Mixing of Cr\(^{51}\)-Labeled Red Cells in Patients with Congestive Heart Failure and Controls

By Robert K. Funkhouser, M.D.

Composite time-concentration curves of labeled erythrocytes in venous blood constructed by pooling data from 11 patients without circulatory failure showed a curve of mixing lasting 25 min. Data from 9 patients in congestive heart failure showed variable mixing lasting at least as long as in the control patients.

The mixing of intravenously injected radioactively labeled erythrocytes with the total erythrocyte content of the blood has been shown to be complete for practical purposes in 2 to 4 min.\(^1\) This finding, based on time-concentration curves in arterial blood, has been difficult to reconcile with the findings of investigators of the peripheral vascular circulation, using the technic of peripheral counting. For example, it is found that equilibration of \(^{131}\)I-labeled human serum albumin requires approximately 10 min. in the superficial circulation of the normal foot.\(^2\)

This report gives the result of a study of \(^{51}\)Cr-labeled erythrocyte mixing made in the course of an experiment on the blood volume in congestive heart failure. While the study of time-concentration curves from venous samples in individual patients indicated no significant deviation from the horizontal after 3 min. from the time of injection, a composite curve constructed from data from each group as a whole shows a falling concentration of labeled cells of minor degree for 25 to 35 min. after injection in control patients. This decrease amounts to approximately 5 per cent.

METHODS

The 9 experimental patients had severe congestive heart failure. All manifested edema, increased venous pressure, hepatomegaly and orthopnea. The 11 control patients had disease of various kinds not known to affect the blood volume. Both groups were selected from patients on the medical wards of Lakeside Hospital.

At the time of study, the cells in a sample of the patient's blood were labeled with \(^{51}\)Cr according to the method of Gray and Sterling,\(^3\) and were injected with a minimum of delay. Assays demonstrated that less than 1 per cent of the radioactivity was in the suspending saline. Following injection of the labeled cells, blood samples were drawn from an indwelling no. 16 or no. 18 gage needle in an antecubital vein. Assays of radioactivity were carried out on 3 ml. samples of whole blood in the liquid state, using a conventional well-type scintillation counter. The cell:plasma ratio of each sample was determined after counting, within 1 hour of withdrawal. The anticoagulant was dry heparin.

Construction of Composite Time-Concentration Curves. The composite time-concentration curves shown in figures 1 and 2 were constructed by pooling all the data from the control patients and from the failure patients respectively. For each subject in both groups a mean final concentration value was calculated representing all samples drawn after 35 min. Thirty-five minutes was arbitrarily chosen in the belief that mixing would be complete by this time. The deviation of the concentration of each sample during the entire period of study from this mean value was found in microcuries per milliliter. Each deviation from this final value was plotted against the time of its withdrawal to form a composite graph. This type of graph presents group data so that general trends can be seen which might otherwise be obscured by individual variations within a small number of points. An ordinate scale has been so selected as to emphasize the scatter of the experimental data. The method is similar to that used by Erlanger in his discussion of the mixing and disappearance of protein bound dyes.\(^4\)

RESULTS

Figure 1 is the composite time-concentration curve for the control patients. A fall in concentration during the first 25 minutes after injection, followed by a phase during which there is no apparent further decline in concent-
MIXING OF Cr$^{51}$-LABELED RED CELLS

FIG. 1. Plot of the deviations from final mean concentration of the individual blood samples in the control patients.

Fig. 2. Plot of the deviations from final mean concentration of the individual blood samples in the patients with congestive heart failure.

The scatter of the points is clearly visible. The scatter of the points gives a coefficient of variation of approximately 1 per cent. This scatter is no larger than would be expected on the basis of counting statistics and pipetting errors and apparently does not reflect spontaneous fluctuations in the true concentration of Cr$^{51}$-labeled cells.

The individual mean concentration values used as a reference in constructing the composite curve ranged from .0209 to .0439 μc./ml with a mean of .0309 μc./ml. The intercept of the “mixing” portion of the curve is at about .0015 μc./ml, above the intercept of the horizontal mean, a deviation of approximately 5 per cent.

