The Life Span of the Red Blood Cell and the Red Blood Cell Volume in the Chicken, Pigeon and Duck as Estimated by the Use of Na2Cr5O4

With Observations on Red Cell Turnover Rate in the Mammal, Bird and Reptile

By GERALD P. RODNAN, FRANKLIN G. EBAUGH, JR., AND M. R. SPIVEY FOX

With the technical assistance of DORIS M. CHAMBERS

THE PRESENT REPORT is concerned with the applicability of intravenously injected Na2Cr5O4 in the study of erythrocyte survival in the chicken, duck and pigeon, and more generally with the comparative aspects of an apparent direct relationship between an animal's total metabolic rate and the life span of its red blood cells.

It has been demonstrated in man that radioactive chromate binds rapidly with the hemoglobin of the red cells when added in vitro and in vivo, and that the radioactivity released from destroyed cells in vivo is not re-utilized in red blood cell tagging. It has been further shown that in vitro labeling with Na2Cr5O4 does not impair the survival of auto-transfused cells, provided that the concentration of chromium metal is kept below 10 μg. per ml. whole blood. A disadvantage of this technic for the estimation of red cell life span has been that of the apparent slow elution of chromium from the circulating cells in vivo, so that in the human, approximately half the radioactive material initially present has left the labeled circulating red cell after 55–90 days.

METHODS

Groups of 6 each of adult New Hampshire chickens, weighing 2.8–4.0 Kg., Palmetto Strain white Carneau pigeons, weighing 0.38–0.45 Kg., and white Pekin ducks, weighing 2.2–3.2 Kg., were fed a stock diet of Nutrena Chick Starter. All birds appeared healthy and maintained or gained slightly in weight during the course of study. A single dose of Na2Cr5O4, containing 320–420 μg. of chromium metal and 40–60 μc. of radioactivity was injected intravenously into the wing vein. Blood specimens of from 0.1 to 0.5 ml. were obtained from the opposite wing vein the same day or the day following the Cr5O4 injection, and at intervals thereafter as indicated under experimental results. The blood was pipetted into screw top counting vials, and, in the case of the initial samples, was washed at least twice with 6 ml. of 0.85 Gm. per cent saline in order to remove extracorporeal radioactivity before final counting. Two weeks following the intravenous injection of the chromium41, unwashed samples were used, since it was found that there was no significant difference in count between these and washed specimens.

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

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* Obtained from Palmetto Pigeon Plant, Sumter, S. Carolina.
† Courtesy of the National Zoological Park, Washington, D. C.
‡ Nutrena Mills, Minneapolis, Minnesota.
**TABLE 1.—Hematologic Values for the Chicken, the Pigeon, and the Duck**

<table>
<thead>
<tr>
<th>No. animal</th>
<th>Sex</th>
<th>Hemo-globin (gram %)</th>
<th>Hct. (%)</th>
<th>RBC (X10^6/μl)</th>
<th>MCH (μg)</th>
<th>MCHC (%)</th>
<th>Serum Iron (% uptake of intravenously administered Na₂Cr₂O₇)</th>
<th>RBC volume</th>
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* Employed in erythrocyte survival study.

The emissions of chromium⁴¹ were counted in a well-type scintillation counter* with a thallium-activated sodium iodide crystal 3/4 in diameter. The background ranged from 110–120 cpm, and 1 μc. of chromium⁴¹ distributed in a 5 ml. volume gave 90,000 cpm. The determination of the radioactivity of each sample was based on duplicate counts of 12,800 to 25,600. Hematocrits were determined by the capillary tube technic of Strumia et al.¹⁸ Radioactivity was expressed as counts per minute per milliliter of packed red cells, corrected for physical decay of the isotope (half-life, 26.5 days), by comparison with a standard solution of Na₂Cr₂O₇.

The percentage of the total intravenously injected radioactivity taken up by the red cells of the chicken and pigeon was calculated, using values for the red blood cell volume of 24 ml. per kilogram of body weight in the rooster, 12 ml. per kilogram in the hen, and 33 ml. per kilogram in the pigeon. These estimates of red cell volume represent the highest values found for the hen and rooster and the average value for the pigeon as determined in the present study (table 1.).

