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An improved method for the measurement of red cell survival using non-radioactive chromium

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SUMMARY An improved technique for the measurement of red cell survival is described in which non-radioactive ^{50}Cr is used in patients for whom radioactive labels are not advisable. The ^{50}Cr was measured by neutron activation analysis using a high resolution germanium (lithium) diode gamma-ray spectrometer. In adult controls, the values obtained were in the range accepted as normal for the ^{51}Cr method, and double labelling experiments showed close agreement. The application of this technique to the measurement of red cell survival in pregnant women suspected of having haemolytic anaemia is described.

The generally recognised technique for the measurement of red cell survival time is by the infusion of cells labelled with ^{51}Cr and the measurement of this nuclide in serial blood samples taken subsequently (Ebaugh *et al.*, 1953). However, the use of radioactive tracers is not advisable in pregnant women and children. In 1968, Donaldson *et al.* described an alternative method, in which cells labelled with inactive chromium enriched in ^{50}Cr (normal abundance 4·3%) were reinfused, and the chromium content of subsequent blood samples was determined by neutron activation analysis. The induced ^{51}Cr was measured using sodium iodide detectors. This method required the labelling of 200 ml of blood and was later modified by Uchiyama *et al.* (1975), who used smaller quantities and more sensitive instrumentation. Glomski *et al.* (1976) described its use in children with various haemolytic anaemias using volumes of blood that are appropriate for the paediatric age group.

We describe a further modification of the technique, in which the precision is increased by expressing the results as the $^{50}Cr/^{59}Fe$ ratio, the iron being the natural inherent red cell iron activated by the neutron flux. Its application to the measurement of erythrocyte survival in normal and haemolysing pregnant women is described, and a fingerprick technique for the collection of blood samples from children, with recommendations on the minimal amounts of blood needed for labelling, is also given.

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Patients and methods

PATIENTS

Informed consent was obtained from all 10 subjects used in the study. The control group consisted of two men, two premenopausal women, and two women in the last trimester of pregnancy. The patient group consisted of three women in the last trimester of pregnancy who were suffering from congenital haemolytic anaemia and a 67-year-old man with hereditary spherocytosis.

BLOOD LABELLING AND SAMPLING

The stable chromium was prepared by electromagnetic separation at Harwell. The chromium, in the chemical form Cr_2O_3, was converted to sodium chromate and dissolved in pyrogen-free water before use. The isotopic composition was: ^{50}Cr 96·80%, ^{52}Cr 2·98%, ^{53}Cr 0·18%, ^{54}Cr 0·04%.

Venous blood (50 ml) was anticoagulated with ACD, and the packed red cells were incubated with stable ^{50}Cr as sodium chromate (500 μg) to give 10 μg/ml, a concentration not thought to damage red cells, at room temperature for 30 minutes, washed twice with normal saline, and resuspended with saline before re-introduction into the patient.

Blood samples were taken into lithium heparin tubes before injection as a control, at 45 minutes post-injection, and thereafter at regular intervals for 28 days. There was no chromium contamination from the standard steel needles used for venepuncture. At Harwell approximately 0·2 ml of

blood from each specimen was transferred into clean silica capillary tubes (6 cm × 3 mm) and dried initially at 67° C to avoid losses, and finally at 110° C. Both ends of the tube were then sealed using 'heat shrink' PTFE tubing, which will withstand neutron activation. For the 'fingerprick' technique, samples of capillary blood were collected directly into the silica capillary tubes, dried as above, and then dispatched to Harwell for activation.

NEUTRON ACTIVATION AND RADIOACTIVE MEASUREMENTS

The irradiations were carried out at a thermal neutron flux of 6×10^{12} n/cm²/s in either the DIDO or PLUTO Harwell reactors for 10 days, and the short-lived activation products were allowed to decay for two weeks before counting was started.

Induced radioactivity of the samples was measured on a germanium (lithium) detector gamma-ray spectrometer controlled by a data acquisition system (Salmon and Creevy, 1971). Figure 1 shows a typical gamma-ray spectrum of a sample of irradiated blood labelled with ^{50}Cr. The chromium and iron contents were determined by means of the GASP programme (Salmon, 1965) using a linear regression analysis in which the pulse-height distribution was compared with those from individual radiochemically pure samples of each component nuclide. This technique is inherently more accurate than the more commonly used peak area measurements.

