Technical Papers

Biophysical Methods for the Assay of the Life Span of Red Blood Cells[1]

Harold C. Box, Worthington G. Schenk, and Charles E. Wiles[2]

Surgical Laboratory, Meyer Memorial Hospital, Buffalo, New York

Radioactive iron, principally Fe^{59}, has been used frequently as a tracer in the study of red cell dynamics. This isotope which can be incorporated into the erythrocyte *in vivo* remains in the cell during its life span. However, upon the death of old cells, the iron is almost quantitatively reincorporated into new erythrocytes thus rendering uncertain the interpretation of many investigations. The rapid reutilization of iron has been demonstrated at this laboratory during the course of our investigations of the fate of transfused red cells. In an effort to avoid this difficulty we have been led to a consideration of other isotopes as erythrocyte tracers. The work of Shemin and Rittenberg (*1*) indicates that N^{15} can be satisfactorily incorporated into the erythrocyte *in vivo*. An experimental study was undertaken to compare the relative reutilization of Fe^{59} and N^{15} after the destruction of the tagged erythrocytes.

The red cells of the dog are easily tagged by administering an intravenous dose of Fe^{59} as a buffered solution of $FeCl_3$. The radioactivity in the red cells thus tagged is determined by first ashing a few cc of red cells and electroplating the iron out of solution following essentially the method described by Peacock *et al.* (*2*). The activity (in counts per minute) of the sample can then be measured with a conventional Geiger counter and the activity per cc of red cells calculated. In order to determine the total circulating activity, red cell mass measurements were made by the T-1824 dye technique.

The reutilization of iron was amply demonstrated in our laboratory when whole blood from a donor dog tagged with Fe^{59} was transfused to an untagged dog. The donor's blood type did not match that of the recipient with the result that the recipient underwent a severe hemoclastic crisis following the transfusion. The destruction of the labeled cells and the subsequent reincorporation of the iron in newly formed cells is illustrated in Fig. 1 where the total circulating activity in the recipient is plotted against the day of the experiment. The days are counted from the day of the transfusion. The total activity measured in the recipient immediately after the transfusion is arbi-

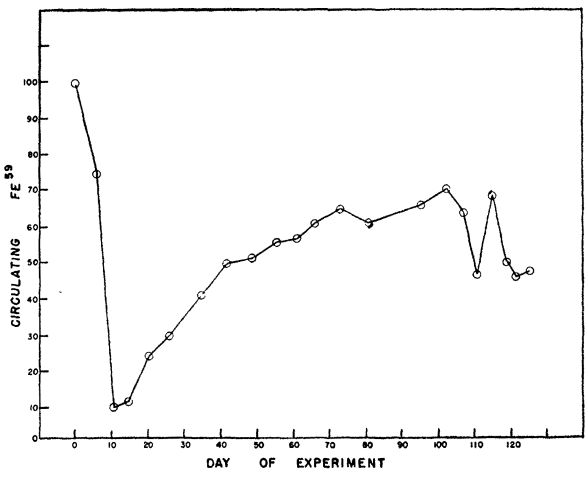

FIG. 1.

trarily taken as 100%. The ordinate has been corrected for the natural decay of Fe^{59} (half-life of 46.3 days) and for the effect of sampling losses. It is evident that 11 days after the transfusion nearly all the donor cells had been removed from circulation. Subsequently, however, approximately two-thirds of the iron in these cells was incorporated in new cells and restored to circulation. The work of Cruz *et al.* (*3*) further illustrates the reutilization of iron in the dog.

This characteristic makes the use of iron unsuited as a tracer for following transfused red cells over an extended period of time since one cannot determine what part of the activity is due to radioiron reincorporated in newly formed cells. The comparison of Fe^{59} and N^{15} in this regard was accomplished by double tagging of the erythrocytes of a healthy adult mongrel dog with N^{15} and Fe^{59}. A week after the tagging with the radioiron, 19 g of N^{15}-tagged glycine prepared after the method of Schoenheimer and Ratner (*4*) were given orally in small doses over a 2-day period. The animal was offered only water during this time to insure maximum absorption of the glycine. Twenty-six days after this feeding (sufficient time to allow incorporation of both elements into newly formed red cells) a hemoclastic crisis was artificially produced. Four doses of 0.3 g of acetylphenylhydrazine were administered over a 4-day period by subcutaneous injection. Measurements of the red cell mass by the T-1824 dye method before and after the injections showed that the red cell mass had been decreased approximately 64%.

