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Erythropoietin (Ep) Production and Kupffer Cell Alterations following Nephrectomy, Hypoxia, or Combined Nephrectomy and Hypoxia[1] (40413)

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Erythropoietin (Ep) is a glycoprotein hormone which controls erythrocyte production in higher animals (1). Although the kidney is the main site of Ep production in the adult, extrarenal sources of Ep also exist. Bilateral nephrectomy (B Nx) does not completely abolish the Ep response to hypoxia in adult rodents (2) and Ep has been detected in the serum of anephric humans (3). In addition, the kidney is not required for fetal and neonatal elaboration of this principle. B NX does not significantly alter Ep production following hypoxia in fetal goats (4), fetal sheep (5), or in neonatal and weanling rats (6, 7). However, when subtotal hepatectomy and B Nx are performed simultaneously, the Ep response to hypoxia in fetal sheep is almost completely abolished (5). The liver is the primary site of both Ep production and erythropoiesis during fetal and neonatal life (1) and is the main locus of extrarenal Ep elaboration in the adult (1, 2).

The reticuloendothelial system (RES) has been implicated as the extrarenal cellular site of origin of Ep. An increase in plasma Ep levels is noted in anephric rats following the induction of RES hyperplasia by colloidal carbon (8) or thorotrast (9). Cells of the RES have been shown to produce hematopoietically active substances. Thus the Colony Stimulating Factor has been localized in Kupffer cells (10) as well as in blood monocytes (11). Immunofluorescent labeling for Ep in liver sections of carbon injected neonatal rats further implicates the Kupffer cell as the site of Ep synthesis and/or storage (12). In the present study, quantitative measurements of various hepatocellular elements are performed under conditions which favor extrarenal Ep production and detection, i.e., bilateral nephrectomy and hypoxia.

Materials and methods. Male Long-Evans rats (130–150 g) were used in all procedures. Six experimental groups were established as follows: (a) Nephrectomy. These rats were subjected to either B Nx or unilateral nephrectomy (13) (U Nx) under light ether anesthesia and examined 3, 6, 12, 18, and 24 hr later; (b) Hypoxia. These rats were exposed to either 6, 12, or 24 hr of hypobaric hypoxia at 0.4 atm of air; (c) Combined Nx and hypoxia. At 0, 6, 12, and 18 hr after either B Nx or U Nx, these animals were exposed to 6 hr of hypoxia; (d) Bilateral Ureteral Ligation (BUL). The ureters were ligated as close to the kidneys as possible and the animals were examined 6, 12, 18, and 24 hr later; (e) Combined BUL and Hypoxia. These rats were exposed to 6 hr of hypoxia at 0, 6, and 18 hr after BUL; (f) Sham nephrectomy. Incisions of equal size and position as those required for B Nx were performed on these rats at 3 hr and 6 hr prior to examination.

Four procedures were employed in this study: (a) Ep Assay-Serum from each experimental group was pooled and assayed in the exhypoxic polycythemic mouse (14); (b) Gamma counting and scanning-Radioactive technetium sulfur colloid (TSC) (Tech Colloid Kit, Mallinckrodt Co., N.Y.) was administered iv in dosages varying from 420 to 440 μCi/150 g rat. Five min after injection of TSC, imaging was performed for a 4 min period with the Elscint CE 1 γ camera with pinhole collimator and Minim computer. A 25% window was used with a 64 cm^2 region of interest. Counts were recorded along with

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the scintigraphs on polaroid film. Whole organ liver counts were performed for 30 sec with the Spectroscaler IIIA Picker well counter 15 min after the original injection of TSC. The animals were scanned sequentially following the same order as the surgery. The number of rats in each experimental group was small (6–8) and the variation in the time elapsing between the completion of surgery and the initiation of the scanning procedure did not exceed 10 min for any two given animals. The data in the tables represent the means for three to four trials per each experimental group. (c) Autoradiography-Tritiated thymidine (1 μCi/g body wt) was administered ip 1 hr prior to sacrifice. After routine histological preparation, hepatic cell labeling was determined (13), and (d) Planimetry-Liver tissue was perfused with 2.5% Millonig buffered glutaraldehyde, postfixed in 1% Millonig buffered OsO_4, and dehydrated in ethanol prior to DER 332/732 embedding. Sections were stained with uranyl acetate and poststained with citrated lead hydroxide prior to viewing. Random photographs of various hepatic cells were taken at 2000–2500 X and cell outlines were traced with a Gelman planimeter to determine cell areas (15). Equal numbers of experimental and normal cells were measured and the results are expressed as Experimental: Normal cell relative areas. Values significantly greater than one are indicative of hypertrophy. All autoradiographic and planimetric procedures were performed as blinds, using slides coded by number, to eliminate any subjectivity in the measurements.

