Prolongation of RBC Survival in the Hypophysectomized Rat (35850)

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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 results in a prolongation of RBC survival similar to that seen in the above-noted experimental conditions.

Materials and Methods. Male buffalo rats, weighing 200–275 g, were hypophysectomized by the 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. Adequacy of hypophysectomy was established for each animal by direct examination of the pituitary fossa, and by weighing appropriate target organs (adrenals, testes). Fifty microcuries of glycine-2-^{14}C (sp act 19–26 mCi/mmole, New England Nuclear Corp., Boston, Mass.) was injected intravenously into 5 hypophysectomized rats 90 days postoperatively, 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). The generation of ^{14}CO from RBC hemoglobin heme is obtained in this method by suitable corrections for the ^{14}CO arising from turnover of nonhemoglobin hemes and that due to persistence of label in the glycine pool (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. Four days later, blood was removed from the donors by aortic puncture, washed 3 times in isotonic saline, the hematocrit was reconstituted to 40–50%, and 1–2 ml of the RBC suspension was injected intravenously into normal or hypophysectomized (90 days postoperative) rats. The excretion rate of ^{14}CO in these hosts was then followed from 4 to 120 days after the original injection of glycine-2-^{14}C.

Results. In all hypophysectomized rats used in this study, the pituitary fossa contained no gland remnants at sacrifice. Adrenal weights averaged 54% less [4.8 $\pm$ 0.2 (SE) mg/100 g of body wt vs 10.5 $\pm$ 0.7; $p<0.001$] and testicular weights 85% less (1.6 $\pm$ 0.1 mg/g of body wt vs 10.7 $\pm$ 0.3; $p<0.001$) than that of comparable normal animals; body weight of the operated animals stabilized at 15–20% less than preoperative weights, while that of the normal controls increased by more than 100% over the same period of time. Forty-two days postoperative, the reticulocyte count was significantly reduced (0.3 $\pm$ 0.1% vs 2.2 $\pm$ 0.1; $p<0.001$), indicating continuance of erythroid hypoplasia, corresponding to the initial postoperative "polycythemic phase" (7). However, at 90 days postoperative, the reticulocyte count had returned to levels not significantly different from the unoperated controls (1.8 $\pm$ 0.2%; $p>0.10$), but with a significantly lower hematocrit (33.5 $\pm$ 0.7% vs 47.4 $\pm$ 1.0; $p<0.001$). Feeding of a soft, supplemented diet was crucial to these experiments since

TABLE I. RBC Survival in Normal and Hypophysectomized Rats.

Parameter	Normal ($n = 3$)	Hypophysectomized ($n = 5$)	p[a]
Random hemolysis (%/day)	0.48 ± 0.02[b]	0.53 ± 0.05	>0.50
Mean potential life-span (days)	66.3 ± 1.2	78.7 ± 0.7	<0.001
Spread of life-spans 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	>0.50
Mean overall RBC life-span (days)	56.9 ± 1.0	64.6 ± 0.8	<0.001

[a] p value from t test.
[b] Mean ± SE.

comparable animals fed a pelleted diet alone showed a continual loss in body weight, no significant incorporation of glycine-2-^{14}C into RBC hemoglobin heme (6), and ultimately died.

Parameters of RBC survival in normal and hypophysectomized rats directly injected with labeled glycine are summarized in Table I. There was a 19% increase in the mean potential life-span (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 life-spans about this mean potential life-span ($p<0.005$) (Fig. 1). This resulted in a prolongation in mean overall RBC survival (including both senescence and random destruction) of 13.5% ($p<0.001$).

Results of the cross-transfusion studies are shown in Table II. RBC survival was normal in normal hosts injected with labeled RBC from hypophysectomized donors. In contrast, 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 (Fig. 2). Normal and hypophysectomized donor RBC had comparable survival in hypophysectomized hosts.

In no study was an alteration in the "early labeled peak" seen which would suggest the presence of "ineffective erythropoiesis" in

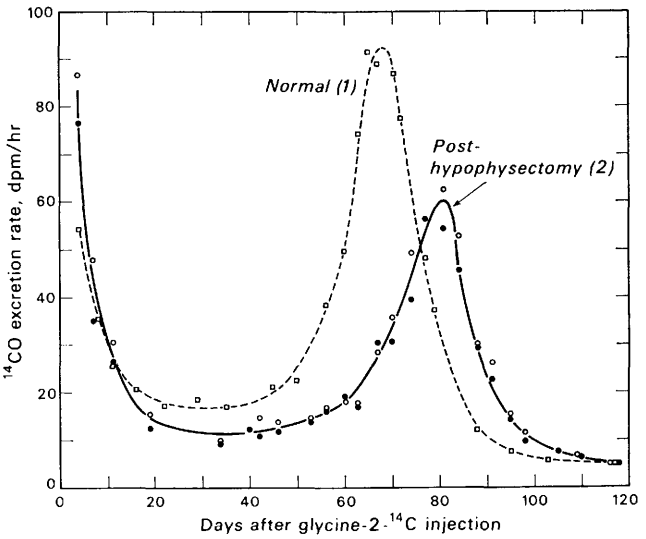

FIG. 1. Endogenous ^{14}CO excretion rate in one normal buffalo rat (□ - -); and in 2 rats injected 90 days posthypophysectomy (○, ●—). Note the prolongation of senescence, as evidenced by a shift in the "late peak" in the latter animals by about 14 days.