Periodic determinations of the red cell mass, Fe^{59} radioactivity, and N^{15} excess in the red cells were made. For this analysis 45 cc of whole blood were required. The red cell mass determination required 20 cc of blood, but the same aliquot could be used for the radioiron determination. The analysis of the remaining 25-cc aliquot for N^{15} was somewhat more difficult

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since this is a stable isotope. Hemin crystals were first obtained from the whole blood. By means of a micro-Kjeldahl process the nitrogen was extracted from the hemin in the form of an ammonium salt which was then treated in an evacuated system with hypobromide so as to release the nitrogen in gaseous form. The gas was compressed into sample bottles suitable for introduction into a mass spectrometer by means of which the ratio of $N^{14}N^{15}$ to $N^{14}N^{14}$ was determined. The probability of forming a $N^{15}N^{14}$ molecule is proportional to $2n_{14}n_{15}$ where n_{15} and n_{14} are the numbers of heavy and light nitrogen atoms in the sample. Likewise, the probability of forming a $N^{14}N^{14}$ molecule is proportional to $n_{14}n_{14}$. The ratio of $N^{14}N^{15}/N^{14}N^{14}$ will be therefore $2n_{15}/n_{14}$. It follows that the percentage of N^{15} in the sample is

$$\% N^{15} = \frac{100n_{15}}{n_{14}+n_{15}} = \frac{100}{2/r+1}$$

where r is the ratio measured on the mass spectrometer. The percentage of N^{15} excess is obtained by subtracting 0.368 (the percentage of N^{15} occurring naturally in matter) from this number. These procedures are essentially those described by Rittenberg *et al.* (*5*).

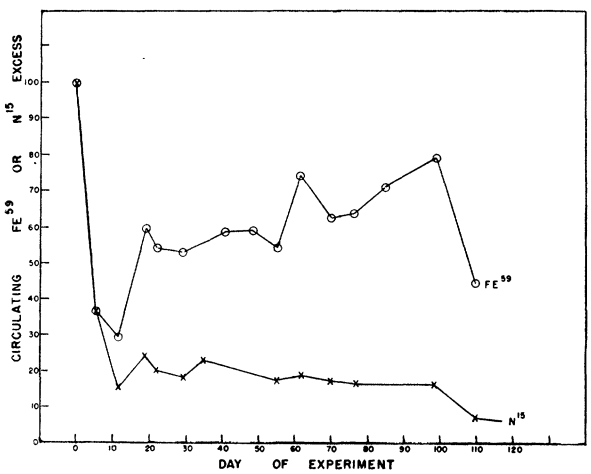

FIG. 2.

The results of the experiments are shown in Fig. 2. The circulating radioactivity as deduced from red cell mass determinations and radioactivity measurements was calculated at intervals of several days and is shown by the upper curve in the figure. The amount of activity just before the injection of the acetylphenylhydrazine was arbitrarily chosen as 100%. As usual, allowance has been made for sampling loss and the natural decay of the iron. The amount of circulating iron decreases sharply with the destruction of the dog's erythrocytes, but again there is remarkable reutilization as the dog regenerates its red cell mass.

In the case of the nitrogen it is the product of the percentage excess of N^{15} and the red cell mass which is of interest. This we refer to as the circulating N^{15} excess in Fig. 2 where it is plotted as a percentage of the initial value. Evidently the destruction of the red cells results in a permanent loss of N^{15} since there is no measurable reutilization.

It is clear that by using an isotope of nitrogen rather than of iron to follow the fate of transfused red cells, the problems resulting from reutilization can be avoided. Moreover, since it is a stable element it is not subject to the restrictions of a long half-life radioactive tracer such as C^{14}—nor is it hampered by the uncertainties of the agglutination technique sometimes used to follow red cells which is a statistical method and depends upon the strength of the serums used. It must be noted, however, that extension of this technique to tracing of transfused cells in human beings, although feasible, will be difficult because of the high excess of N^{15} required in the donor. Because of the inherent difficulties and uncertainties of other methods of following transfused red cells and in view of the results of this experiment, it is believed that N^{15} can become an important tool in the nation's vital research program for improved blood preservation.

References

1. SHEMIN, D., and RITTENBERG, D. *J. Biol. Chem.*, **166**, 627 (1946).
2. PEACOCK, W. C., *et al. J. Clin. Invest.*, **25**, 605 (1946).
3. CRUZ, W. O., HAHN, P. F., and BALE, W. F. *Am. J. Physiol.*, **135**, 595 (1942).
4. SCHOENHEIMER, R., and RATNER, S. *J. Biol. Chem.*, **127**, 301 (1939).
5. RITTENBERG, D., *et al. J. Biol. Chem.*, **127**, 291 (1939).

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The Effect of Streptomycin on Oxygen Uptake and Viability of Resting Suspensions of *Escherichia coli*

Thomas Fite Paine, Jr., and Louise Stone Clark

Departments of Medicine at the Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

The inhibitory effect of streptomycin on the respiration of "resting" bacterial suspensions has been noted by several observers (*1–4*). Work in this laboratory has indicated that there is a discrepancy between the killing effect of streptomycin on bacterial cells and respiratory inhibition by this drug. The experiments reported in this preliminary note may be of interest from the standpoint of studying the effects of metabolic inhibitors on resting cell suspensions.

Simultaneous experiments, using the same suspension of *E. coli* (841),[1] were set up to compare the effects of streptomycin on oxygen uptake and viability. The results of such an experiment are presented in Fig. 1.

[1] The organisms were grown for 24 hr with aeration at 37° C in a synthetic medium prepared as follows: $(NH_4)_2SO_4$ 2.5 g, KH_2PO_4 2.0 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, glucose 10.0 g (autoclaved separately), tap water 200 ml, distilled water to 1000 ml, pH adjusted so that after autoclaving pH was approximately 7. After harvesting, the cells were washed twice with sterile distilled water, suspended in sterile distilled water, and refrigerated at 4° C for 24 hr before use.