The Kinetics of Hematopoiesis in the Light Horse I. The Lifespan of Peripheral Blood Cells in the Normal Horse

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ABSTRACT

Three Standardbred horses were given 0.2 mg (1 mCi) of ^{75}selenomethionine intravenously and a second group of three were given 10 mCi of tritiated diisopropylfluorophosphate (0.5 mg) intravenously. Observations on labelled cells were continued for 250 days after radioselenium injection and 160 days after tritium injection.

The lifespan of erythrocytes using ^{75}selenomethionine was 155 ± 10 days and 148 ± 7.8 days using tritiated diisopropylfluorophosphate. There was no significant difference at the 10% level between the lifespans, using these labels. The uptake of radioselenium into erythrocytes reached a mean maximum of 11.5% at 82 ± 18.9 days after injection of the label. There was an elution of from 17.6% of the injected dose of tritium label down to 7.5% eight days after injection of this isotope. From this study both of these labels appear to be satisfactory for determining the erythrocyte lifespan of the horse.

The mean time of the curve of mean radioselenium activity of peripheral blood leukocytes for the three horses was 5.54 days and the lifespan of these cells was seven days. The mean lifespan of peripheral blood leukocytes of the three horses after in vivo labelling with tritiated diisopropylfluorophosphate was 17.3 hours. No specific labelling was found in bone marrow or peripheral blood cells at this level of tritium labelling by dipping emulsion autoradiography.

The mean lifespan of equine platelets for three horses using radioselenomethionine was 9.2 days and the mean lifespan of equine platelets using tritiated diisopropylfluorophosphate was 6.6 days in a group of three horses.

RÉSUMÉ

Trois chevaux "Standardbred" reçurent une injection intra-veineuse de 0.2 mg (1 mCi) de ^{75}séléno-méthionine; on inocula à trois autres, par la même voie, 0.5 mg (10 mCi) de di-isopropyl-fluorophosphate contenant du tritium. Après l'injection de radio-sélénium, on effectua des observations sur les cellules étiquetées, pendant 250 jours; après celle de tritium, on en effectua pendant 160 jours.

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La durée de la vie des hématies étiquetées avec la ^{75}séléno-méthionine s'avéra de 155 ± 10 jours; celle des hématies étiquetées avec le di-isopropyl-fluorophosphate fut de 148 ± 7.8 jours. On ne décela pas de différence appréciable dans la durée de la vie des hématies, lorsqu'on utilisait les deux traceurs à une concentration de 10%. L'absorption du radio-sélénium par les hématies atteignit un sommet moyen de 11.5%, au bout de 82 ± 18.9 jours après l'injection de ce traceur. Il se produisit une élimination de la quantité de tritium injectée, allant de 17.6% à 7.5%, dans les huit jours qui suivirent l'injection de cet isotope. Il semble que les deux traceurs utilisés au cours de cette étude seraient satisfaisants pour déterminer la durée de la vie des érythrocytes du cheval.

La courbe chronologique de l'activité moyenne du radio-sélénium des leucocytes du sang périphérique de trois chevaux s'établit à 5.54 jours; la durée de la vie de ces cellules fut de sept jours. La durée moyenne de la vie des leucocytes du sang périphérique des trois chevaux auxquels on avait injecté du di-isopropyl-fluorophosphate fut de 17.3 heures. L'autoradiographie de frottis plongés dans une émulsion de NTB_2 ne révéla aucun étiquetage spécifique des cellules de la moelle osseuse ou du sang périphérique, lorsqu'on utilisait le tritium à cette concentration.

La moyenne de vie des plaquettes des trois chevaux auxquels on avait injecté de la radio-séléno-méthionine fut de 9.2 jours; celle des plaquettes des trois autres chevaux auxquels on avait injecté du di-isopropyl-fluorophosphate contenant du tritium fut de 6.6 jours.

INTRODUCTION

A number of studies have dealt with the lifespan of equine erythrocytes. While most of the interest is in studies on light horses much of the work reported has been on ponies and on heavy breeds. To a considerable extent the results of these investigations vary with the type of isotopic label used. Cohort labels are those which require synthesis into the red cell during erythropoiesis, examples of which are radioiron, selenomethionine (^{75}Se-M) and radiocarbon glycine (^{14}C-glycine). Cohort labels do not elute from red cells but may be reutilized and result in overestimation of the "true" lifespan. Population labels are those which attach to circulating red cells of all ages, examples of which are radiochromium (^{51}Cr), radiophosphorous — diisopropylfluorophosphate (DF^{32}P) and tritiated diisopropylfluorophosphate (^{3}H-DFP). Because of the elution ^{51}Cr tends to underestimate the "true" lifespan by about half (28) while DF^{32}P and ^{3}H-DFP are felt to be stable labels. In man where the red cell lifespan has been reliably established at 120 days the "normal" red cell lifespan determined with ^{51}Cr is about 60 days. Despite this discrepancy ^{51}Cr remains the most useful label for clinical purposes because lifespan estimates obtained using ^{51}Cr can immediately be determined as normal or indicative of shortened red cell survival. At present ^{51}Cr can only be used in a relative sense in light horses because the "true" lifespan has not been established.