Results. The results are listed in Tables I–IV. Neither unilateral nephrectomy (Table I), bilateral ureteral ligation (Table IV), combined unilateral nephrectomy and hypoxia (Table III), nor hypoxia alone (Table II) had significant effects on the hepatic cell parameters studied, although hypoxia applied for 6,

TABLE I. SERUM Ep AND HEPATIC CELL MEASUREMENTS AT VARIOUS TIME INTERVALS AFTER UNILATERAL NEPHRECTOMY, BILATERAL NEPHRECTOMY, OR SHAM NEPHRECTOMY.[a]

Groups	[^{3}H]Tdr labeled cells/1000 total cells ± SEM		TSC uptake cts/cm^2 ± SEM	TSC uptake cts/g ± SEM	Exptl: Normal Cell Rel. Areas ± SEM		Serum Ep (I.U.) ± SEM
	K cells	P cells			K cells	P cells	
Normal	2.13 ± 0.18	1.15 ± 0.18	3295 ± 419	6201 ± 395	1.00 ± —	1.00 ± —	0.07 ± 0.01
Sham Nx—3 hr	2.08 ± 0.15	1.19 ± 0.11	3401 ± 683	6351 ± 491	1.06 ± 0.05	0.99 ± 0.08	0.06 ± 0.01
Sham Nx—6 hr	2.18 ± 0.21	1.09 ± 0.16	3199 ± 572	6306 ± 596	1.01 ± 0.07	1.09 ± 0.06	0.07 ± 0.02
B Nx—3 hr	1.99 ± 0.16	1.15 ± 0.15	3426 ± 399	6382 ± 241	1.06 ± 0.03	0.99 ± 0.05	*
U Nx—6 hr	2.10 ± 0.09	1.11 ± 0.09	3623 ± 140	6515 ± 462	———	———	0.07 ± 0.02
B Nx—6 hr	2.05 ± 0.12	1.19 ± 0.13	4688 ± 226	8001 ± 509	1.08 ± 0.04	1.10 ± 0.06	*
B Nx—12 hr	2.19 ± 0.08	1.26 ± 0.23	4253 ± 261	8024 ± 403	1.07 ± 0.03	1.03 ± 0.02	*
B Nx—18 hr	2.29 ± 0.09	1.18 ± 0.14	3580 ± 231	6227 ± 286	1.06 ± 0.05	1.09 ± 0.07	*
U Nx—24 hr	2.16 ± 0.12	1.09 ± 0.13	3095 ± 462	4960 ± 351	1.02 ± 0.04	0.96 ± 0.08	0.06 ± 0.01
B Nx—24 hr	2.33 ± 0.10	1.40 ± 0.41	5252 ± 689	8021 ± 317	1.10 ± 0.04	1.18 ± 0.08	*

[a] Each experimental group consisted of 6–8 rats. The means indicate the result of 3–4 experimental trials. A minimum number of 3000 cells were measured per experimental group with means calculated from 3 to 4 trials per group.

* Undetectable. U = unilateral; B = bilateral; Nx = nephrectomy.

TABLE II. SERUM Ep AND HEPATIC CELL MEASUREMENTS AFTER 6, 12, OR 24 HR OF HYPOXIA AT 0.4 atm OF AIR.[a]

Groups	[^{3}H]Tdr labeled cells/1000 total cells ± SEM		TSC uptake cts/cm^2 ± SEM	TSC uptake cts/g ± SEM	Exptl:Normal Cell Rel. Areas ± SEM		Serum Ep (I.U.) ± SEM
	K cells	P cells			K cells	P cells	
Normal	2.13 ± 0.18	1.15 ± 0.18	3295 ± 419	6201 ± 395	1.00 ± —	1.00 ± —	0.07 ± 0.01
Hypoxia—6 hr	2.30 ± 0.08	1.59 ± 0.52	2906 ± 246	6319 ± 231	1.15 ± 0.03	1.03 ± 0.01	1.31 ± 0.38
Hypoxia—12 hr	2.16 ± 0.11	1.27 ± 0.19	2881 ± 191	6101 ± 243	1.17 ± 0.06	1.01 ± 0.08	0.91 ± 0.20
Hypoxia—24 hr	2.06 ± 0.12	2.08 ± 0.31	3006 ± 202	6364 ± 704	1.20 ± 0.03	1.01 ± 0.05	0.88 ± 0.23

[a] Each experimental group consisted of 6–8 rats. The means indicate the results of 3–4 experimental trials. A minimum number of 3000 cells were measured per experimental group with means calculated from 3 to 4 trials per group.

