decay curves; the curves of the mean values are nearly identical for the 60 day interval.

The per cent uptake of the injected radiochromium by the red cells is low (10–12 per cent), hence the efficiency of the in vivo approach is low when compared to highly efficient in vitro methods (80–95 per cent).\(^3\)\(^4\)

The large fraction of Cr\(^{51}\) that is extravascular ultimately contributes a major portion of the Cr\(^{51}\) found in the urine. About 35 per cent of the injected radioactivity is excreted in the urine within 48 hours; minimal excretion occurs in the feces. Seventy-five per cent of the plasma Cr\(^{51}\) is precipitated in the protein phase with trichloracetic acid. It is not known what physical state characterizes the

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**FIG. 1.**—The survival of normal human erythrocytes tagged in vivo with Na\(_2\)Cr\(^{51}\)O\(_4\) as compared with the in vitro tagging method.
removing 25 per cent; however, compatible erythrocytes do not become tagged when incubated with the plasma from a person that has received Na₂Cr⁵⁺O₄ intravenously. This latter fraction may represent tri-valent Cr⁵⁺ or a colloidal form of a Cr⁵⁺ salt.

**Comment**

Although an interest in the comparison of erythrocytic decay curves produced by the in vivo and in vitro tagging methods initiated the study of the in vivo technic, the latter technic has a practical clinical application in certain patients. The method of tagging erythrocytes in vivo with Cr⁵⁺ has been of value in studying the life span of cells in patients in whom the in vitro method could not be used as a result of either clumping of erythrocytes due to a cold agglutinin active at room temperature, or when hemolysis was easily produced by mechanical trauma in vitro.

**Summary**

1. The deviation from a rectilinear form of the complex curvilinear curves of erythrocyte decay produced by the in vitro tagging of the cells with Na₂Cr⁵⁺O₄ is not a result of the in vitro tagging procedure per se.

2. Human erythrocytes can be tagged in vivo with Na₂Cr⁵⁺O₄. One can determine the in vivo survival of such cells if the gamma detecting unit is sufficiently sensitive and the amount of radiochromium used is sufficient. Such decay curves are similar to those produced by the in vitro Cr⁵⁺ tagging method.

3. The in vivo method permits a study of the life span of erythrocytes in patients who have a very active cold agglutinin or in whom the cells are easily hemolyzed by mechanical trauma.

**Summario in Interlingua**

1. Previe studios per iste e altere autores estabiva que le decadentia de erythrocytos etiquetate in vitro con Na₂Cr⁵⁺O₄ seque un complexe curva curvilinee. Le question se poneva si o non un tal deviation ab le expectate curva rectilinee es le effetlo del processo de etiquettage in vitro.

2. Il es possibile etiquettar erythrocytos human in vivo con Na₂Cr⁵⁺O₄. Le supervivencia del cellulas assi etiquetate es determinabile in vivo si le apparato detector de radiation gamma que es usate possede un sufficiente sensibilitate e si le quantitate de chromium radioactive que es injicite es sufficientemente grande. Le curvas de degeneration assi obtenite es simile al curvas obtenite post etiquettage in vitro. Ergo le deviation de iste ultime curvas ab le forma rectilinee non es debite al etiquettage in vitro per se.

3. Ultra iste valor theoretic, le metodo del etiquettage in vivo es etiam de importantia practic in studiar le supervivencia erythrocytic in patientes qui ha un activissime agglutinin frigide o in qui le cellulas es facilemente hemolysate per trauma mechanic de maniera que le metodo del etiquettage in vitro non es usable.
REFERENCES


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