Thus in one study of red cell survival in horses of undefined breed a "half life" of 14.96 ± 1.98 days was found using ^{51}Cr (21) (half life x 1.44 = lifespan). In another report using ^{51}Cr five Shetland ponies were found to have a mean lifespan of 136 ± 17 days (17). Two Thoroughbred stallions had red cell lifespans of 140 and 160 days when 14C-glycine was used as the label (7) and ten crossbreed Percherons had a mean lifespan of 147 ± 8.2 days using DF^{32}P (16). It has been shown that the red cell mass and ferrokinetic values differed significantly between the Thoroughbred and other breeds of horses (15). Thus there is reason to believe that the red cell lifespan also varies between breeds and therefore normal values for the light horse are based on unacceptably few observations. This study was designed to determine the "true" red cell lifespan in the light horse so that further studies could follow on erythrocyte kinetics in hemorrhagic and hemolytic anemias. The labels used ^{3}H-DFP (population) and ^{75}Se-M (cohort) were selected to have minimal elution or relabelling characteristics and the animals (Standardbred) to be representative of the light or hot blooded racing breeds.

^{75}Se-methionine in man has been shown to label leukocytes and platelets as well as red cells (18). Similarly ^{3}H-DFP has been used to label platelets (9). Thus while the main emphasis of this work has been on erythrokinetics, the lifespans of the leukocytes and platelets were also determined.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Six normal, healthy, mature Standardbred horses weighing from 350 to 380 kg were used. Three horses (A, B and C) were given an intravenous injection of 10 ml of ^{75}Se-M[1] into the right jugular vein (1 mCi of total radioactivity and specific activity of 5 mCi/mg). Three horses (C, D and E) were given an intravenous injection of 2 ml of ^{3}H-DFP[1] (10 mCi of total radioactivity and specific activity of 19.6 mCi/mg) via the right jugular vein.

BLOOD COLLECTION PROCEDURES

All blood samples were taken from the left jugular vein by syringe and then added to anticoagulant vials. Evacuated 10 ml and 5 ml vials[2] containing K_2EDTA were used for red cell and platelet determinations. Polycarbonate centrifuge tubes (50 ml) containing 0.2 ml of a 5% solution of K_2EDTA were used to collect 10 ml of blood for leukocyte separation. A control sample was taken one hour prior to injection of ^{75}Se-M. Samples for the separation of peripheral blood cells and plasma were taken at two, four, six, eight, 12, 16, 20 and 24 hours post isotope injection, then at eight hour intervals on days 2 and 3, at 12 hour intervals on days 4, 5, 6 and 7, then once a day until day 14 and then once a week for 250 days. For the experiment using ^{3}H-DFP, blood samples were taken for the separation of peripheral blood cells and plasma at one hour preinjection, at five, ten, 15 and 30 minutes after injection of the isotope, and at one, two, three, four, five, six, eight, ten, 12, 16, 20 and 24 hours; then at eight hour intervals on day 2, once a day on days 3 and 4, then daily until day 14 and then weekly for 160 days.

SEPARATION OF PERIPHERAL BLOOD CELLS FOR COUNTING OF RADIOACTIVITY

Erythrocytes

A small aliquot of a 10 ml sample was used to determine the packed cell volume (PCV)[3] and hemoglobin (Hb)[4] level. The whole blood was allowed to sediment under gravity for 30 minutes at room temperature and then the supernatant suspension of leukocytes and platelets in plasma was pipetted from the erythrocyte sediment (3). The erythrocytes were then suspended in three washes of homologous unlabelled plasma and allowed to settle spontaneously each time. The ^{75}Se-M labelled erythrocytes were then packed by centrifugation at a relative centrifugal force (rcf) of 2510 for ten minutes and the whole red cell mass from 10 ml of blood was used for the measurement of ^{75}Se activity in a gamma counter.[5]

The ^{3}H-DFP labelled red cell sediment was resuspended a fourth time to the original volume with homologous unlabelled plasma and the PCV of this suspension was determined. One ml of this suspension was pipetted into a dialysis tubing bag[6] which was dried, pelleted and burned in a Packard 300 Sample Oxidizer.[7] The ^{3}HOH thus formed was taken up into a solution of 5 ml of Insta-Gel™[7] and 5 ml of toluene and the radioactivity was counted in a Packard 3380 Liquid Scintillation Spectrometer.[7] The percentage uptake of isotope into the red cell mass was calculated using the formula:

$$\text{Percentage Uptake} = \left(\frac{\text{CPM/ml of packed erythrocytes X red cell mass}}{\text{Total } ^{75}\text{Se or } ^{3}\text{H activity injected}}\right) \text{X } 100.$$

The radioactivity of erythrocytes for lifespan determinations were expressed as CPM/ml of erythrocytes corrected for body weight and PCV.

Leukocytes

Thirty-five ml of 0.00178% saponin[8] in 6% dextran 6% dextrose[9] at 4°C was added to 10 ml of whole blood in a 50 ml centrifuge tube and allowed to stand for ten minutes. The lysed erythrocytes were separated from the leukocytes by centrifugation at an rcf of 2510 for ten minutes

[1]Amersham/Searle, Arlington, Illinois.

[2]Vacutainers, Becton Dickinson Co., Canlab, Toronto.

[3]Clay Adams micro hematocrit, Canlab, Toronto.

[4]Cyanomethemoglobin method.

[5]Autogamma counter, Nuclear Chicago Corp.

[6]Dialysis Tubing, Fisher Scientific Co., Toronto, Ontario.

[7]Packard Instrument Co., Downer's Grove, Illinois.

[8]BDH Chemicals, Poole, England.