In the double labelling experiments, determinations of the ^{51}Cr from the radioactive label were made using the normal hospital procedure (Dacie and Lewis, 1975), and the total gamma disintegrations were counted in a well-type NaI crystal. The ^{51}Cr was allowed to decay before activation in the reactor and measurement on the germanium (lithium) system.

The results were expressed conventionally as $T_{\frac{1}{2}}$ ^{50}Cr (the time taken for disappearance of half the chromium from the blood) rather than the mean cell life, as recommended by the International Committee for Standardization in Haematology (ICSH) (1971), in order to compare these results with those of previous authors. All results are the mean of five determinations unless otherwise stated.

Results

The overall results on six control subjects and four patients with haemolytic anaemia are shown in Table 1. The half-times obtained by measuring the absolute chromium content per unit of blood, as in the conventional ^{51}Cr method, are compared with those calculated from the chromium content relative to iron where the iron acts as an internal standard. There is close agreement between the two methods except in one control patient (No. 1) who was the first subject investigated before the technique was perfected.

The results of a double labelling study using ^{51}Cr and ^{50}Cr on an adult male suffering from hereditary spherocytosis are shown in Figure 2. There is close agreement between the two methods, the $T_{\frac{1}{2}}$ for the three plots shown being 14·3 ± 0·9, 13·7 ± 0·8, and 14·0 ± 0·6 days, respectively.

Figures 3a and 3b show the results obtained during the third trimester of a normal pregnancy together with the curve on a pregnant patient suffering from pyruvate kinase deficiency. The latter demonstrates well the presence of a double population of cells in this condition (Nathan *et al.*, 1968).

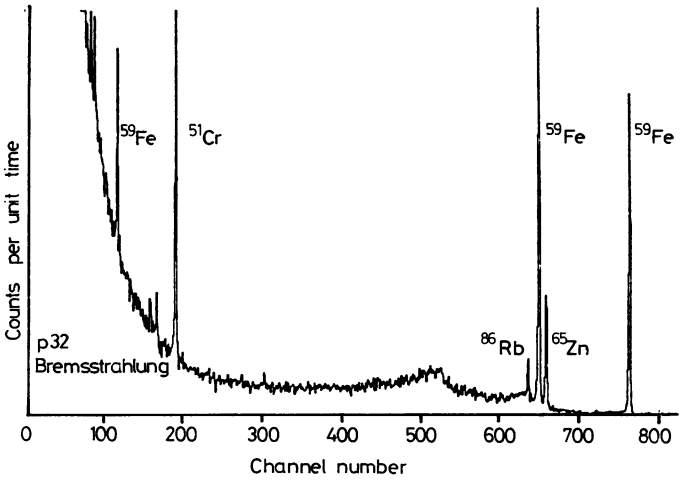

Fig. 1 *Gamma-ray spectrum of neutron-activated peripheral blood labelled with stable ^{50}Cr.*

Table 1 *Half-times in controls and haemolysing patients comparing absolute chromium-50 content with chromium-50 content relative to iron-59*

No.	*Subject*	*Condition*	*Half-time by absolute ^{50}Cr content ± SD*	*Half-time by ^{50}Cr content relative to ^{59}Fe ± SD*
1	Normal male	Control	17·5 ± 3·4	23·7 ± 3·2
2	Normal male	Control	25·1 ± 3·8	28·1 ± 3·7
3	Normal male	Control	21·8 ± 1·7	21·6 ± 1·4
4	Normal male	Control	30·7 ± 3·8	30·3 ± 0·9
5	Pregnant	Control	37·1 ± 7·4	35·3 ± 4·9
6	Pregnant	Control	38·8 ± 2·5	39·0 ± 1·5
7	Pregnant	Hereditary spherocytosis	25·7 ± 1·9	20·3 ± 0·7
8	Pregnant	Hereditary spherocytosis	17·2 ± 2·2	17·2 ± 0·7
9	Pregnant	Pyruvate kinase deficiency	—	15·0 ± 0·7
10	Male	Hereditary spherocytosis	—	13·7 ± 0·8

