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PROLONGATION OF RBC SURVIVAL IN THE HYPOPHYSECTOMIZED RAT

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DISCLAIMER

INTRODUCTION

Previous experiments in reptiles (1), amphibians (2), and hibernating mammals (3-5) have shown a prolongation of red blood cell survival with decreasing environmental temperature. It has generally been accepted that this effect is related to the attendant decrease in oxygen consumption, although the method(s) by which this is achieved can only be speculated upon.

Hypophysectomy also results in a decrease in oxygen consumption. The question arose, therefore, whether hypophysectomy also results in a prolongation of RBC survival similar to that seen in hibernating mammals and in poikilotherms exposed to reduced environmental temperature. Erythrocyte survival was studied in hypophysectomized rats using the endogenous production of ^{14}CO in the breath following injection of glycine-2-^{14}C (6).

MATERIALS AND METHODS

Male buffalo rats weighing 200-275 grams were hypophysectomized by the ~~transoral~~ PARAPHARYNGEAL route (obtained from Simonsen Laboratory, Gilroy, California). They were fed a diet of pulverized rat food (White Diet, Laboratory Rat and Mouse Food, Feedstuffs Processing Co., San Francisco, Calif.), supplemented by 2-3 times/week feeding of soft fruit and vegetables. Red blood cell survival studies were started 90 days post-hypophysectomy, at which time the initial period of erythroid hypoplasia (7) had subsided, and when body weights had been stable for more than 6 weeks. Adequacy of hypophysectomy was established for each animal at the end of the study by direct examination of the pituitary fossa with a dissecting microscope, and by weighing appropriate target organs (adrenals, testes). Fifty microcuries of

glycine-2-^{14}C (specific activity 19-26 mCi/mM, New England Nuclear Corp., Boston, Mass.) was injected intravenously into 5 hypophysectomized rats 90 days post-operatively, under light ether anesthesia. Parameters of RBC survival were then determined from analysis of the resulting excretion rate of ^{14}CO in the expired air over the next 120 days, using methods as described previously (6).

Cross-transfusion experiments were performed by injecting 250 μCi of glycine-2-^{14}C intravenously into normal and 90 day post-hypophysectomy adult male buffalo rats. Seventy-two hours later, blood was removed from the donors by aortic puncture, washed 3 times in isotonic saline, the hematocrit adjusted to 40-50%, and 1-2 ml. of the RBC suspension was injected intravenously into compatible normal or hypophysectomized (90 days post-operative) rats. The excretion rates of ^{14}CO in these hosts was then followed from 4 to 120 days following the original injection of glycine-2-^{14}C.

RESULTS

In all hypophysectomized rats, the pituitary fossa was clear of pituitary remnants at the completion of each study. Adrenal weights averaged 54% less, and testicular weights 85% less than that of comparable normal animals. The body weight of the operated animals was maintained throughout the experiments (90 to 210 days post-operatively) at about 15-20% less than their pre-operative weight, while that of the normal controls increased by more than 100% over the same period of time. At 42 days post-operative, there was a significant reduction in reticulocyte count, indicating a period of erythroid hypoplasia (7). However, at 90 days post-operative, the

reticulocyte count had returned to levels not significantly different from the unoperated controls ($p > 0.10$). A 21% and 29% reduction in mean venous hematocrit was seen 42 and 90 days postoperatively, respectively. These alterations in target organs and blood values are summarized in Table I. Feeding of a soft, supplemented diet was crucial to these experiments since comparable animals fed a pelleted diet alone continued to show a loss in body weight, and showed no significant incorporation of glycine-2-^{14}C into RBC hemoglobin heme (6).

Parameters of RBC survival in normal and hypophysectomized rats directly injected with labeled glycine are summarized in Table II. There was a 19% increase in the mean potential lifespan (time of senescent death) from 66 days in the normals to 79 days in the operated animals ($p < 0.001$), and a 26% increase in the spread of lifespans about this mean potential lifespan ($p < 0.001$). This resulted in a prolongation in mean overall RBC survival (including both senescence and random destruction) of 13.5% ($p < 0.001$). A graphic example of the results obtained in 2 hypophysectomized rats and a single control animal is shown in Figure 1.