[9]Dextran 75, Abbot Laboratories, North Chicago, Illinois.

at 10°C. The supernatant was then poured off and the leukocyte button was resuspended in 15 ml of normal saline at 4°C. This suspension was again centrifuged at an rcf of 2510 for ten minutes and the supernatant poured off. The leukocytes were then resuspended in a small volume of homologous unlabelled plasma and smears were prepared (3). The ^{75}Se-M labelled leukocyte button was washed with normal saline and sedimented in a gamma counting vial and the ^{3}H-DFP labelled leukocyte button was washed and sedimented in a dialysis tubing bag[6], dried, pelleted and burned in the sample oxidizer, the ^{3}HOH being taken up into a liquid scintillation solution of 6.7 ml toluene, 3.3 ml methoxyethanol and containing 0.0005 gm Permablend fluor[7] per litre of mixture.

Platelets

Ten ml of whole blood was centrifuged at 5650 rcf for approximately two minutes. The platelet rich supernatant was pipetted from the top of the sediment of erythrocytes and leukocytes. This platelet rich plasma was centrifuged at 2510 rcf for ten minutes and the supernatant plasma pipetted from the platelet button. The platelets were resuspended in homologous unlabelled plasma and smears were prepared. They were then centrifuged again at 2150 rcf for ten minutes. The ^{75}Se-M labelled platelets were centrifuged in a gamma counting vial and the final button was left in this vial for the measurement of ^{75}Se activity. The ^{3}H-DFP labelled platelets were centrifuged in gamma counting vials lined with a dialysis tubing bag. The bag with the washed platelet button was then removed from the gamma counting vial, dried and burned in a sample oxidizer and the ^{3}HOH taken up into liquid scintillation fluid of methoxyethanol, toluene and Permablend.

Counting of Radioactivity

^{75}Se activity was counted in a Nuclear Chicago Auto Gamma counter[10] with a base setting of 0.020 and a window of 0.140 MeV. These settings gave a background count of 355 counts per minute. Each sample was counted for 20,000 counts or 20 minutes. A standard was counted after each 50 samples and results were corrected for nuclear decay back to the time of injection.

^{3}H activity was counted on a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3380[7] using a preset window for tritium.

[10]Nuclear Chicago, Des Plaines, Illinois.

Bone Marrow Biopsies

Bone marrow samples were obtained by sternal puncture at one day preinjection and at one and nine hours, two, three, four, five and seven days after injection of ^{3}H-DFP. One half ml of EDTA anticoagulant (2.0 mg/ml) in plasma was placed in a 35 ml syringe before aspiration of the biopsy. Bone marrow smears were made using the method of Dacie and Lewis (8).

Autoradiographs were made from bone marrow smears, whole blood smears, and smears made from separate suspensions of platelets and leukocytes in homologous unlabelled plasma. Autoradiographs were made in a darkroom with a 25 watt safelight with Wratten 2B filter[11] four feet away by dipping the slides in NTB_2 emulsion[11] at a temperature of 43°C. They were air-dried and placed in light-tight bakelite boxes with dessicant[12] capsules and allowed to develop for 30 days at a temperature of 40°C. They were developed for four minutes in D19[11] and fixed in hypo. The smears were stained in May Grunwald-Geimsa (12) and coverslips were mounted in Diatex[13].

Excretion of Isotopic Labels

Feces and urine were collected from all horses for 25 days after isotope injection. These samples were individually quantitated and a representative sample taken for determination of radioactivity.

[11]Canadian Kodak Co., Toronto, Ontario.

[12]Driaire Humicap Dessicator, Driaire Inc., East Norwalk, Conn., U.S.A.

[13]Canlab, Toronto, Ontario.

RESULTS

Whole Blood

The mean ^{75}Se activity of whole blood from three horses (A, B and C) is shown in Fig. 1 and the mean ^{3}H activity for whole blood from three horses (D, E and F) is shown in Fig. 4. Corrections were made for changes in PCV and blood volume based on body weight (red cell mass = 3.98% of body weight at a PCV of 42.7% (14).

Percentage Labelling of Erythrocytes

The mean percentage uptake of ^{75}Se activity in erythrocytes of horses A, B and C is shown in Fig. 2 and the mean erythrocyte ^{3}H activity for horses D, E and F is shown in Fig. 5.

Erythrocyte Lifespan

The mean survival of erythrocytes from horses A, B and C labelled with ^{75}Se-M are shown in Fig. 3 and Table I. The lifespan was calculated by the method of Neuberger

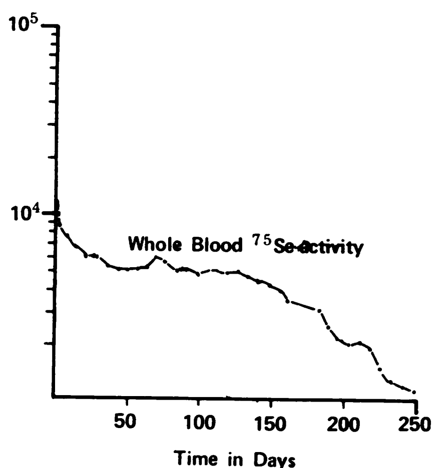

Fig. 1. The mean ^{75}Se-activity of equine whole blood from three horses following in vivo labelling with ^{75}Se-selenomethionine.