**Blood Volume Determination**—1–2 ml. of heparinized whole blood was incubated for one hour at room temperature with 15–25 μc. of Na₂Cr₂O₇. The cells were then washed.

* Nuclear Instrument Company, Chicago, Ill.
twice with saline to remove extracorporeal radiochromium and a known volume of these labeled cells injected into a wing vein. Twenty minutes after injection, a blood sample was obtained from the vein of the opposite wing, and the circulating red blood cell volume determined by the following formula:

\[
\text{RBC Volume} = \frac{\text{Total counts per minute injected}}{\text{CPM/ml. RBC in blood sample}}
\]

**Experimental Observations**

*RBC Uptake of Intravenously Injected Na\textsubscript{2}Cr\textsuperscript{51}O\textsubscript{4}—*An hour after injection of the Na\textsubscript{2}Cr\textsuperscript{51}O\textsubscript{4}, approximately 50 per cent of the radioactivity of whole blood was in the plasma. On the second day, from 2–8 per cent of this activity was in the plasma, on the fifth day less than 1 per cent, and by the eighth day there was no significant amount of radioactivity in the plasma.

The uptake of radioactivity by the erythrocyte mass was 8–10 per cent of the total intravenous dose in the hens, 13–19 per cent in the 3 roosters, and between 15 and 25 per cent in the 6 pigeons (table 1).

**Red Blood Cell Survival**

*Chicken.* The decline in the radioactivity of the red blood cells of the chicken, as corrected for physical decay of chromium\textsuperscript{51}, is depicted in figure 1. The 100 per cent value in figure 1 represents the amount of radioactivity present per ml. of packed red blood cells, 30 minutes to 1 hour after injection of the sodium chromate. The hematocrit remained constant in the individual birds during the course of the 7-week experiment. The blood drawn for sampling totaled approximately 4–5 ml. for each bird for each series of measurements, and no correction was made for this loss, which represented less than 5 per cent of the bird’s total blood volume.

Inspection of the curve of radioactivity of the circulating red cells of the chicken (fig. 1) reveals little or no decrease in radioactivity during the first 2

![Graph](image-url)
days and a subsequent rate of decline of radioactivity neither linear nor logarithmic which reached a value of 2 per cent of the initial radioactivity by the 35th day after injection of Na₂Cr⁵¹O₄. No difference in red blood cell survival between roosters and hens could be demonstrated in this small series of birds. One rooster (7 in table 1) was anemic, having an hematocrit of 33.5 per cent with a total red cell volume of 17 ml. per kilogram. The erythrocyte survival in this animal was not significantly different from the other two roosters studied.

Pigeon. Survival data of the in vivo chromium⁵¹ labeled erythrocytes of 6 pigeons are depicted in figure 1. No correction was made for blood lost through sampling since the total drawn (2-3 ml.) equaled less than 10 per cent of the total volume, and hematocrits did not fall during the period of study. It may be noted that in three of these birds there was little or no fall in red cell radioactivity during the first week following injection of the Na₂Cr⁵¹O₄. Following this period however, the radioactivity declined in a curvilinear fashion together with that of the other three animals, so that by the 45th day post-injection there was 3 per cent or less of the initial activity remaining in the red cells. No difference was noted in the survival of red cells from male and female birds.

Duck. The estimate of erythrocyte survival in 6 ducks (4 male and 2 female) (fig. 1) is based on data in which the initial or “100 per cent” value was determined on the day following injection of the radiochromium. The amount of
blood drawn in sampling was 3–4 ml. per animal, and there was no change in hematocrit during the course of the study. There ensued a smooth, slightly curvilinear decline in radioactivity. Less than 2 per cent of the initial activity remained in the circulating red blood cells on the 42nd day post-injection. In order to assess the reproducibility of the method, the measurement of red cell survival was repeated in two of the original ducks. The values in this second study were found to agree closely with those of the first (fig. 2). In the case of the repeat study, the initial or “100 per cent” value was determined on the second day after injection.

