

Rate of clearance by the spleen of heat-damaged erythrocytes

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SYNOPSIS The rate of uptake in the spleen of heat-damaged red blood cells labelled with ^{99m}Tc has been measured by radioisotope scanning in 38 patients. The values obtained for the half-clearance time into the spleen using this method have been compared with the values obtained simultaneously by blood sampling for the rate of loss of radioactivity from the circulation. It was found that the uptake into the spleen was approximately three times faster than the measured rate of loss of radioactivity from the blood. The reasons for this are discussed and it is suggested that the scanning technique is more reliable as a measure of this aspect of splenic function.

Measuring the half-clearance time from the blood of heat-damaged erythrocytes labelled with a radioisotope has become established as a method for obtaining information on splenic function (Crome and Mollison, 1964; Kimber and Lander, 1964; Marsh *et al*, 1966; Marsh and Stewart, 1970; Fischer *et al*, 1971; Pettit *et al*, 1972). The usual method is to collect a blood sample three minutes after the injection of the damaged red cells and further samples at intervals thereafter, the radioactivity of each sample being then expressed as a fraction of the activity of the three-minute sample. The heat-damaged erythrocytes are, as a rule, taken up selectively by the spleen, although on occasions a fraction of the cells may be taken up by the liver and by the rest of the reticuloendothelial system. It has been suggested (Fischer *et al*, 1971) that this latter is, in fact, the usual occurrence and that it results in a clearance curve comprised of two components: the first component is assumed to be primarily caused by removal by the spleen of cells which have become sphered during the heating process and the slow component by the removal of fragmented cells by the liver and the rest of the reticuloendothelial system. Thus clearance of damaged cells from the blood will not necessarily imply that they have been cleared by the spleen. Moreover the quality of the data seldom permits analysis of the first component

of the clearance curve with sufficient accuracy for this alone to be used for evaluation of splenic function.

In this paper we present details of a method which may be used to make a direct determination of the rate of uptake of heat-damaged erythrocytes by the spleen in order to provide a more direct and valid method for evaluation of splenic function. The method has been used on a series of 38 consecutive patients undergoing routine spleen scans. They included patients with polycythaemia vera, myelofibrosis, chronic granulocytic leukaemia, chronic lymphocytic leukaemia, lymphosarcoma, and haemolytic anaemias. The results have been compared with the measured half-clearance time from the blood.

Method

Approximately 15 ml of blood were collected in ACD from each patient before the study and the samples were centrifuged at 1500 *g* for 5 minutes. The packed red cells were then heat-damaged for exactly 20 minutes at 49.5°C. They were then incubated for 5 minutes at room temperature with 1.5 mCi of ^{99m}Tc. A volume of freshly prepared 1% solution of SnCl₂ in saline was then added (approximately 20 µg/ml red cells) and the mixture was allowed to stand for a further 5 minutes. The cells were then washed twice in 9 g/l NaCl, resuspended with an equal volume of saline, and reinjected.

Following re-injection of the labelled damaged red cells, blood samples were taken at 3, 10, 20, 40, and

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60 minutes, the time of injection being noted accurately. The radioactivity per unit volume of whole blood was subsequently measured and the half-clearance time of disappearance of the radioactivity from the circulation was measured.

In order to measure the uptake into the spleen directly, quantitative scans were made of the spleen using a dual detector scanner and scanning at a speed of 200 cm/minute. The first scan began as soon as possible after injection and each scan took approximately 5 minutes so that a total of five scans could be obtained in about 45 minutes. Each scan was analysed using the method described by Pettit *et al* (1971) and the percentage of the injected radioactivity in the spleen was measured. This quantity is greater than the true uptake of the damaged cells since it includes radioactivity remaining in the circulating blood present in the spleen. A correction for this can be applied. If M is the measured uptake and V the splenic red cell volume, then U , the true uptake, is given by $U = 100(M - V)/(100 - V)$. The splenic red cell volume was determined separately using the method of Hegde *et al* (1973) so that this correction could be applied. The data for each patient were fitted using a least squares procedure (Marquard, 1964) to the curve $U = A(1 - e^{-KT})$ where T is the time after injection at the mid-point of each scan and A is the final percentage of the radioactivity which is taken up by the spleen. The value obtained for K was then used to calculate the half-clearance time of the radioactivity into the spleen.