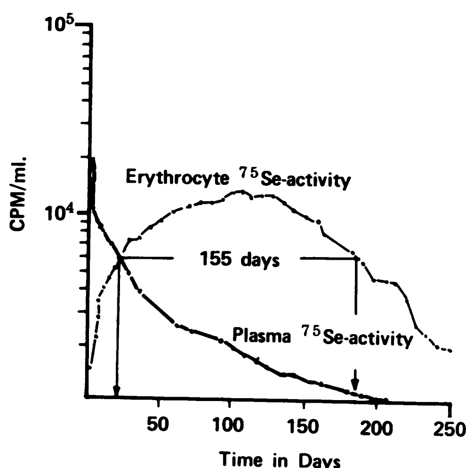

Fig. 3. The mean ^{75}Se-activity of equine erythrocytes and plasma from three horses following in vivo labelling with ^{75}Se-selenomethionine.

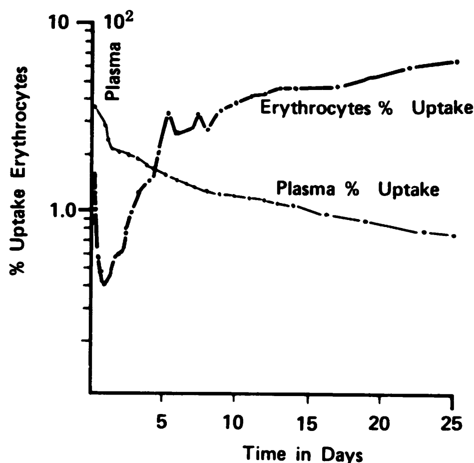

Fig. 2. The mean percentage uptake of ^{75}Se-activity into equine erythrocytes and plasma from three horses following in vivo labelling with ^{75}Se-selenomethionine.

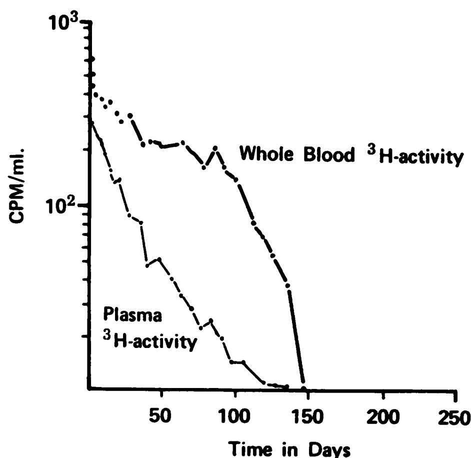

Fig. 4. The mean ^{3}H-activity of equine plasma and whole blood from three horses following in vivo labelling with ^{3}H-DFP.

TABLE I. The Mean Survival of Erythrocytes from Six Horses

Horse	^{75}SeM Label				^{3}H-DFP Label			
	A	B	C	Mean + S.D.	D	E	F	Mean + S.D.
Red Cell Lifespan, d........	167	150	147	155 ± 10.08	144	157	143	148 ± 7.80
Red Cell Turnover %/d......	0.599	0.667	0.680	0.649 ± 0.043	0.694	0.640	0.699	0.678 ± 0.033
Mean PCV %...............	42.6 ± 5.0	50.7 ± 5.0	38 ± 4.2		43 ± 6.6	38 ± 4.5	29 ± 6.2	
Mean body weight kg........	399	413	394		414	490	412	

and Niven (20). The mean survival of erythrocytes from horses D, E and F labelled with ^{3}H-DFP are shown in Fig. 6 and Table I. The lifespan of ^{3}H-DFP labelled red cells was determined from a regression line calculated by the least squares method.

Leukocytes

The mean ^{75}Se activity of leukocytes for the three horses A, B and C is given in Fig. 7. There was a lag of two days before ^{75}Se activity appeared in peripheral leukocytes. The lifespan of the curve according

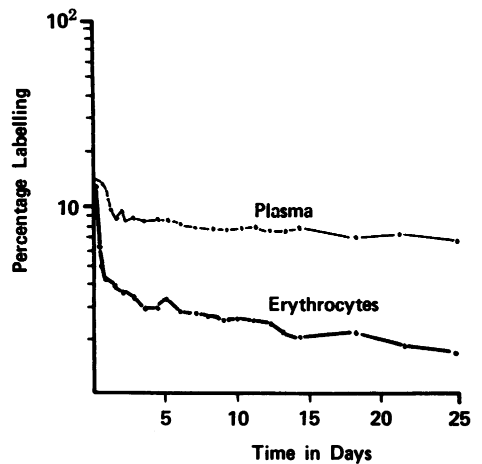

Fig. 5. The mean percentage labelling of ^{3}H-activity of equine plasma and erythrocytes from three horses over 25 days following in vivo labelling with ^{3}H-DFP.

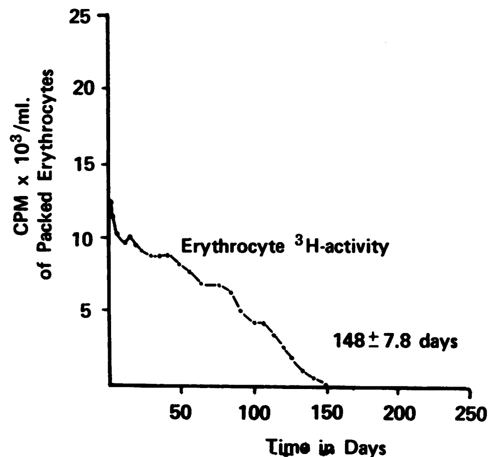

Fig. 6. The mean ^{3}H-activity of packed equine erythrocytes from three horses following in vivo labelling with ^{3}H-DFP.

to the method of Neuberger and Niven (20) was seven days and the mean time of the curve was 5.54 days. The mean percentage uptake of injected ^{75}Se activity into leukocytes calculated at the peak of activity was 0.137% of the total activity injected.