Radioactivity of the Egg. A few of the chickens and ducks laid eggs while under study. No appreciable difference was noted in the red blood cell survival curves of the non-layers as compared with the laying birds. It is of interest that two chicken eggs (boiled before counting) obtained 7 and 13 days following intravenous administration of Na₂Cr₂O₇ were found to contain significant amounts of radioactivity, 87 per cent of which was in the yolk, 8 per cent in the white, and 5 per cent in the shell. The activity of the yolk was concentrated in the innermost portion. Similarly, in the case of a duck, eggs laid up to 6 days after the administration of Na₂Cr₂O₇ were markedly radioactive (with a concentration in the yolk), but by the 11th day after injection, there was so significant radioactivity in the eggs.

Blood Volume

Chicken. The total red cell volume was estimated in 4 hens and 3 roosters by the technic of in vitro tagging with Na₂Cr₂O₇, as described above. Expressed as ml. of red blood cells per kilogram body weight (including feathers), the values for the hens were 8, 9, 11, and 12, and for the roosters, 17, 23, and 24 (table 1).

Pigeon. The red blood cell volume in the pigeon was found to be 31 and 33 ml. per kilogram in 2 cocks and 34 ml. per kilogram body weight in each of the 2 hens (table 1).

Duck. Two drakes were found to have red blood cell volumes of 29 and 31 ml. per kilogram body weight, and two females 25 ml. per kilogram (table 1).

Discussion

Survival of the Avian Erythrocyte

The relative importance of senescent death (linear decay) and random destruction (exponential) in effecting the removal of the tagged erythrocytes could not be determined from this study, since it is possible that there was elution of chromium from the circulating cells, as has been found in the case of the human erythrocyte. Such a loss of tag from the circulating cells would lead to falsely low estimates of the red cell life span. In addition, the plateauing of the radioactivity of the red cells in the case of three of the pigeons, and to a lesser extent the chicken, suggests that an unknown amount of the intravenously injected chromium may have continued to enter the circulating RBCs during the initial period of study. This additional labeling may have been effected either through the continued tagging of red cells in the peripheral circulation or through entry into the circulation of erythrocytes labeled while undergoing maturation in the
bone marrow. Inasmuch as the concentration of plasma Cr^{48} activity fell rapidly during this early period, the latter would appear to be the more likely probability. The data do not exclude the additional possibility that the intravenously injected chromium selectively tagged younger red cells.

The number of days which elapse from time of the intravenous injection of the radiochromium to the time of virtual disappearance of radioactivity from the blood represents therefore a maximum life span of the labeled erythrocytes. This figure exceeds the mean (intravascular) life span by an amount, the magnitude of which depends on the labeling of red cells still in the marrow at the time of injection and/or the continued tagging of circulating red cells by radioactive chromate in the plasma.

**Chicken.** Ottesen\(^2\) found that 5 days elapsed before red cells labeled with P\(^{32}\) were released to the circulation from the marrow. If this be subtracted from the maximum life span value of 35 days (fig. 1), the resulting 30 days may be taken as the estimate of the mean (intravascular) life span. This figure for the mean life span is very close to the value of 28 days noted in the chicken by Hevesy and Ottesen,\(^3\) Ottesen,\(^2\) and Shemin.\(^4\)

**Pigeon.** The maximum erythrocyte life span in the adult pigeon as determined with the radiochromium tag was 35–45 days. If the initial plateauing of the circulating radioactivity, which was observed in the case of three of the birds (fig. 1) be the result of the entry of cells labeled while still in the marrow and/or the continued tagging of additional cells in the peripheral blood, as discussed above, then 35 days, the elapsed time from the beginning fall in radioactivity to the virtual disappearance, would be the more reasonable estimate of mean (intravascular) life span. We have found no other studies of the life span of the pigeon erythrocyte with which these values may be compared.

**Duck.** The smooth decline in the radioactivity of the duck erythrocytes suggests that fewer labeled erythrocytes entered the circulation after injection than was the case for the chicken and pigeon. The estimated maximum life span of 42 days for the duck erythrocyte is the same as Brace and Altland's figure\(^5\) in two male white Pekins given glycine-2-C\(^14\), and contrasts sharply with the mean life span of 11.7 days suggested by McConnell, Portman, and Rigdon.\(^6\) These latter workers transfused young Pekin ducks with radioactive selenohemoglobin-labeled red blood cells obtained from two donor ducks which had been previously injected with sodium selenate. They found a linear decline lasting 11.7 days in the radioactivity of the radioselenium tagged blood. It should be noted, however, that the use of the blood from donor birds would allow for the possibility of transfusion incompatibilities or red cell damage in vitro, either of which would result in abnormally rapid removal of the tagged cells in vivo.

