obtained are considerably lower than previously accepted values based on chemical analysis of cadavers. The presence of a significant sodium pool, not detected by isotope dilution techniques, was confirmed.

The techniques described in this paper involving the neutron irradiation of living subjects have been approved by an ethical committee of Birmingham University. Clinicians and physicists were represented on the committee, which included the Radiation Protection Officers of the University and of the United Birmingham Hospitals. The work involving cadavers was done with the permission of the Inspector of Anatomy at the Home Office.

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Red Cell Survival Time in Man Measured by ^{50}Cr and Activation Analysis

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Radioactive isotopes have been used extensively in clinical practice as tracer substances. Chromium-51 as sodium chromate has been used for the measurement of the red cell survival time since Gray and Sterling (1950) demonstrated that hexavalent chromium was a good erythrocyte label. The present work was designed to determine whether non-radioactive sodium chromate might also be used to measure the red cell survival time, the chromium content of the serial blood samples being determined *in vitro* by neutron activation analysis.

When an element is irradiated with neutrons it may become radioactive, emitting gamma-rays or ionizing particles with energies characteristic of the nuclide produced; moreover, the radioactivity is proportional to the original quantity of the element irradiated. Thus after irradiating a tissue or fluid such as blood, which contains many elements, it is possible to quantify those elements emitting gamma-rays from measurement of the number and energy of the gamma-rays emitted. The technique has been used to analyse the naturally occurring isotopes present in tissues. It is also possible to add stable isotopes to act as tracers, the amounts in the final samples being determined by neutron activation analysis. This technique has been applied infrequently in clinical practice (Lowman and Krivit, 1963), and we believe this is the first report concerning the use of chromium-50 (^{50}Cr) or the application of the principle to the determination of the red cell survival time.

Methods

The method was based on the standard procedures using radioactive sodium chromate (Donohue *et al.*, 1955). From the patient 100–200 ml. of blood was taken, anticoagulated with acid-citrate dextrose, and incubated with non-radioactive

sodium chromate at room temperature. The amount of sodium chromate used was such that the concentration of elemental chromium was less than 10 $\mu\text{g./ml.}$ of red blood cells, a concentration believed not to affect red cell survival (Ebaugh *et al.*, 1953; Read *et al.*, 1954; Donohue *et al.*, 1955). The red cells were then washed twice in normal saline and resuspended in saline before intravenous infusion. Blood samples were taken subsequently from the patient every few days for up to 30 days for chromium analysis.

Five patients were studied. Their diagnoses are given in the Table. In four the red cell survival time was also determined by means of sodium ^{51}Cr chromate, simultaneously with chromium-50 in three of the four. The $T_{1/2}$ ^{51}Cr was measured by a standard technique; the radioactivity due to chromium-51 in the serial blood samples was estimated by means of a well-type scintillation counter. (The $T_{1/2}$ ^{51}Cr is the time taken for the radioactivity of the blood to reach 50% of its original value.) In two patients the erythrocyte survival time was measured simultaneously with di-isopropyl phosphorofluoridate with incorporated phosphorus-32 (DF^{32}P). This label was given intravenously in a dose of 10 μCi and the β emission of washed haemolysed red cells counted in an M6 liquid β counter. DF^{32}P does not elute from the red cell and therefore gives a measure of true red cell life-span (Eernisse and van Rood, 1961).

Comparison of Red Blood Cell Isotope Labels

Case No.	Diagnosis	Isotope	$T_{1/2}$ (Days)
1	Congenital spherocytosis	^{50}Cr	12
		DF^{32}P	19
		^{51}Cr	18
2	Autoimmune haemolytic anaemia	DF^{32}P	13
		^{50}Cr	8
3	Autoimmune haemolytic anaemia	^{50}Cr	19
		^{51}Cr	20
4	Autoimmune haemolytic anaemia	^{50}Cr	10.5
		^{51}Cr	9
5	Hypertension	^{50}Cr	31.5
		^{51}Cr	30.5

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However, for ease of comparison results have been expressed as $T_{1/2}^{DF^{32}P}$.