The mean ^{3}H activity of leukocytes for horses D, E and F is shown in Fig. 8. There was a biphasic exponential disappearance of ^{3}H activity for peripheral leukocytes. The early decline of activity was very rapid with a half time of 12 hours. This rate of disappearance changed after the first day to a more gradual slope with a half time of 2.5 days.

The mean labelling level of leukocytes for horses D, E and F was 0.044% of the injected radioactivity calculated on the first sample (five minutes) after isotope injection.

PLATELETS

The mean ^{75}Se activity for platelets for horses A, B and C is given in Fig. 7. There was a lag period of two days, then a sharp increase in platelet ^{75}Se activity. The life-span of the curve according to the method of Neuberger and Niven (20) was 9.2 days.

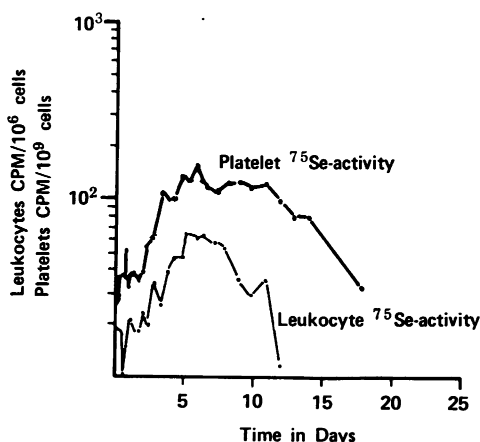

Fig. 7. The mean ^{75}Se-activity of leukocytes and platelets from three horses following in vivo labelling with ^{75}Se-methionine.

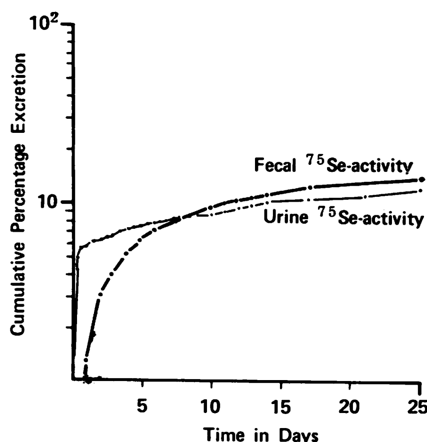

Fig. 9. The mean cumulative percentage excretion of ^{75}Se-activity from urine and feces from three horses given ^{75}Se-selenomethionine intravenously.

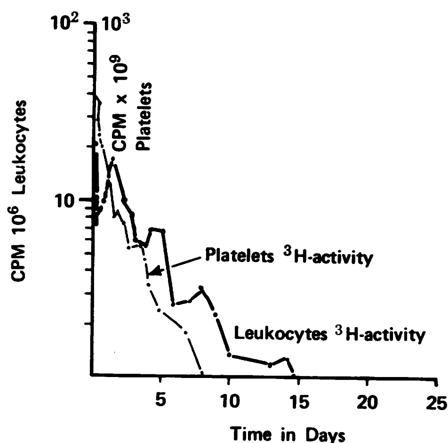

Fig. 8. The mean ^{3}H-activity of circulating equine platelets and leukocytes from three horses following in vivo labelling with ^{3}H-DFP.

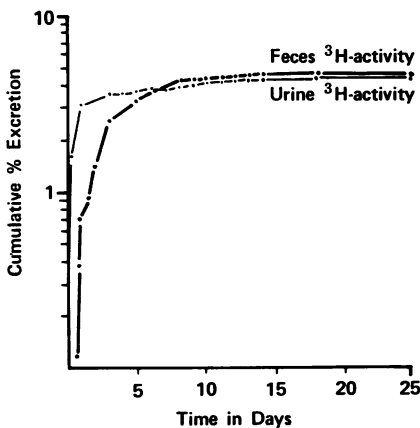

Fig. 10. The mean cumulative excretion percentage in urine and feces from three horses following the intravenous injection of ^{3}H-DFP.

The ^{3}H activity of platelets for horses D, E and F is given in Fig. 8. The regression line of this curve has a half time of 4.6 days and a lifespan of 6.6 days. The mean recovery of platelets from 10 ml of whole blood for horses A, B and C was 66.7% and for horses D, E and F was 62.2%.

The mean percentage uptake of ^{75}Se-M activity in platelets calculated at the peak of activity was 0.102% of the total isotope injected. The mean labelling level of platelets with ^{3}H-DFP was 0.242% of the injected isotope calculated on the first sample (five minutes) after isotope injection.

AUTORADIOGRAPHY

No specific labelling was found in whole blood, leukocyte, platelet and bone marrow smears.

EXCRETION OF ISOTOPIC LABELS

The mean results of radioactive counting on feces and urine from horses A, B and C are given in Fig. 9 and for horses D, E and F in Fig. 10.

DISCUSSION

Of the two red cell labels used in the present study ^{75}Se-selenomethionine (^{75}Se-M) is a cohort label which is synthesized into the globin chains of hemoglobin in the erythrocytes during the early stages of red cell development (23). For the purpose of erythrocyte labelling where only tracer dosages are used ^{75}Se-M may be regarded as replacing methionine.

