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Extra-renal Erythropoietin Production in the Baboon* (33634)

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Erythropoietin (ESF) is a hormone active in the regulation of erythropoiesis and it is believed to be produced mainly in the kidney (1–3). The kidney as a sole source of erythropoietin was first proposed by Jacobson *et al.* (1). Naets (2) substantiated Jacobson's findings in the dog. While the kidney plays a major role as a site for erythropoietin production or activation, the question arises where in the kidney is erythropoietin produced and are there extrarenal sources for ESF. Studies in rodents by Mirand *et al.* (4–6) in 1957 and later studies by others (7–9) have confirmed that there is experimental evidence for an extrarenal source for erythropoietin. Recently Mirand *et al.* showed the presence of ESF in the plasmas of anephric humans prior to renal homotransplantation (10–12). This has been verified, too, by Naets and Wittek (13) and Erslev *et al.* (14). It is the purpose of this report to relate results that demonstrate that renoprival baboons revealed elevations of plasma ESF activity in response to a bleeding stimulus as intact baboons subjected to similar bleeding episodes.

Materials and Methods. Eight adult healthy male and female chacma baboons (22–18 kg body wt.) housed at the Stellenbosch-Johns Hopkins Primate Project in Bellville, Cape Province, South Africa were used. Three experimental groups were employed. In group I, 2 animals were bled 150–200 ml by femoral arterial puncture at various times during a 10-day observation

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TABLE I. Urea Levels in Intact and Renoprival Baboons.*

Treatment	Animal groups	Days post-bilateral nephrectomy									
		0	1	2	3	4	6	7	8	9	10
Intact and bleeding	I. N32		30								
	N33		45								
Nephrectomy	II. O37	—	76	84	76	140					
	M33	—	89								
Nephrectomy and bleeding	III. M34	—	79	106	99	124		244	252	268	
	N35	—	88	400							
	J38	—	82	180	188		476	470			548
	J39	—	45	188	224		368				

* Urea (total—mg/100 ml) normal range (30–45 mg/100 ml). Nephrectomized animals were peritoneally dialyzed every 2–3 days. Values are those present prior to dialysis.

period. In group II, 2 additional baboons were studied without hemorrhage following bilateral nephrectomy. At 2- to 3-day intervals the animals in groups II and III underwent peritoneal dialysis. In group III, 4 baboons were studied following bilateral nephrectomy. These animals were bled in the same manner as group I animals at similar periods during a 10-day period. Periodic venous blood samples were obtained for hematocrit and total urea determinations performed in the manner previously described (10). During all procedures animals were lightly tranquilized to facilitate ease in handling by intramuscular injections of Sernylan (Parke Davis Co.) less than 0.5 mg/kg.

Plasmas were also periodically obtained from individual baboons during the observation periods and frozen. All specimens were assayed at the same time in adult Ha/ICR Swiss mice rendered polycythemic (15, 16). Five to seven mice were used to determine the ESF activity for each plasma sample. Each test mouse received subcutaneous injections of 0.5 ml of plasma daily for 3 successive days. On the fourth day, they were injected intravenously with 1 μCi of ^{59}Fe in 0.5 ml of saline. Twenty-four hr later they were bled from the dorsal aorta, and the radioactivity of the blood sample was measured in a well-type scintillation counter. The percentage incorporation of radioactive iron into the circulating red cells was then calculated (17). Assay mice with hematocrit levels of less than 60% at the end of the experiment were discarded.

Results. In Table I, total urea values in the nephrectomized animals demonstrate that despite repeated peritoneal dialysis a mild azotemia was initially present, and progressively increased to value over 100 mg/100 ml in all animals by 4 days postnephrectomy with or without hemorrhage.

In the renoprival state hematocrit values after 7 days were below 20%, as seen in Fig. 1. The response to hemorrhage in the intact and nephrectomized animals was similar. Only 1 nephrectomized animal (M34, Group III) showed an increase in hematocrit on 1 day after hemorrhage.

Figure 1 also summarizes the ESF responses in the intact and renoprival baboons with and without bleeding episodes. Group I intact animals responded to hemorrhage with anticipated elevations in ESF activity. The percentage of 24 hr ^{59}Fe uptake in polycythemic mice in the group ranged from 2.10 to 17.22% after bleeding. Normal, intact, nonbled baboon levels of plasma ESF ranged from 0.63 to 0.84. Group II animals who were nephrectomized and not bled showed some ESF activity in their plasma following nephrectomy. Percentage 24 hr ^{59}Fe uptakes up to 4.20% were obtained. The ^{59}Fe uptakes of 1.89 and 4.20% were obtained the day of bilateral nephrectomy and are above the normal levels of ESF observed in the nonbled nephrectomized and intact baboons. During