Naturally occurring chromium contains only 4% chromium-50, and preliminary calculations showed that, with the neutron flux and irradiation time available, enriched material would be necessary to give sufficient sensitivity. Accordingly the non-radioactive sodium chromate used had incorporated chromium-50, obtained from the Electromagnetic Separation Group, Harwell, enriched to 97% abundance. The chromium-50 was activated by thermal neutrons to the radioactive isotope chromium-51 as shown in the equation

For the purpose of activation 5-ml. samples of blood in polyethylene containers were centrifuged and the plasma was discarded. After freeze-drying the red cells were exposed in the reactor¹ to a neutron flux of 10^{12} thermal neutrons per sq. cm. per second for from 12 to 20 hours. The blood samples were then removed from the reactor and left for two weeks to allow decay of the short half-life radioactive nuclides, such as sodium-24 (radioactive half-life 15 hours) and potassium-42 (radioactive half-life 12.5 hours). The ^{51}Cr content of the activated blood was determined by pulse height analysis of the gamma-ray spectra of the blood samples, a Laben 128-channel analyser or a TMC 100-channel Gammascopie being used. Preliminary experiments showed that the polyethylene containers were not radioactive two weeks after removal from the reactor.

In a further preliminary experiment radioactivation analysis was performed on four blood samples to which had been added in vitro 0, 1, 2, and 4 $\mu\text{g.}$ of ^{50}Cr respectively. A linear correlation was obtained between the measured amounts of ^{51}Cr produced by activation and the amounts of ^{50}Cr added to the blood samples. The typical gamma-ray spectrum of an activated blood sample is shown in Fig. 1. The amount of ^{51}Cr

FIG. 1.—Typical gamma-ray spectrum of activated blood to which chromium-50 had been previously added. The various radioactive isotopes present are identified by their characteristic peaks at different gamma-ray energies.

present was taken from the area under the peak at 0.32 MeV (the energy of the single gamma-ray emission of ^{51}Cr). The fall in the ^{50}Cr content of serial blood samples from each patient was then determined by similar measurements of the ^{51}Cr peaks produced by activation, and the $T_{1/2}^{50\text{Cr}}$ calculated as the time in days for the ^{50}Cr content to fall to 50% of the original amount. In Fig. 1 can also be seen two peaks due to iron-59. The peak at the low energy end of the spectrum is due partly

to the Compton effect but also to Bremsstrahlung from the β particles emitted from phosphorus-32. Other elements make smaller contributions to the gamma-ray spectrum (Johnson *et al.*, 1968).

The methodology is discussed in greater detail elsewhere (Johnson *et al.*, 1968). When ^{50}Cr and ^{51}Cr were used simultaneously, the radioactivity in the blood samples due to ^{51}Cr was first estimated and allowed to decay before the samples were placed in the reactor for activation.

Results

The results on the five patients are shown in the Table. In Cases 1 and 2 the $T_{1/2}^{50\text{Cr}}$ and $T_{1/2}^{DF^{32}P}$ were measured simultaneously. In Case 1 the $T_{1/2}^{51\text{Cr}}$ was estimated six weeks after the $T_{1/2}^{50\text{Cr}}$; there had been considerable spontaneous improvement in the interval, with a rise in the haemoglobin level and a fall in the reticulocyte count. In Cases 3, 4, and 5 the $T_{1/2}^{50\text{Cr}}$ and $T_{1/2}^{51\text{Cr}}$ were measured simultaneously, and the erythrocyte survival curves for Case 5 are shown in Fig. 2.

FIG. 2.—Survival of autologous red cells labelled with chromium-50 and chromium-51, measured simultaneously.

Discussion

The apparent lack of correlation between the $T_{1/2}^{50\text{Cr}}$ and the $T_{1/2}^{DF^{32}P}$ is due to elution of chromium but not of DFP from the red cell (Cline and Berlin, 1963). In Case 1 the discrepancy between the $T_{1/2}^{50\text{Cr}}$ and the $T_{1/2}^{51\text{Cr}}$ was explained by remission in the haemolytic process. There was good correlation between the $T_{1/2}^{50\text{Cr}}$ and $T_{1/2}^{51\text{Cr}}$ in the patients where these were measured simultaneously. It appears, therefore, that the use of stable chromium-50 and activation analysis forms a satisfactory alternative to radioactive chromium-51 for studies of red cell life-span.