TABLE III. SERUM Ep AND HEPATIC CELL MEASUREMENTS AT VARIOUS TIME INTERVALS AFTER UNILATERAL OR BILATERAL NEPHRECTOMY AND FOLLOWED BY 6 HR OF HYPOXIA AT 0.4 atm OF AIR.[a]

Groups	[^{3}H]Tdr labeled cells/ 1000 total cells ± SEM		TSC uptake cts/cm^2 ± SEM	TSC uptake cts/g ± SEM	Exptl:Normal Cell Rel. Areas ± SEM		Serum Ep(I.U.) ± SEM
	K cells	P cells			K cells	P cells	
Normal	2.13 ± 0.18	1.15 ± 0.18	3295 ± 419	6201 ± 395	1.00 ± —	1.00 ± —	0.07 ± 0.01
U Nx—0 hr	2.27 ± 0.19	1.02 ± 0.16	2161 ± 420	5861 ± 721	1.05 ± 0.04	1.07 ± 0.07	0.63 ± 0.07
U Nx—6 hr	2.33 ± 0.15	1.14 ± 0.09	2932 ± 316	6325 ± 809	1.00 ± 0.09	1.15 ± 0.11	———
U Nx—18 hr	2.31 ± 0.22	1.13 ± 0.12	3296 ± 237	6003 ± 749	1.12 ± 0.10	1.06 ± 0.08	0.56 ± 0.11
B Nx—0 hr	4.24 ± 0.16	2.10 ± 0.47	11,668 ± 911	13,889 ± 937	1.38 ± 0.08	1.05 ± 0.06	0.13 ± 0.04
B Nx—6 hr	4.21 ± 0.34	1.50 ± 0.26	9307 ± 731	10,018 ± 829	1.26 ± 0.10	1.03 ± 0.07	0.11 ± 0.03
B Nx—12 hr	3.39 ± 0.40	2.08 ± 0.32	5093 ± 425	8780 ± 308	1.08 ± 0.06	1.07 ± 0.05	0.07 ± 0.01
B Nx—18 hr	2.40 ± 0.18	2.71 ± 0.35	3997 ± 308	4885 ± 769	1.19 ± 0.05	1.09 ± 0.03	0.05 ± 0.03
B Nx—24 hr	2.09 ± 0.17	1.45 ± 0.53	3609 ± 413	6143 ± 455	1.20 ± 0.04	1.03 ± 0.08	*

[a] Each experimental group consisted of 6–8 rats. The means indicate the results of 3–4 experimental trials. A minimum number of 3000 cells were measured per experimental group with means calculated from 3 to 4 trials per group.

* Undetectable. U = unilateral; B = bilateral; Nx = nephrectomy.

TABLE IV. SERUM Ep AND HEPATIC CELL MEASUREMENTS AT VARIOUS TIME INTERVALS AFTER BILATERAL URETERAL LIGATION OR AT 0, 6, AND 18 HR AFTER BILATERAL URETERAL LIGATION FOLLOWED BY 6 HR OF HYPOXIA AT 0.4 atm OF AIR[a]

Groups	[^{3}H]Tdr labeled cells/ 1000 total cells ± SEM		TSC uptake cts/cm^2 ± SEM	TSC uptake cts/g ± SEM	Exptl: Normal Cell Rel. Areas ± SEM		Serum Ep (I.U.) ± SEM
	K cells	P cells			K cells	P cells	
Normal	2.13 ± 0.18	1.15 ± 0.18	3295 ± 419	6201 ± 395	1.00 ± —	1.00 ± —	0.07 ± 0.01
BUL—6 hr	2.09 ± 0.11	1.19 ± 0.07	3460 ± 569	5893 ± 923	0.97 ± 0.06	1.01 ± 0.04	0.07 ± 0.02
BUL—12 hr	2.17 ± 0.13	1.14 ± 0.13	3249 ± 221	6144 ± 668	———	———	0.05 ± 0.01
BUL—18 hr	2.13 ± 0.08	1.25 ± 0.13	3724 ± 703	6020 ± 581	1.04 ± 0.01	1.06 ± 0.07	0.05 ± 0.01
BUL—24 hr	2.20 ± 0.14	1.21 ± 0.11	3816 ± 651	5687 ± 975	1.07 ± 0.02	1.04 ± 0.03	0.07 ± 0.02
BUL—0 hr + hyp.	2.01 ± 0.16	1.19 ± 0.08	3011 ± 399	5343 ± 419	1.12 ± 0.03	1.03 ± 0.05	1.06 ± 0.31
BUL—6 hr + hyp.	2.16 ± 0.13	1.08 ± 0.14	2991 ± 248	5416 ± 379	1.13 ± 0.05	1.09 ± 0.11	0.78 ± 0.26
BUL—18 hr + hyp.	2.07 ± 0.15	1.19 ± 0.17	2548 ± 474	5773 ± 501	1.10 ± 0.03	1.06 ± 0.09	0.60 ± 0.12

[a] Each experimental group consisted of 6–8 rats. The means indicate the result of 3–4 experimental trials. A minimum number of 3000 cells were measured per experimental group with means calculated from 3–4 trials per group. Hyp. = hypoxia for 6 hr at 0.4 atm of air. BUL = bilateral ureteral ligation.