Tritiated diisopropylfluorophosphate (^{3}H-DFP) is a population label which acts by binding to hydroxyl groups on the serine residues of proteins, particularly cholinesterase (22). In the case of the erythrocyte, binding takes place both on the cell membrane and on intracellular binding sites (25). The cohort label ^{75}Se-M was used to measure the erythrocyte production time as well as the lifespan and the population label ^{3}H-DFP was used solely to measure the peripheral lifespan of erythrocytes. Tritiated-DFP was used in preference to radiophosphorous DFP because the short nuclear half-life (14.3d) of ^{32}P limits the period of observation to about 60 days. In the horse with an expected lifespan of more than twice 60 days this label results in an unacceptably long period (about 87 days) of extrapolation to obtain the full lifespan.

The lifespan of erythrocytes of 155 $\pm$ 10 days using ^{75}Se-M and 147 $\pm$ 7.8 days using ^{3}H-DFP is similar to the 140 and 160 day estimate found by Cornelius *et al* (7) in two Thoroughbreds and to the 147 $\pm$ 8.2 days found in 13 Percheron crossbreeds by Marcelese *et al* (16). There was no significant difference between the values found using DFP and ^{75}Se-M at the 10% level of significance. The possibility of reutilization of ^{75}Se-M prompted the statement (18) that "Se-M was not a satisfactory label for erythrocytes, leukocytes and platelets." The lack of significant difference between the values obtained with ^{75}Se-M (supposedly reutilized) and ^{3}H-DFP where no reutilization takes place is difficult to explain in view of the very prolonged uptake of ^{75}Se-M by erythrocytes (82 $\pm$ 18.9 days). This could be explained by the level of reutilization being too low to significantly affect the lifespan distribution of the erythrocytes. The prolonged uptake could also be due to the ^{75}Se-M being recycled from catabolism of labelled plasma proteins into the red cell globins at a relatively long time after injection, rather than reutilization of label from senescent red cells.

The initial elution of ^{3}H-DFP from red cells from 17.62% down to 7.5% of the injected dose was similar to that described in man (1, 11), in the rat (27) and in dogs (6). For the present study both ^{75}Se-M and ^{3}H-DFP appear to be satisfactory labels for the measurement of erythrocyte lifespan in the horse.

LEUKOCYTES

The mean lifespan of the peripheral blood leukocytes in the three horses (A, B and C) was seven days as determined by the method of Neuberger and Niven (20), which is the time interval between the 50% level of peak activity on the ascending and descending slopes of the leukocytes radio-activity curve (Fig. 7). The mean time of the same curve was calculated to be 5.54 days. This latter calculation gives in effect the center of gravity of the leukocyte labelling curve. Both of these parameters are based on the production time as well as

the peripheral lifespan of the blood leukocytes. It can be seen from Fig. 7 that there was a lag time of two days before the ^{75}Se-M labelled cells began to appear in the peripheral blood. It was shown by Valli *et al* (26), using tritiated thymidine in calves, that the intercompartmental transit time of developing granulocytes in the marrow is about one day. If the same times apply to the horse we can assume that the band and segmented neutrophils do not take up this label and the first labelled leukocytes to appear in the peripheral blood were labelled in the marrow at the metamyelocyte stage. This leukocyte curve and the times derived from it are, therefore, quite similar to the leukocyte curves reported from studies using the nuclear label. tritiated thymidine, in man, the dog and the calf. It has been assumed (13) that in the dog the myelocyte is the last dividing stage in the marrow and therefore is the most mature cellular compartment able to take up tritiated thymidine, although recent studies in the calf (26) and man (30) indicate some proliferative potential for the metamyelocyte. In the present experiment, metamyelocytes apparently did label heavily with ^{75}Se-M as compared with the light nuclear labelling (10%) obtained with tritiated thymidine in calves (26). Thus, ^{75}Se-M-labelled peripheral blood neutrophils should appear two days after ^{75}Se-M injection, while thymidine-labelled peripheral blood neutrophils should appear three days after thymidine injection. Therefore, the mean time of the curve derived from ^{75}Se-M data should be a day shorter than the mean time determined using tritiated thymidine. The mean time of peripheral blood labelled leukocytes labelled *in vivo* with tritiated thymidine was reported to be 6.92 days in calves (26) and about seven days in man (24). It would appear therefore that the mean times of labelled peripheral leukocyte curves for the calf and man are in very close agreement with the present estimate in the horse if one day is added to the 5.54 day estimate to allow for the difference in arrival times characteristic of the two labels.

It can be seen from Fig. 7 that during the first two days following injection of the isotope, before the peripheral blood leukocyte level began to rise sharply, that there was a low level of radioactivity in the leukocytes. This could be interpreted to mean that there was a low level of labelled serum proteins adherent to the surfaces of the cells or this could mean there was ^{75}Se activity in peripheral lymphocytes. Lymphocytes are known to be able to synthesize both DNA and cytoplasmic components in the circulation (29) and can take up ^{75}Se-M (5). Most granulocyte kinetic studies assume a low labelling index in lymphocytes and interpret the leukocyte curve as primarily due to activity in neutrophils (24). This assumption is probably justifiable in species where neutrophils predominate in the peripheral blood (man, dog and horse) but is less accurate in calves where lymphocytes predominate and have a high labelling index (26). In the present study, the general bell-shaped curve of the peripheral blood leukocyte ^{75}Se activity is strongly indicative that long-lived lymphocytes are not contributing significantly to this curve, otherwise the descending line of the curve would be greatly extended.