TABLE II. RBC Survival in Cross-Transfusion Studies.

Donor of RBC: Host for RBC: Parameter	Normal Normal ($n = 4$)	Hypophysectomized Normal ($n = 3$)	Normal Hypophysectomized ($n = 3$)	Hypophysectomized Hypophysectomized ($n = 3$)
Random hemolysis (%/day)	0.66 ± 0.05[a]	0.65 ± 0.01	0.36 ± 0.02[c]	0.44 ± 0.02[d]
Mean potential life-span (days)	64.6 ± 0.4	64.6 ± 0.5	72.3 ± 0.4[b]	77.8 ± 2.3[b]
Spread of life-spans about MPLS (days)	8.6 ± 0.6	8.1 ± 0.2	8.8 ± 0.1	9.9 ± 0.1
Mean overall RBC life-span (days)	52.8 ± 0.8	52.9 ± 0.1	63.8 ± 0.2[b]	65.9 ± 1.5[b]

[a] Mean ± SE.
[b] $p < 0.001$.
[c] $p < 0.01$.
[d] $p < 0.025$.

affected animals.

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), conditions in which oxygen consumption is reduced. To date, no consistent prolongation of RBC survival has been reported in animal (or human) subjects with altered metabolic rate (8). Berlin *et al.* (9) studied hypophysectomized rats to determine whether their anemia was due to shortened RBC survival or decrease in erythropoietic rate. Their study indicated that the latter was operative, since RBC survival was *not shortened.* They noted that the hypophysectomized rats actually had prolonged RBC survival, with normals having a mean life-span of 57 days and hypophysectomized rats a value of 64 days. Cline and Berlin (10) reported normal

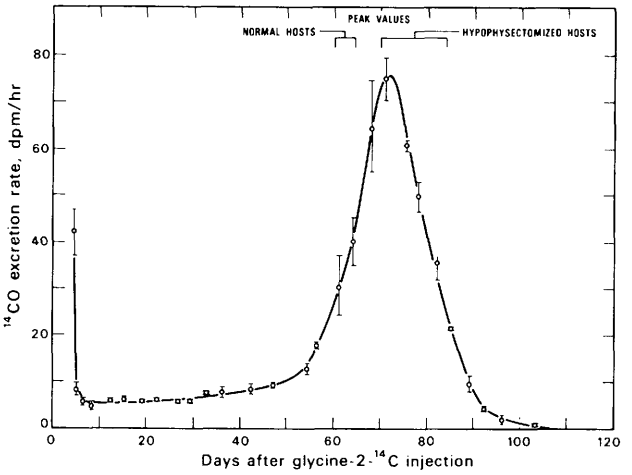

FIG. 2. ^{14}CO excretion rate in 3 rats 90 days posthypophysectomy injected with labeled RBC from a normal compatible donor on day 4 (glycine-2-^{14}C injected into donor on day 0); (vertical lines) the range of values in the 3 animals; (O) the mean value. Above the "late peak" are brackets showing 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"). Destruction of a small proportion of RBC on the day of injection was seen in all cross-transfusion experiments, and is due to the inevitable trauma imposed by handling of these cells.

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 increased overall RBC survival in hypophysectomized rats is due primarily to prolongation of the phase of senescent death. Cross-transfusion studies indicate that the prolongation in survival is not due to an intrinsic alteration in the RBC, since RBC formed in hypophysectomized donors survive normally in normal hosts. That the defect is extrinsic to the RBC is shown by the prolonged survival of normal RBC in hypophysectomized hosts (Table II).

Altered RBC senescence in hypophysectomized rats sheds some light on those "extrinsic" factors influencing this process. It is probable that RBC senescence is related to the time at which enzymatic processes in the RBC are no longer capable of repairing 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 reduced, prolonging the time at which such regenerative processes are exhausted. This is also suggested by the following observations:

1. Activity of certain RBC enzymes, notably those of the hexose monophosphate shunt [glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconic dehydrogenase (6PGD)] decrease markedly with cell age *in vivo* (11). However, RBC stored at 4° in ACD for as long as 7 months show no decline in G6PD or 6PGD (12), suggesting that *in vitro* aging is inhibited by lowered temperature, similar to the results obtained in intact animals (1–5).

2. Thyroid hormone (T3) increases the activity of the hexose monophosphate shunt in RBC (13), which may increase the turnover of these critical RBC enzymes (*vide supra*). Absence of this effect in hypophysectomized or hypothyroid animals might thus lead to life-span prolongation by maintaining adequate levels of these enzymes for a longer period of time.

3. RBC in intact rats exposed to 100% O_2 tend to show accelerated senescence—the higher the O_2 partial pressure, the lower the mean potential life-span (14). Further, older RBC are more liable to immediate hyperoxia-induced destruction than are young RBC (14).

4. RBC survival in a series of vertebrate species was noted to be inversely related to O_2 consumption—the higher the O_2 consumption, the lower the mean potential lifespan (15).

Summary. Red blood cell survival was prolonged in hypophysectomized rats. While the rate of random hemolysis was decreased in some hypophysectomized hosts, in all directly injected and cross-transfused hypophysectomized rat hosts, there was a significant prolongation of the phase of senescent death. In contrast, RBC from hypophysectomized donors survived normally in normal hosts. These experiments are further evidence of a relationship between RBC aging and metabolic rate, and suggest an intimate involvement with the calorigenic hormones.

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