*Duplicate determinations only

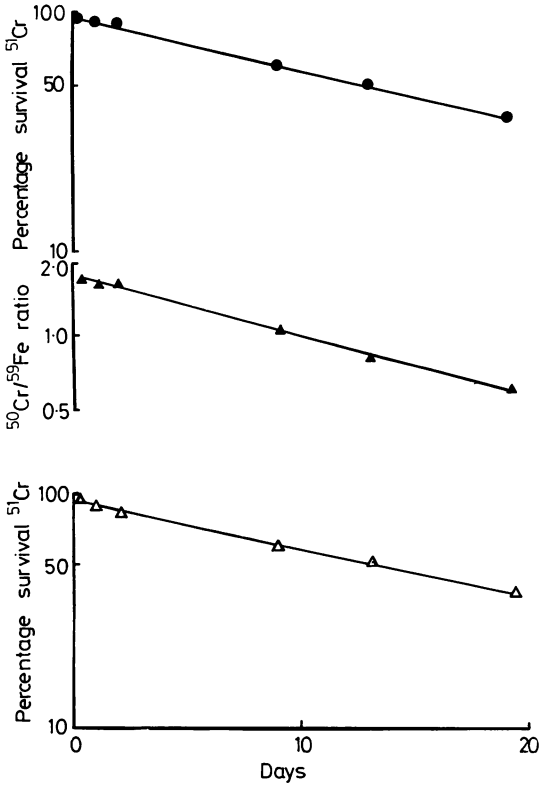

Fig. 2 *Double labelling experiment using ^{51}Cr and ^{50}Cr. Results obtained at Harwell (● and ▲) $T_{\frac{1}{2}}{}^{51}Cr$ = 14·4 ± 0·9, $T_{\frac{1}{2}}{}^{50}Cr$ 13·7 ± 0·8 days. Results obtained at local hospital (△) $T_{\frac{1}{2}}{}^{51}Cr$ = 14·0 ± 0·6 days.*

The chromium/iron ratio is a satisfactory way of expressing the results and possibly superior, as shown by the smaller standard deviations. The $T_{\frac{1}{2}}$ ^{50}Cr for the haemolysing group are all reduced, as would be expected, and of interest is the relatively long half-time observed in the two normal pregnant women. Further studies are needed to assess the normal life span at various stages of pregnancy by this method.

Discussion

Radioactive chromium was initially introduced as a measure of the red cell mass but was subsequently swiftly adopted for red cell survival studies (Ebaugh *et al.*, 1953; Necheles *et al.*, 1953). Comparison of the corrected survival curves with the simultaneous use of labelled di-isopropyl phosphofluoridate showed good concordance. Nevertheless the use of even small doses of radioactivity is contraindicated in infancy and pregnancy, and so the introduction of the use of stable chromium with neutron irradiation *in vitro* promised an alternative approach to this problem. Unfortunately, the method and techniques of Donaldson *et al.* (1968) and Johnson *et al.* (1969) necessitated the use of 100-200 ml of blood, but subsequent refinements have enabled this amount to be substantially reduced. The observations reported here parallel those of Uchiyama *et al.* (1975), who labelled 50 ml of blood with a concentration of chromium at or below the level of 10 μg/ml of red cells, a concentration thought not to affect the subsequent red cell life span. This paper demonstrates that the chromium/iron ratio, by providing an internal standard, has improved the replicate precision and gives good correlation with the radioactive chromium results. This method also obviates the need for weighing samples, and for repeated PCV estimations during the survival.