The close agreement of the chromium\(^{51}\) estimates of the erythrocyte survival of the chicken and duck with values secured by independent methods suggests that the avian red cells were not damaged by the in vivo tagging with Na\(_2\)Cr\(^{36}\)O\(_4\). It would also appear that elution of chromium\(^{51}\) from the circulating cells, if such did occur, did not influence appreciably the estimation of the maximum RBC life span, and that there was no significant re-utilization of radiochromium released from destroyed cells.
*Comparative Aspects of Erythrocyte Turnover Rates*

Utilizing data compiled from the literature and from the present study, we have listed in table 2 estimates of the red blood cell turnover (% red cell mass replaced per day = 100/RBC survival in days) and basal heat production (Cal./Kg. body weight) for a variety of animal species. The determinations of red cell life span are based on data obtained by a wide variety of technics in many laboratories. Many of these values are probably somewhat greater than the mean cell life span and more nearly approximate the potential or maximum life span. In those animals in which there appears to be little or no random destruction of the red blood cells, but only senescent or linear decay (man, dog, mouse, dog, rabbit, pig, cat, etc.) the mean cell life span would be very close to the potential span. In those animals with significant degrees of random destruction of the red cells, (cat, rabbit, pig) the mean red cell life span would be shorter than the potential or maximum life span. In any event, despite the uncertainties.

**Table 2.—Red Blood Cell Survival and Basal Heat Production in a Variety of Animal Species**

<table>
<thead>
<tr>
<th>Species</th>
<th>RBC life span (days)*</th>
<th>RBC turnover (% RBC volume replaced/day)</th>
<th>References</th>
<th>Animal weight (Kg.)</th>
<th>Basal heat production (Cal./Kg./24 hrs.)</th>
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<td>0.47**</td>
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<td>41</td>
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* Values presented are those for healthy mature animals save for exceptions, as noted.
† Figure in brackets represents an average value of the various available estimates of RBC life span.
‡ Estimated red blood cell life span of single animal kept at “room temperature.” Red cell survival 65, 95 and 112 days in 3 animals kept in cold room (4.5 C.) for periods of 35 to 82 days, and then returned to room temperature.
§ Pigs employed in this study were young, growing animals.
†† Data concerning animal weight and basal heat production represent average values in groups of animals other than those utilized in study of red blood cell survival.
‖ Average value for group of non-hibernating animals kept at 28 C. temperature.
** Values presented for weight (without carapace) and basal heat production are those of animal (tortoise no. 3) kept at 20 C. temperature.
involved in computing the red cell turnover rates of these animals, there is a positive correlation between metabolic rate and red cell turnover ($p < 0.003$ as determined by the rank coefficient of correlation $^4$) (fig. 3).

The marked fluctuation in the metabolic rate and red cell survival of the marmot with changes in environmental temperature $^7$ may account for the discrepancy of correlation between metabolic rate and erythrocyte turnover in this animal. While the data are meager in amount, they indicate a marked prolongation of erythrocyte life span in marmots kept in a cold room (table 2).

Despite considerable clinical experimental study of the effects of alteration in thyroid function upon erythropoiesis, the means by which metabolic activity may influence red cell turnover remain obscure. More accurate appraisal of the relationship between red blood cell turnover and metabolic rate requires additional data which would be obtained using uniform methods in many different groups of animals in which there were simultaneous estimates of metabolic rate.

**Blood Volume in the Bird**

Using Evans blue dye dilution, with equilibration periods ranging from 3 to 10 minutes, various workers have estimated a total *whole blood* volume of 90 ml.
per Kg. body weight in the rooster and 55–56 ml. per Kg. in the hen, with red cell volumes of 40 ml. per Kg. and 16–18 ml. per Kg. respectively. With radiochromium tagged cells, the results indicate lower red cell volume, namely, 17–24 ml. per Kg. body weight in the rooster and 9–12 ml. per Kg. body weight in the hen.