The mean leukocyte ^{3}H activity for the three horses (B, C and D) follows a biphasic exponential disappearance with half times of 12 hours and 2.5 days. Hence, the lifespans of the cells forming these two curves are 17.3 hours ($t_{½}$ of 12 hours x 1.44) and 3.6 days ($t_{½}$ of 2.5 days x 1.44)[14]. The first component represents the sojourn of mature granulocytes in the peripheral blood (4). If this peripheral lifespan of 17.3 hours is subtracted from the second component this gives a maximum sojourn of just under three days in the bone marrow. If there is a transit time in the horse of one day between each cellular compartment of the bone marrow, then the labelling of granulocytes in the bone marrow began at the myelocyte stage. Cartwright *et al* (4) have reported autoradiographic labelling of myelocytes with ^{3}H-DFP but no labelling of earlier granulocyte precursors but it is possible that in the horse with such a low dosage of DFP (0.5 mg.) most of the DFP was taken up in the peripheral circulation and very little of this label was available to the bone marrow. Cartwright *et al* (4) have shown that segmented neutrophils, band neutrophils and metamyelocytes of the bone marrow contain about 25% of the label of the mature granulocytes in the peripheral blood. Since myelopoiesis has been shown to be extravascular (28), it is possible that most mature granulocytes (the marrow granulocyte reserve) which

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[14]Lifespan $= t_{½} \times \frac{1}{2 \log^{e}}$ or $t_{½} \times \frac{1}{.699}$

are just entering the circulation would have better access to this label than the more primitive cells. There was insufficient label present in the marrow autoradiographs to confirm the extent of bone marrow labelling and the distribution of the label between the myeloid and lymphoid cells. As with the leukocytes labelled with ^{75}Se-M, the exponential drop in leukocyte ^{3}H activity was sufficiently short to indicate that few circulating long-lived labelled lymphocytes contributed to the total leukocyte ^{3}H activity. The results of these studies using ^{75}Se-M and ^{3}H-DFP combine to give a production and utilization time of the neutrophil in the light horse of close to seven days and a peripheral lifespan of about 17 hours.

Platelets

The ^{75}Se activity of platelets gives a curve with a lifespan of 9.2 days using the method of Neuberger and Niven (20) (Fig. 7). This estimate agrees closely with the value in some other species using ^{75}Se-M (eight to 12 days in man (19) and 10.6 $\pm$ 3.3 days in man (2)) and less closely with the four to five days in the dog (10). Najean and Ardiallou (19) reported that ^{75}Se-M is an unsatisfactory platelet label due to the reutilization of the label. However, Evatt and Levin (10) found no label in the platelets of rabbits after the injection of highly labelled plasma protein, which suggests that there was no reutilization. In the present experiment, the lifespan of platelets was shorter than that of all of the plasma protein fractions (not reported here) and this supports the concept that no or little reutilization of ^{75}Se-M into the first cohort of labelled platelets occurred. Wintrobe (28) indicated that the transit time of megakaryocytes in the bone marrow is three to four days. Since there were few labelled platelets in the peripheral blood for the first two days after ^{75}Se-M injection (Fig. 7) it could be assumed that mature megakaryocytes did not take up this label, but if the lag time of two days is taken from the production time of four days, it would appear that the platelets are then delivered to the peripheral blood over a two day period. If this delivery period (two days) is subtracted from the apparent lifespan of 9.2 days, this gives a peripheral lifespan of 7.2 days, which agrees closely with the 6.6 day lifespan obtained using ^{3}H-DFP. Since ^{3}H-DFP is a population label, little or no delivery of labelled platelets from the bone marrow to the peripheral circulation can be expected after *in vivo* labelling (9). This is borne out by this study (Fig. 8) and therefore the correction that has been made for the platelet production time (subtraction of two days from the ^{75}Se-M lifespan) appears to be valid and lends further support to an overall platelet lifespan in the horse of about seven days. The correlation coefficient (r) of the regression line for ^{3}H-DFP labelled platelets was 0.67, which could be interpreted as indicating the occurrence of random as well as senescent platelet destruction.

A detailed knowledge of platelet kinetics in the horse is required for studies of equine infectious anemia (E.I.A.) where there is a constant thrombocytopenia in the acute phase (17). Therefore, an understanding of platelet production and lifespan in the normal horse is essential before the thrombocytopenia of E.I.A. can be properly characterized.

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REFERENCES

1. **BOVE, J. R., F. G. EBAUGH and N. H. HANOVER.** The use of diisopropylfluorophosphate^{32} for the determination of in vivo red cell survival and plasma cholinesterase rates. J. Lab. clin. Med. 51: 916-925. 1958.
2. **BRONSKY, I., N. H. SEIGEL, S. B. KAHN, M. R. ROSS and G. PETRON.** Simultaneous fibrinogen and platelet survival with ^{75}Se-selenomethionine in man. Br. J. Haemat. 18: 341-355. 1970.
3. **CARTER, E. I., V. E. VALLI and B. J. McSHERRY.** The separation of peripheral blood cells of the horse. Can. J. comp. Med. 38: 72-74. 1974.
4. **CARTWRIGHT, G. E., J. W. ATHENS and M. M. WINTROBE.** The kinetics of granulopoiesis in normal man. Blood 24: 780-803. 1964.
5. **CHENG, F. H. F., R. E. PETERSON and T. C. EVANS.** Incorporation of ^{75}Se-selenomethionine in human peripheral lymphocytes as an in vitro test for globulin-synthesizing capacity. J. nucl. Med. 10: 63-67. 1963.
6. **CLINE M. J. and N. I. BERLIN.** Measurement of red cell survival with tritiated diisopropylfluorophosphate. J. Lab. clin. Med. 60: 826-832. 1962.
7. **CORNELIUS, C. E., J. J. KANEKO, D. C. BENSON and J. D. WHEAT.** Erythrocyte survival studies in the horse using glycine-C^{14}. Am. J. vet. Res. 21: 1123-1124. 1962.