Results of the cross-transufsion studies are shown in Table III. RBC survival was entirely normal in normal hosts injected with labeled RBC from hypophysectomized donors. In contrast, the phase of senescence was prolonged by 12% ($p < 0.001$) and the rate of random hemolysis was reduced by 45% ($p < 0.01$) in hypophysectomized hosts injected with RBC from normal donors (Figure 2). There was no statistically significant difference between the survival of normal and hypophysectomized donor RBC in hypophysectomized hosts.

In none of these studies were any alterations in the "early labeled peak" for ^{14}CO seen.

DISCUSSION

Red blood cell survival is known to be prolonged in poikilotherms exposed to lowered environmental temperature (1,2) and in hibernating mammals (3-5), all conditions in which oxygen consumption is reduced. To date, no consistent prolongation of RBC survival has been reported in animal (or human) subjects with any alteration in metabolic rate (8). Berlin, Van Dyke and Lotz (9) originally studied the RBC survival in hypophysectomized rats, in an effort to determine whether the anemia of these animals was due to a shortening of RBC survival or a decrease in erythropoietic rate. Their study indicated that the latter was operative, since RBC survival was not shortened. Their data suggested that the hypophysectomized rats actually had prolonged survival, with normals having a mean lifespan of 57 days and hypophysectomized rats a value of 64 days, as determined from the inflection point of the heme specific activity curves. Similarly, Cline and Berlin (10) reported normal RBC survival in the hypothyroid dog, although 4 of their 6 animals had longer RBC survival in the hypothyroid state than when euthyroid, with a single animal showing a prolongation of as much as 23%.

Our own studies confirm these earlier observations, and indicate that the increase in overall RBC survival in hypophysectomized rats is due primarily to prolongation of the phase of senescent death. The cross-transfusion studies confirmed this increased mean

potential lifespan, and indicated that the prolongation in survival was not due to an intrinsic alteration in the RBC, since RBC formed in hypophysectomized donors survived normally in normal hosts. That the defect was extrinsic to the RBC was shown by the prolonged survival of normal RBC in hypophysectomized hosts (Table III).

The finding of prolonged RBC survival in hypophysectomized rats due to some "extrinsic" process(es) sheds some light on those processes influencing RBC survival. It appears probable that senescence is related to the time at which the enzymatic processes in the RBC are no longer capable of repairing oxidative damage to critical membrane-linked compounds (11). If the rate of metabolism is decreased (hypophysectomy, hibernation, reduced environmental temperature, hypothyroidism), it is conceivable that the turnover of such RBC enzymes is also reduced, prolonging the time at which such regenerative processes are exhausted. This is also suggested by the following observations:

1. RBC survival was noted to be inversely related to O_2 consumption in a series of vertebrate species---the higher the O_2 consumption, the lower the RBC survival (12).
2. Animals exposed to 100% O_2 at 197 to 450 torr 14 days after glycine-2-^{14}C injection, tend to have a slight shortening of the mean potential lifespan (accelerated senescence). Although the changes were minimal, results indicated that the higher the total O_2 partial pressure the lower the mean potential lifespan (13).
3. Red cells stored at 79° C in glycerol for up to 21 months remain viable following re-warming and transfusion (14) and RBC stored at 4°C in acid for 7 months show no decline in levels of critical

enzymes, such as glucose-6-phosphate dehydrogenase (11). Presumably, under the effect of reduced temperature *in vitro*, the ageing of these cells is almost completely inhibited, similar to the results obtained *in vivo* as described above (1-5).

SUMMARY

Red blood cell survival was prolonged in hypophysectomized rats. While there was an inconsistent diminution in the rate of random hemolysis in the reported experiments, in directly injected and cross-transfused rats, there was a consistent prolongation of the phase of senescent death of RBC in hypophysectomized hosts. In contrast, RBC from hypophysectomized donors survived normally in normal hosts. These experiments are further evidence of a relationship between RBC ageing and metabolic rate, and suggest an intimate involvement with the calorigenic hormones (thyroxine, corticosteroids).