Portman et al. injected human serum albumin into ducks, obtained blood samples at 15 minutes, and estimated a red cell volume of 38 ml. per Kg. body weight in 150–450 gram birds, and 31 ml. per Kg. in 1100–2000 gram birds. Again, as in the case of the chicken, the use of chromium-labeled cells indicate somewhat lower values for red cell volume, i.e., 25–31 ml. per Kg. for male or female ducks. In four pigeons, two male and two female, the red cell volume obtained by use of chromium-labeled cells approximates 31–34 ml. per Kg. body weight.

The use of a plasma protein tag can be expected to provide falsely high estimates of plasma volume because the proteins do not remain confined to the vascular bed but appear to be in dynamic equilibrium with an extravascular compartment. If this be true in the bird, as in man, then calculating red cell volume indirectly from a measured “plasma” volume and peripheral venous hematocrit would result in a falsely high estimate of red cell volume.

While there are many species of animals in which the female has a lower hematocrit and/or total red cell volume than the male, the chicken is unique in that the hen has a total red cell volume only half that of the male. Ramsay and Campbell have shown that the serum iron of the hen is not lower than that of the rooster and indeed reaches extraordinarily high levels during the egg laying process. These data, plus the normal red blood cell indices of the hen (table 1) indicate that the lower erythrocyte volume in the hen is not due to iron deficiency. The fact that the red blood cell survival of the hen is no shorter than that of the rooster suggests that the difference in total red cell volume is due to a lower rate of red cell production by the marrow and not to any increased destruction of the red blood cells of the hen.

Further study of the concentration of radiochromium in the yolk of the egg of the laying fowl would appear warranted, for this might be a useful tool in learning more about the physiology of egg laying.

**SUMMARY**

Eight to 25 per cent of intravenously injected Na$_2$CrO$_4$ binds firmly with erythrocytes of the chicken, pigeon and duck. Calculation of the maximum life span of these avian red cells was made from the disappearance time of circulating radioactivity. The maximum life span of the chicken erythrocyte was found to be 35 days, of the pigeon erythrocyte 35–45 days, and the duck erythrocyte 42 days. Comparing the life span of avian erythrocytes with those of other animal species, the rate of red cell turnover in the mammals, birds, and reptile (turtle) was found to correlate directly with basal heat production per kilogram body weight.

Using erythrocytes tagged with Na$_2$CrO$_4$ in vitro, the total red blood cell volume was found to be 17–24 ml. per Kg. body weight in the rooster, 9–12 ml. per Kg. in the hen, 25–31 ml. per Kg. in the duck, and 31–34 ml. per Kg. in the pigeon. These values proved somewhat lower than those obtained from the
indirect estimates of red cell volume, using plasma volume figures and periphery blood hematocrit.

**SUMMARIO IN INTERLINGUA**

Inter 8 e 25 pro ceiito del intravenosemente injicite Na2Cr\textsuperscript{51}O\textsubscript{4} se liga firmente con le erythrocytos de gallinas, columbas, e anates. Le maximo del superviventia intravascular del erythrocytos de iste aves esseva calculate super le base del periodo de disparitiomi del radioactivitate circulante. Esseva constatat que le superviventia maximal del erythrocytos de gallinas es 35 dies; illo del erythrocytos de columbas esseva 35 a 45 dies; e illo del erythrocytos de anates esseva 42 dies. Post comparar le duration del vita de iste erythrocytos avian con illo del erythrocytos de altre species animal, il esseva constatat que le rapiditate del reimplaciamento erythrocytic in mammales, aves, e reptiles (tortuca) es directemente correlate con le production de calor metabolic per kilogramma de peso corporee.

Per medio de erythrocytos marcate per Na2Cr\textsuperscript{51}O\textsubscript{4} in vitro, ii esseva constatat que le total volumine erythrocytic es 17 a 24 ml per kg de peso corporee in le gallo, 9 a 12 ml per kg de peso corporee in le gallina, 25 a 31 ml per kg in le anate, e 31 a 34 ml per kg in le columba. Iste valores esseva alique inferior al valores obtenite ab estimationes indirecte del volumine erythrocytic super le base de valores del volumine plasmatic e del hematocrite de sanguine peripheric.

**REFERENCES**


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