Effect of Temperature on Red Cell Survival in the Alligator. (27901)

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In higher vertebrates the erythrocyte life span is predominantly finite with a smaller and variable number of red cells being destroyed by random processes(1). Aging of the erythrocyte can be correlated with progressive depletion of certain enzyme systems (2). The rate of erythrocyte aging for any species appears to be fixed. In homeothermic mammals, the normal red cell life span may be shortened by abnormalities in the external or internal erythrocyte environment, but prolongation of red cell life span beyond the relatively narrow limits for the species has not been documented. In animals whose rate of metabolism is dependent on environmental temperature, evidence has been obtained suggesting that erythrocyte longevity is increased at low metabolic rates. However, in these species, the hibernating marmot(3) and hamster(4) and the frog at 4°C(5), it has not been possible to correct the observed red cell survival curves for changes in total red cell volume due to changes in rate of cell synthesis associated with hibernation or low environmental temperature. For this reason the present study of the effect of temperature on red cell survival was performed in a poikilotherm sufficiently large to permit repeated determinations of red cell mass. The data obtained suggest that in the alligator the erythrocyte life span is inversely related to environmental temperature.

Methods. Immature American alligators (*Aligator mississippiensis*) of either sex weighing between 2 and 3 kg were maintained at either 16-17°C or 31-32°C. After 4 to 8 weeks at constant temperature, 4 animals were given an intravenous injection of 0.05 to 0.2 mg of tritiated diisopropylfluorophosphate (H^3-DFP*; specific activity 1.0 mc/mg) and 2 animals were given 0.04 or 0.20 mg of DFP^{32}* (0.18-0.28 mc/mg). One milliliter blood samples were drawn from the ventral tail vein into heparinized syringes at 7-22 day intervals and the red cells washed free of plasma and leukocytes. Erythrocyte P^{32} content was determined with a Geiger-Müller tube(6). Red cell tritium specific activity was determined as tritiated water in a liquid scintillation spectrometer(7). Correction was made for physical decay of both radio-isotopes and for quenching in the case of tritium. Specific activity was expressed as cpm/ml of red cells.

At the beginning and end of the H^3-DFP red cell survival study total red cell volume was determined in each animal using 5-7 μc of Cr^{51}(8).

The red cell life span was calculated from the least squares line of the rate of change of specific activity with time, corrected for blood sampling and for change in total red cell volume assuming a linear rate of change during the period of observation.

Results. In 2 animals (A and B) maintained at 31°C, the uncorrected erythrocyte life spans obtained from the least squares lines of decrease in H^3-DFP radioactivity are shown in Fig. 1. Corrected for blood sampling and for increase in red blood cell volume associated with growth, the red cell life spans were 184 and 412 days respectively. The fall in specific activity appeared to be linear suggesting a finite life span although an element of random destruction (exponential decline) could not be excluded. Removal of red cells by sampling would introduce an element of exponential decrease in specific activity. An additional factor might be the increase in red cell mass if this were an exponential function rather than linear. The specific activity curves in theory thus represent the sum of linear and exponential functions. In practice, they may be treated as linear functions.

In 2 animals (C and D) maintained at 31° C, the least squares lines of decrease in

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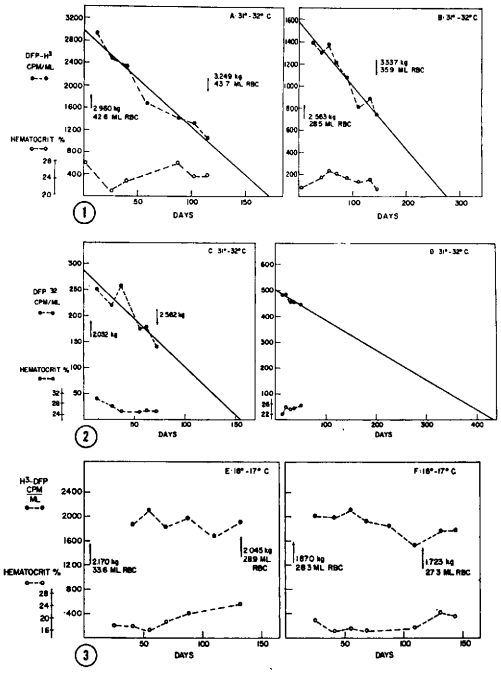

FIG. 1. Red cell specific activity (H^3-DFP) in 2 animals kept at 31°-32°C. Body weights and total red cell volumes were determined at beginning and end of the red cell survival study.

FIG. 2. Red cell specific activity (DFP^{32}) in 2 alligators kept at 31°-32°C. A long extrapolation from the end of survival study (50 days) to the abscissa (432 days) was necessary to determine red cell life span in animal D.

FIG. 3. In 2 animals maintained at 16°-17°C, red cell specific activity (H^3-DFP) did not change significantly for 132 days (E) or 144 days (F). There was a small decrement in body weight and total red cell volume in both animals.