12, and 24 hr evoked some increase in relative Kupffer cell area (Table II) when compared to normal room pressure controls ($p < .03$). Bilateral nephrectomy resulted in some activation of the Kupffer cells (i.e., TSC counts determined 6–12 hr after surgery) (Table I). However, when bilateral nephrectomy was performed just prior to hypoxic exposure (Table III), a dramatic elevation in TSC counts and Kupffer cell [^{3}H]Tdr uptake was noted when compared to normal rats ($p < .02$) (Table I), sham operated controls ($p < .02$) (Table I), or animals subjected to bilateral nephrectomy ($p < .02$) (Table I), or hypoxia ($p < .03$) (Table II) alone. Kupffer cells examined after combined bilateral nephrectomy and hypoxia (Table III) exhibited peak relative areas whereas parenchymal cell relative areas did not change significantly. Bilateral ureteral ligation or combined bilateral ureteral ligation and hypoxia had little effect on Kupffer cell TSC uptake (Table IV). As the time between bilateral nephrectomy and exposure to hypoxia increased, the ability of the liver to accumulate TSC declined as did Kupffer cell [^{3}H]Tdr uptake and Kupffer cell relative areas (Table III). When rats were subjected to unilateral nephrectomy, bilateral nephrectomy, or bilateral ureteral ligation without subsequent exposure to hypoxia, Ep levels were barely detectable (Tables I–IV). Intact rats and animals subjected to bilateral ureteral ligation or unilateral nephrectomy produced significant quantities of Ep when

exposed to different durations of hypoxia (Table III). Ep production decreased as the time between bilateral ureteral ligation and hypoxia increased (Table IV). Ep levels decreased more dramatically as the time between bilateral nephrectomy and hypoxia increased ($p < .02$) (Table III).

Discussion. Scintillation scanning of the liver using TSC has previously been described (16). Colloid clearance is directly proportional to hepatic blood flow (17) and may be influenced by changes in either the number or the activity of the RES cells. Hypoxia has been reported to diminish the bile excretion rate (18) and to decrease both the hepatic and splanchnic circulation (19, 20) although the liver can compensate for oxygen tensions as low as 5–10 mm Hg by increasing its oxygen extraction efficiency (21). The elevated TSC extraction by the liver after combined nephrectomy and hypoxia therefore, is not attributed to a higher hepatic perfusion rate but rather to an increased ability of Kupffer cells to accumulate TSC. The buildup of unexcreted wastes appeared to have little effect on Kupffer cell TSC uptake since bilateral ureteral ligation did not alter hepatic TSC levels. Although stress has been reported to increase liver blood flow (22), sham nephrectomy did not significantly alter hepatic TSC uptake (Table I). Kupffer cell [^{3}H]Tdr uptake, TSC accumulation, and relative areas decreased as did serum Ep levels (23) as the time between bilateral nephrectomy and hypoxia increased (Table III). These studies demonstrate that Kupffer cell function parallels extrarenal Ep production in response to hypoxia. Ep production decreased slightly as the time between bilateral ureteral ligation and hypoxia increased indicating that the accumulation of metabolic wastes may suppress the elaboration of this principle. When rats were maintained at room pressure after nephrectomy or bilateral ureteral ligation (Tables I–IV), Ep levels were low. Since hypoxia is the primary stimulus for both renal and extrarenal Ep production (1), this was to be expected.

Liver tissue regenerating after partial hepatectomy has been shown to produce increased quantities of Ep following bilateral nephrectomy and hypoxia when compared to sham operated, anephric hypoxic controls (24, 25). The ability to regenerate liver tissue declines with age as does the capacity to produce Ep (26). Highest Kupffer cell: parenchymal cell ratios, Kupffer cell relative areas, and hepatic TSC uptake correspond to peak Ep production in both adolescent and adult rats with regenerating livers (13, 24, 26, 27). The young animal has been shown to be a more potent producer of extrarenal Ep than the adult (6). The phagocytic capabilities of the Kupffer cells are also considerably higher in the young when compared to the adult animal (28). Hepatic Ep production parallels Kupffer cell activity in normal as well as in regenerating livers. The present studies suggest that the Kupffer cell may be an extrarenal cellular site of Ep origin.

Summary. Hepatic cells were evaluated using scintillation scanning and counting, autoradiography, and planimetry under conditions favoring extrarenal Ep production. In general, Kupffer cell activity paralleled Ep production whereas the liver parenchymal cells did not manifest significant changes. The studies suggest that the Kupffer cell may be an extrarenal cellular site of Ep production.

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