In all patients studied autologous cells were labelled. The method would, however, allow the simultaneous comparison of the survival of two different populations of erythrocytes. Previous methods of doing this have always required the use of two labels differing chemically and physically and probably with different rates of elution. The method also allows the determination of the red cell survival time without exposing the patient to radiation, a factor which might be important in the investigation of pregnant women and of children.

The disadvantages are the lengthy nature of the analysis, the delay of two weeks to allow decay of short half-life radioactive nuclides, and the cost of enriched material and reactor time. In our study it has been necessary to label 100–200 ml. of patients' blood because of the limitation of the neutron flux available and because of the need to keep the concentration of chromium below a level which would be toxic to the red blood cells. These disadvantages would be less important with higher reactor fluxes, increased radiation times, and automation of counting techniques.

¹ Scottish Research Reactor, East Kilbride, near Glasgow.

Summary

A method is described which uses non-radioactive sodium chromate enriched with chromium-50 (^{50}Cr) for the measurement of the red cell survival time. The chromium content of serial blood samples was measured by activation analysis and its rate of fall was used to calculate the $T_{\frac{1}{2}}^{50}\text{Cr}$. There was good agreement between the $T_{\frac{1}{2}}^{50}\text{Cr}$ and the $T_{\frac{1}{2}}^{51}\text{Cr}$, measured simultaneously. The method would allow simultaneous comparison of the survival times of two different populations of red cells, using two erythrocyte labels which behaved identically. Other advantages and some disadvantages of the technique are discussed.

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Nasal Cancer in Woodworkers in the Furniture Industry*

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The purpose of this paper is to confirm and amplify preliminary reports which suggested that in the Buckinghamshire and Oxfordshire furniture industry there was an excessive risk of adenocarcinoma of the nasal cavity and sinuses among woodworkers (Macbeth, 1965; Acheson, Hadfield, and Macbeth, 1967).

Material and Method

Means of Ascertainment

The material consists principally of two groups of cases of carcinoma of the nasal cavity and accessory sinuses occurring in persons resident in Oxford County Borough, the Administrative County of Oxfordshire, and those parts of Buckinghamshire and Berkshire within the Oxford Hospital Region (Fig. 1). Group 1 consists of patients in whom the diagnosis was made during the decade 1956-65. As the ascertainment is thought to be complete, and the 1961 Census was taken at the midpoint of the decade, these figures have been used in the computation of incidence rates. Group 2 consists of all other patients ascertained in the same area who were diagnosed either before 1 January 1956 or after 31 December 1965. The means of ascertainment used were different in the two groups. In addition to these two groups of cases brief mention is made of eight woodworkers with nasal adenocarcinoma ascertained from other parts of Southern England.

The source of the cases in group 1 was the Oxford Cancer Register. This register was established in 1951, and it is thought that case-finding has been almost complete since 1956. Thus it is noteworthy that none of the additional searches described in the succeeding paragraph uncovered any cases for this decade not previously known to the register.

In group 2 the cases occurring since 1965 were obtained either direct from the hospitals concerned or from the register,

and are thought to be complete for cases diagnosed up to the end of 1967. Attempts to uncover earlier cases were made along the following lines: (1) a search was made of the death registers for High Wycombe and the surrounding countryside, and for Banbury and district back to 1900; (2) a circular letter was sent to the records officers of all London hospitals requesting information about patients treated for nasal cancer who gave an address in the area under study; and (3) a search of the diagnostic index of the Radcliffe Infirmary was made back to its beginning in 1939.

In addition a circular letter was sent to each general practitioner practising in the area, and a systematic search was made of all claims for death benefit (which include a note of the cause of death) received since 1960 by the National Union of

FIG. 1.—Map of area showing numbers of cases of adenocarcinoma ascertained in persons ever employed in the furniture industry according to place of exposure.

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