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FIGURE LEGENDS

Figure 1:

Endogenous ^{14}CO excretion rate (dpm/hour, ordinate) versus time after glycine-2-^{14}C injection (days, abscissa) in one normal buffalo rat (dotted lines, open squares) and in 2 rats injected 90 days post-hypophysectomy (solid lines, open and closed circles). Note the prolongation of senescence, as evidenced by a shift in the "late peak" in the latter animals by about 14 days.

Figure 2:

^{14}CO excretion rate (ordinate and abscissa as in Figure 1) in 3 rats 90 days post-hypophysectomy injected with labeled RBC from a normal compatible donor on day 4 (glycine-2-^{14}C injected in donors on day 0). Vertical lines indicate the range of values in the 3 animals, while the open circles indicate the mean value. Shown above the "late peak" are brackets showing the range of times at which the "late peak" was maximal in studies in which RBC from normal or hypophysectomized donors were injected into normal hosts ("normal hosts") or hypophysectomized hosts ("hypophysectomized hosts"). The excess destruction of a small proportion of injected RBC on the day of injection was seen in all cross-transfusion experiments, and is due to the inevitable RBC trauma imposed by handling of these cells.

TABLE I

HEMATOLOGIC AND AUTOPSY DATA IN NORMAL AND HYPOPHYSECTOMIZED RATS

Parameter	Normal Rat	Hypophysectomized Rat	"p" †
Hematocrit (%)	47.4 ± 1.0 (5)*		
42 days post-op		37.3 ± 0.1 (5)	< 0.001
90 days post-op		33.5 ± 0.7 (5)	< 0.001
Reticulocytes (%)	2.2 ± 0.1 (5)		
42 days post-op		0.3 ± 0.1 (5)	< 0.001
90 days post-op		1.8 ± 0.2 (5)	> 0.10
Adrenal Weight (mg/100 grams body wt.)	10.5 ± 0.7 (3)	4.8 ± 0.2 (16)	< 0.001
Testicular Weight (mg/gram body weight)	10.7 ± 0.3 (3)	1.6 ± 0.1 (16)	< 0.001

*All values are Mean ± S.E. Number of animals studied in parentheses.

†"p" value from "t" test.

TABLE II

RBC SURVIVAL IN NORMAL AND HYPOPHYSECTOMIZED RATS

PARAMETER	THREE NORMAL RATS	FIVE HYPED RATS	"p" †
Random hemolysis (%/day)	0.48 ± 0.02*	0.53 ± 0.05	N.S.
Mean potential lifespan (days)	66.3 ± 1.2	78.7 ± 0.7	< 0.001
Spread of lifespans about MPLS (days)	8.0 ± 0.2	10.1 ± 0.3	< 0.005
Fractional incorporation of glycine into RBC heme (%)	0.324 ± 0.052	0.296 ± 0.011	N.S.
MEAN OVERALL RBC LIFESPAN (days)	56.9 ± 1.0	64.6 ± 0.8	< 0.001

†"p" value from "t" test.

*Mean ± S.E.

TABLE III

RBC SURVIVAL IN CROSS-TRANSFUSION STUDIES

Parameter	n=4 Donor of RBC: Normal Host for RBC: Normal	n=3 Donor of RBC: Hyped Host for RBC: Normal	n=3 Donor of RBC: Normal Host for RBC: Hyped	n=3 Donor of RBC: Hyped Host for RBC: Hyped
Random Hemolysis (%/day)	0.66 ± 0.05†	0.65 ± 0.01	0.36 ± 0.02**	0.44 ± 0.02*
Mean Potential Lifespan (days)	64.6 ± 0.4	64.6 ± 0.5	72.3 ± 0.4***	77.8 ± 2.3***
Spread of Lifespans about MPLS (days)	8.6 ± 0.6	8.1 ± 0.2	8.8 ± 0.1	9.9 ± 0.1
Mean Overall RBC Lifespan (days)	52.8 ± 0.8	52.9 ± 0.1	63.8 ± 0.2***	65.9 ± 1.5***

***"p" 0.001

**"p" 0.01

*"p" 0.025

†Mean ± S.E.

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