8. **DACIE, J. V. and S. M. LEWIS.** Practical Hematology, 4th Ed. London: J. & A. Churchill. 1970.
9. **EBBE, S., F. STOHLMAN, J. DONOVAN and D. HOWARD.** Platelet survival with tritium labelled diisopropylfluorophosphate. J. Lab. clin. Med. **68**: 233-243. 1966.
10. **EVATT, B. J. and J. LEVIN.** Measurement of thrombopoiesis in rabbits using ^{75}Se-selenomethionine. J. clin. Invest. **48**: 1615-1626. 1969.
11. **HJORT, P. F., H. PAPUTCHIS and B. CHENEY.** Labelling of red blood cells with radioactive diisopropylfluorophosphate ($DF^{32}P$): Evidence for an initial release of label. J. Lab. clin. Med. **55**: 416-424. 1960.
12. **LILLIE, R. D.** Histologic Technique and Practical Histochemistry. First Edition. Toronto: McGraw-Hill. 1948.
13. **MALONEY, M. A. and H. M. PATT.** Granulocyte transit from bone marrow to blood. Blood **31**: 195-201. 1968.
14. **MARCELESE, N. A., R. M. VALSECCHI, H. D. FIGUEIRAS, H. R. CAMBEROS and J. E. VALERA.** Normal blood volumes in the horse. Am. J. Physiol. **207**: 223-227. 1964.
15. **MARCELESE, N. A., H. D. FIGUEIRAS, R. M. VALSECCHI, A. A. FRAGA, H. R. CAMBEROS and J. E. VALERA.** Erythro-kinetics in the horse. Am. J. Physiol. **209**: 727-730. 1965.
16. **MARCELESE, N. A., H. D. FIGUEIRAS, R. M. KREMENSHUZKY, R. M. VALSECCHI, H. R. CAMBEROS and J. E. VALERA.** Red cell survival time in the horse, determined with diisopropylphosphofluoridate-^{32}P. Am. J. Physiol. **211**: 281-282. 1966.
17. **McGUIRE, T. C., J. B. HENSON and S. E. QUIST.** Viral induced hemolysis in equine infectious anemia. Am. J. vet. Res. **30**: 2091-2097. 1969.
18. **McINTYRE, P. A., B. EVATT, B. A. HODKINSON and V. SCHEIFFEL.** Selenium-75 selenomethionine as a label for erythrocytes, leukocytes and platelets in man. J. Lab. clin. Med. **75**: 472-480. 1970.
19. **NAJEAN, Y. and N. ARDAILLOU.** The use of ^{75}Se-Metionine in the **in vivo** study of platelet kinetics. Scand. J. Haemat **6**: 395-401. 1969.
20. **NEUBERGER, A. and J. S. F. NIVEN.** Haemoglobin formation in rabbits. J. Physiol., Lond. **112**: 292-310. 1951.
21. **OBARA, J. and H. NAKAJIMA.** Lifespan of ^{51}Cr-labelled erythrocytes in equine infectious anemia. Jap. J. vet. Sci. **23**: 207-210. 1961.
22. **O'BRIEN, R. D.** Organophosphates and carbamates. In Metabolic Inhibitors, Vol. III. R. M. Honchster, Ed. New York: Academic Press. 1963.
23. **PENNER, J. A.** Investigation of erythrocyte turnover with Selenium-75-labelled methionine. J. Lab. clin. Med. **67**: 424-438. 1966.
24. **PERRY, S., H. A. GOODWIN and T. S. ZIMMERMAN.** The physiology of the granulocyte. Part 2. J. Am. med. Ass. **203**: 1025-1032. 1968.
25. **SEARS, D. A. and R. I. WEED.** Diisopropylfluorophosphate is not a specific label for the red cell membrane. Blood **34**: 376-379. 1969.
26. **VALLI, V. E. O., T. J. HULLAND, B. J. McSHERRY, G. A. ROBINSON and J. P. W. GILMAN.** The kinetics of haematopoiesis in the calf. I. An autoradiographical study of myelopoiesis in normal, anaemic and endotoxin treated calves. Res. vet. Sci. **12**: 535-550. 1971.
27. **VAN PUTTEN, L. M.** The lifespan of red cells in the rat and the mouse as determined by labelling with DFP^{32} **in vivo**. Blood **13**: 789-794. 1958.
28. **WINTROBE, M. M.** Clinical Hematology. Sixth Ed. Philadephia: Lea and Febiger. 1967.
29. **WU, A. M., J. E. TILL, L. SIMINOVITCH and E. A. McCULOCH.** Cytological evidence of a relationship between normal and hemopoietic colony forming cells and cells of the lymphoid system. J. exp. Med. **127**: 455-464. 1968.
30. **YOSHIDA, Y., A. TODO, S. SHIRAKAWA, G. WAKISAKA and H. UCHINO.** Proliferation of megaloblasts in pernicious anemia as observed from nucleic acid metabolism. Blood **51**: 292-303. 1968.