Results and discussion

The figure shows a comparison of the results obtained by measurement of the half-clearance time from the blood and measurement of half-clearance time into the spleen. It can be seen that the rate of clearance into the spleen is approximately three times faster than the measured rate of clearance from the blood. This is probably caused by a combination of two factors. First, in their analysis of the blood clearance curve, Fischer *et al* (1971) found that the half-clearance time of the fast component ranged between 3 and 7 minutes. This is similar to the splenic clearance time found in our study, which thus supports the assumption that only the first component of the curve relates to splenic activity. Secondly, as some trapping of sphered cells will occur in the spleen before the first blood sample is taken, the importance of the fast (splenic) component in the measured blood clearance curve is reduced. The measured half-clearance time from the circulation may thus be longer than the half-clearance time into the spleen.

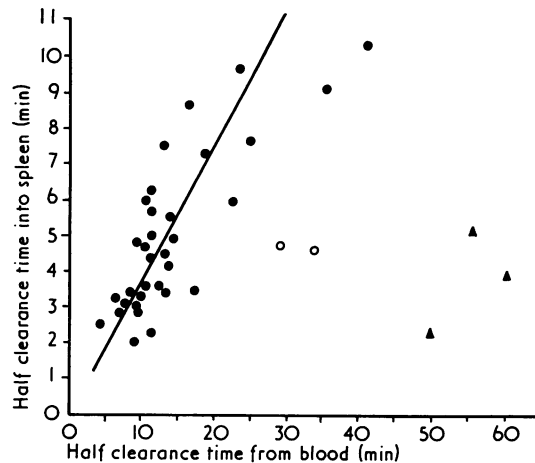


Figure Comparison of half-clearance time of heat-damaged red cells from circulation and into spleen. See text for explanation of outlying results (\blacktriangle and \circ).

Fischer *et al* (1971) found that an average of 68% of the radioactivity was associated with the fast component; we have found that the uptake in the spleen was normally between 60% and 90%, with an average uptake of 75% in the spleen at the end of the study. There was no correlation between the percentage uptake by the spleen and the clearance time into the spleen. The scatter of the points about the regression line is presumably caused by the variation in the amount of uptake in the spleen. The three data points which are well to the right of the regression line (shown in the figure as triangles) were obtained in three patients in whom the percentage of the radioactivity taken up into the spleen was reduced to approximately one-third of the normal value. These patients had been diagnosed, respectively, as chronic lymphocytic leukaemia, myelosclerosis, and neutropenia (probably preleukaemia). Thus, although the clearance time measured by scanning is in the normal range, the clearance time measured from the blood samples is longer than normal because of the greater importance of the slow component in these cases.

This effect may also occur if the first blood sample is taken later than 3 minutes, since then the uptake of the damaged cells in the spleen before the first blood sample is taken will be increased. As mentioned above, this results in the importance of the fast component of the measured blood clearance being reduced and therefore there will be a longer half-clearance time. This source of error probably accounts for the other two data points to the right of the regression line (open circles in the figure) since with these two patients the first blood sample was

not taken until 4 and 5 minutes, respectively, after injection; subsequent samples were taken at the usual times.

These observations suggest that simple analysis of the rate of clearance of damaged red cells from the circulation can give misleading results and that it is necessary to achieve a more accurate analysis of the clearance curve into its constituent components. In practice this may be difficult as it is seldom possible to obtain sufficient blood sample in order to measure this aspect of splenic function reliably; it is better, in principle, to make a direct measurement of activity within the spleen by means of a quantitative scanning method as described in this paper.

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