DFP^{32} activity extrapolated to 155 and 437 days respectively, (Fig. 2). Because of physical decay of P^{32}, it was not possible to follow erythrocyte specific activity beyond 50-70 days. Consequently it was necessary to make a large extrapolation in the case of animal D. The estimate of 437 days for the red cell life span in this animal is therefore questionable. In animal C, where a long extrapolation was not necessary, a life span of 260 days could be calculated by correcting for blood sampling and for an estimated increment in blood volume comparable to the increment in blood weight (26%). These results are shown in Fig. 2.

Two animals (E and F) were maintained at a temperature of 16-17°C for 7 months. They did not eat during this period and lost weight, in both instances amounting to approximately 6% of their initial mass. In both animals, red cell tritium specific activity did not fall significantly during the period of observation—132 and 144 days after isotope administration (Fig. 3). In both instances, the standard error of the slope of the least squares line of specific activity *vs.* time was equal in magnitude to the slope of this line. It was concluded that there had been little or no dilution of labelled erythrocytes by unlabelled cells and therefore no red cell synthesis. In animal E, a red cell life span could be calculated from the decrement in red cell volume during the period of observation assuming a linear decrease with time. The erythrocyte life span thus calculated and corrected for blood sampling was 1320 days. In animal F, the decrement in total red cell volume could be accounted for wholly by the blood samples removed. If the error in determination of blood volume is approximately 3% then the erythrocyte life span in this animal is at least 2500 days and may be infinite.

Discussion. Tritiated DFP with a long physical half-life appears to be the best available label for measuring the survival of long lived and nucleated erythrocytes. Data obtained in mammals(9) and in amphibia(5) suggest that after a short initial period DFP does not elute from the intact cell. After labelling, DFP is converted to diisopropylphosphate and is not reutilized after destruction of the labelled cell. In contrast, chromium[51] has a relatively short physical half life, and the pattern of its elution from the red cell of hibernating mammals or from lower vertebrates is unknown. Labelled glycine which is the other commonly employed erythrocyte marker, remains in an active metabolic pool and may be reincorporated by nucleated erythrocytes.

From data obtained in the present study in alligators and from a previous study in frogs(5), it appears that in these species of poikilotherms, red cell synthesis ceases or is significantly decreased at low environmental temperatures. This is analogous to the diminished protein synthesis in reptiles and in amphibians on cold exposure(10).

Data obtained in these studies suggest that in the alligator erythrocyte survival is temperature dependent and inferentially dependent on metabolic rate. The Q_{10} for almost all enzyme systems lies between 1.5 and 5, with the majority being under 3. A change of red cell life span of a comparable order of magnitude was achieved by a 14 to 15°C change in environmental temperature.

Summary. Immature American alligators were maintained at temperatures of 16°-17° C or 31°-32°C for periods of 6 to 7 months. Red cell life spans were measured with P^{32} or tritium labelled diisopropylfluorophosphate. Calculated red cell life spans corrected for changes in total red cell volume were prolonged by a factor of at least 3 at the lower temperature. Erythropoiesis was markedly depressed at low environmental temperature.

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Persistent Infection of Human Carcinoma and Primary Chick Embryo Cell Cultures with Simian Virus 40. (27902)

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Newborn hamsters injected with extracts of cell cultures prepared from the kidneys of apparently healthy rhesus monkeys developed subcutaneous neoplasms at the site of inoculation several months later(1). The oncogenic substance in the rhesus monkey kidney cell cultures was subsequently identified as the vacuolating virus or simian virus 40 (SV-40)(2,3). Before means for detecting the virus were known, SV-40 had been administered to man as a contaminant of poliomyelitis and adenovirus vaccines and as a contaminant of the respiratory syncytial virus tested experimentally in man. Antibody to SV-40 developed in some of the recipients (4-6). The serum titers were low and conclusive evidence that the virus could grow in human cells was lacking until Schein and Enders(7) demonstrated that primary cultures of a number of human tissues supported growth of the virus. No cytopathic changes were noted on first passage to these cultures but after transfer in kidney cell cultures, proliferating foci that became progressively larger were seen. Koprowski *et al.*(8) also cultured SV-40 in primary human buccal mucosa and skin organ cultures and noted morphologically abnormal changes in the cells accompanied by distinct chromosomal differences.

This paper describes the shedding of SV-40 in the fluid of 2 continuous and presumably transformed lines of human carcinoma, HeLa and HEp-2, and in primary chick embryo cell cultures, over periods of time ranging from 6 to 8 months.

Materials and methods. SV-40 virus. SV-40 strain A426 was recovered from a tumor induced by injecting a newborn hamster with an extract of rhesus monkey kidney cell culture(2).

Cercopithecus monkey kidney (CMK) and chick embryo (CE) cell cultures. Primary monolayers of these cells were prepared in