Toxicological data indicate that only a negligible quantity of administered chromium will cross the placental barrier (Visek *et al.*, 1953). Furthermore, the chromium in the concentrations involved appears to be non-toxic (Anwar *et al.*, 1961). The method, therefore, seems to be ideally suited for the

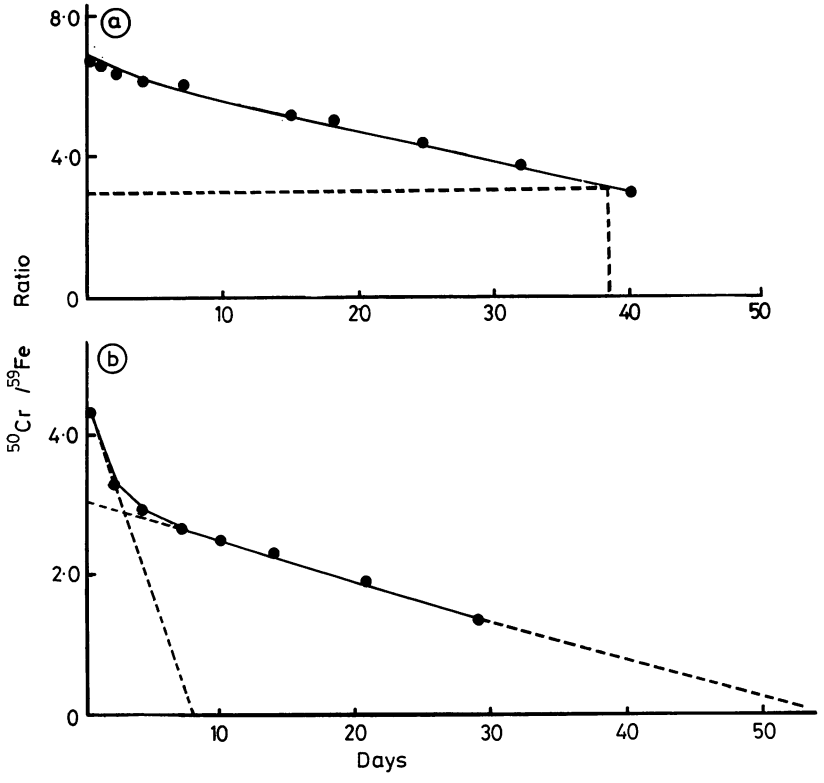

Fig. 3a *Red cell survival in normal pregnancy. Each point represents the mean of five determinations. Standard errors for each point are too small to be shown graphically (mean 0·07) $T_{\frac{1}{2}}{}^{50}Cr$ = 39·0 ± 1·5 days.*

Fig. 3b *Red cell survival in pregnancy complicated by congenital pyruvate kinase deficiency. The curve demonstrates a double population of cells. Population I 60% (MCL = 56 days). Population II 40% (MCL 3·5 days). $T_{\frac{1}{2}}{}^{50}Cr$ = 25·1 ± 1·2 and 0·7 ± 0·2 days respectively (double exponential fit).*

investigation of red cell survivals. Further, there is no reason why it cannot also be applied to red cell mass determination, and, as stated earlier, the method is ideally suited for investigating the complex haematological changes that occur during pregnancy.

Analysis of samples can be undertaken on volumes as low as 0·2 ml, which makes sequential sampling by the fingerprick technique, especially in infancy, much easier. Table 2 shows recommended amounts of blood needed for labelling throughout childhood and adolescence which are slightly smaller than those used by Glomski *et al.* (1976). Because of their small volume all the samples can be irradiated together, thus avoiding inaccuracies due to variations in neutron flux with time. The main drawbacks of the method remain the delay in obtaining the result and the cost. Two to three weeks must elapse after irradiation to allow for the decay of the shorter lived induced nuclides, and so overall about two months must elapse between the beginning of the estimation and the final result. The current cost of the irradiation and analysis at Harwell is about £125 per patient but this should be balanced against the saving in doctors' and technicians' time, and we feel strongly that any means by which exposure to irradiation can be limited is a distinct advantage.

Table 2 *Typical blood volumes of children during growth calculated from mean body weights and volumes of chromium-50-labelled blood needed for infusion*

	Mean weight (kg)	*Blood volume (ml)*	*Volume of labelled blood for infusion (ml)*
Premature	—	100-150	1-1·5
Birth	3·2	300	2·7
1	10·1	720	5
2	12·6	900	8
4	16·5	1200	11
6	21·9	1560	14
8	27·3	1950	18
10	32·6	2330	21
12	38·3	2730	25
14	48·8	3490	32
16	58·8	4200	38
18	63·1	4510	41

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