Figure 2 is the composite time-concentration curve for the patients with congestive failure. During the first 35 min. after injection there is greater scatter of the points than seen in the control patients. Thereafter, the scatter of the points is of approximately the same magnitude as that seen throughout figure 1. This increased scatter may be due to a greater variation among the several individual time-concentration curves of venous blood of the patients in heart failure as well as to an increased scatter of individual points about this curve in any individual patient.

Although there is a greater scatter during the first 35 min., inspection of figure 2 indicates that in general there is a declining concentration during this period with questionably continuing fall thereafter. In view of the possibility of diminishing concentration after 35 min., it is unfortunate that sampling was not continued for a longer period in the patients with congestive failure. The present data does not permit a conclusion as to whether mixing is complete in 25 min. or perhaps takes an hour or more in such patients. The reference means used in constructing figure 2 range from .0131 to .0445 μc./ml. with a mean of .0243 μc./ml.

DISCUSSION

The ideal substance for a study of blood mixing would be a material which, while mixing homogeneously in blood, would remain entirely within the vascular tree. Such a substance would give a horizontal time-concentration curve after mixing was complete, and no confusion would be created by the simultaneous effects of the curve of disappearance of the labeled material.

Cr$^{51}$-labeled erythrocytes closely approximate this ideal substance. The rate of disappearance is only 1 to 3 per cent/day. It is not certain, however, whether a small fraction of the labeled cells, injured or uninjured in the labeling process and the injection, might disappear from the circulation during the first few minutes after injection. Therefore, even when using Cr$^{51}$-labeled cells, a possibility remains that the “mixing” curve is actually due to disappearance.

Hahn and his co-workers have emphasized that the mixing of erythrocytes with blood may be quite different from that of plasma-soluble substances such as dyes and labeled protein. Nevertheless, complete mixing of
erythrocytes in 2 to 4 min. is inconsistent with microscopic observations of the circulation. These observations indicate that there are capillary circuits that contain stationary erythrocytes for 10 min. or longer at a time, in a number of different organs of a wide variety of animal species. The finding that erythrocyte mixing requires 25 to 35 min., then, is more consistent with these observations.

The increased scatter of the early points of figure 2 is consistent with the finding of Nylin\textsuperscript{6} that the mixing of P\textsuperscript{32}-labeled erythrocytes as reflected by the arterial concentration of P\textsuperscript{32} shortly after injection, was delayed and variable in patients with congestive heart failure. If the diminishing concentration after 35 min. is significant, and if it is indeed due to continued mixing, it would introduce a systematic error in the determination of blood volume in congestive heart failure, tending to make the blood volume appear lower than the true value. In view of the greater variation among individuals as to the slope of the early curve, a greater random error would also be expected in subjects with congestive heart failure.

**Summary**

Evidence has been presented to show that mixing of labeled erythrocytes in blood has required at least 25 min. in a group of hospitalized patients free of edema. There was greater variation in individual mixing curves in patients with congestive failure than in the control patients. No definite statement can be made on the basis of the present data as to the termination of the mixing period in patients with congestive heart failure.

**Acknowledgment**

I wish to express my gratitude to Miss Barbara Jambour for painstaking technical assistance and to Dr. A. S. Littell who suggested the method used in the construction of the figures.

**SUMMARIO IN INTERLINGUA**

Es presentate datos que indica que le immixtion de marcate erythrocytos in le sangui de un gruppo de hospitalisate patientes sin edema requireva al minus 25 minutus. Le curvas individual de immixtion exhibiva plus pronunciate variationes in patientes con insufficientia congestive que in patientes de controlo. Super le base del presente datos il non es possibile formular definite conclusiones in re le termination del periodo de immixtion in patientes con congestive insufficientia cardiac.

**REFERENCES**

Mixing of Cr$^{51}$-Labeled Red Cells in Patients with Congestive Heart Failure and Controls
ROBERT K. FUNKHOUSER

Circ Res. 1957;5:579-581
doi: 10.1161/01.RES.5.5.579

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1957 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/5/5/579