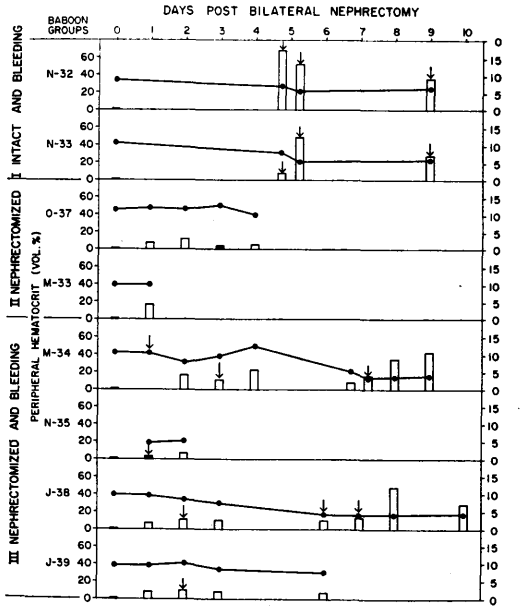

FIG. 1. Erythropoietin plasma levels in the intact and renoprival baboons: The scale to the left measures peripheral hematocrit (vol %), shown as (●—●). The scale to the right measures plasma ESF levels as percentage of 24 hr ^{59}Fe uptake in polycythemic mice, shown by open bars. Solid bars represent values not significantly greater than control values as carried out on day (0) as determined by Student's t test ($p<0.05$); all other values are significant. The arrow (↓) indicates that the baboon was bled 150–200 ml; ESF sample was taken 1.5 hr later, on day of bleeding.

this time, hematocrit levels were 40–48%, well within the normal range. Mild azotemia (Table I urea 76–89 mg/100 ml) was present concurrently.

Group III animals (Fig. 1) were bilaterally nephrectomized and bled in a manner similar to the intact group I animals. Two of the nephrectomized animals (M34 and J38) showed ESF increases in response to bleeding. The ESF activity in the plasma of some of these animals was high. The ^{59}Fe uptakes of 11.97% (J38) were obtained even 8 days postnephrectomy in the presence of azotemia; this is 25 times above control level. Elevated ESF levels were present in some of the baboons within 2 days postnephrectomy and in all 4 baboons up to 10 days postnephrectomy. In 2 of 4 baboons who had repeated hemorrhages, progressively higher ESF levels were attained. High levels of ESF activity (0.42–11.97% ^{59}Fe uptakes) were seen in the nephrectomized-bled animals, in some instances nearly similar to those levels of ESF activity (2.10–17.22% ^{59}Fe uptakes) in intact-bled animals. All of the higher ESF values in anephric bled baboons were noted at hematocrit levels below 17%. The concomitant presence of azotemia was without detectable effect.

Discussion. In this study, and others (18) we observed that the intact baboon is able to release ESF in response to bleeding. Also, anephric baboons show ESF elevations without any stimulation, suggesting the presence of an extrarenal ESF source similar to that recently confirmed in humans by Mirand *et al.* (10–12), Naets and Wittek (13), and Erslev *et al.* (14). This observation was further substantiated by the increases in ESF that we observed in anephric animals in response to bleeding (group III, Fig. 1). These elevated ESF levels in anephric-bled animals are nearly comparable to intact bled animals.

Previously in renal allotransplants in man, monkeys, and baboons elevated ESF activity was related to ischemic rejection episodes (18–20). It is unknown at present in what manner extrarenal ESF site or sites may respond to renal ischemia, although on the basis of present experimental results and previous clinical observations (10–13) there appears to be no doubt that extrarenal ESF site or sites exist(s). However, ESF may be activated from extrarenal site or sites only after an animal or man has been nephrectomized. This might be analogous to the increased production of sex steroids by the adrenals of castrated animals and to the compensatory hypertrophy of the reticuloendothelial system that follows splenectomy. Where the extrarenal site or sites for ESF are located are at present unknown. The liver might be a likely site. To what extent various species or individuals in a species possess an extrarenal source for ESF is not known. There are, however, apparent species differences. Although intact or renal allotransplanted dogs

release ESF in response to bleeding, the renoprival dog maintained in physiological electrolyte and nitrogen balance for up to 30 days does not release ESF in response to hemorrhage (21). It appears that the dog does not have an extrarenal site for ESF.

These studies demonstrated that renoprival baboons revealed elevations in ESF activity in response to bleeding stimulus in a nearly similar manner and degree as intact baboons subjected to similar repeated bleeding stimuli. Anephric baboons, nonbled, also exhibit detectable ESF activity above control levels. We do not believe that the erythropoietic response obtained from anephric baboon plasma in polycythemic mice is due to angiotensin, but rather due to ESF because of the small amount of plasma given the polycythemic mouse and the route in which it was given. In the studies of Gould *et al.* (22), on the presence of renin-like activity in ESF preparation, they conclude that renin, renin substrate, and angiotensin II in small amounts would not induce any measurable erythropoietic activity in assay animals.

Summary. Erythropoietin activity has been studied in eight intact and renoprival baboons. These studies demonstrate that renoprival baboons revealed elevations of plasma ESF activity in response to bleeding stimulus as intact baboons subjected to similar repeated bleeding stimuli. It appears that humoral regulation of erythropoiesis persists in anephric baboons.

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