THE ROLE OF THE KIDNEY IN ERYTHROPOIESIS*

By JEAN-PIERRE NAETS

(From the Clinique Médicale et Laboratoire de Médecine Expérimentale de l'Hôpital Brugmann et de la Fondation Reine Elisabeth, Université libre de Bruxelles)

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The pathogenesis of anemia in chronic and acute renal insufficiency has not yet been fully understood. Emerson and Burrows (1) and Chaplin and Mollison (2), using the Ashby technic, have shown the decreased red cell survival in chronic and acute nephritis. Later other workers (3–6) noted the same defect, and Muirhead and co-workers (7, 8) studying nephrectomized rabbits and dogs pointed out a hemolytic picture. Loge, Lange and Moore (9) in 1950, using radioactive iron, observed a reduced uptake in the red cells of uremic patients; these findings were confirmed by others using Fe^{59} with high specificity (4–6, 10). This reduction of the erythropoietic function was also demonstrated by the depletion of erythroblasts from the marrow in uremic patients (6, 11, 12) and by the severe erythroblastopenia commonly associated with acute renal failure (6, 13). These observations show that uremic anemia results from a double mechanism: a short red cell survival and a depression of erythropoiesis.

It was postulated without evidence that toxic products resulting from renal failure impaired marrow red cell production. This view was supported by *in vitro* experiments; Sacchetti (14) observed reduction of mitosis and Markson and Rennie (15) reported inhibition of erythroblast maturation after adding uremic plasma to marrow culture.

Recently Jacobson, Goldwasser, Fried and Plzak (16) showed the absence of erythropoietic factor response in hypoxic nephrectomized rats and assumed that the kidney was the source of an erythropoietic factor.

In preliminary notes (17–19) we reported that bilateral nephrectomy of dogs abolished erythropoiesis while bilateral ureter ligation did not impair this function despite the same elevation in blood urea nitrogen and the same degree of malnutrition. Details of these experiments and further data are described in this paper.

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MATERIALS AND METHODS

Forty-four mongrel dogs weighing between 15 and 25 Kg. were used. Fifteen dogs were subjected to bilateral nephrectomy and nine to bilateral ureter ligation or unilateral nephrectomy with ureter ligation of the opposite kidney. One dog was submitted to unilateral uretral ligation and, 15 days later, to removal of the opposite normal kidney. The controls consisted of nine normal dogs and three unilaterally nephrectomized dogs.

Nephrectomy and ureter ligation were done by lumbar approach. Ureters were ligated and cut. Bilateral nephrectomy and ureter ligation after unilateral nephrectomy were done in two steps in order to avoid fatalities; the second operation was carried out 15 to 30 days after the first one. Unilateral ureter ligation following a previous nephrectomy was preferred to bilateral ureter ligation after observation that the first procedure reduced mortality and that unilaterally nephrectomized dogs had a normal erythropoiesis. For convenience, no distinction will be made among dogs submitted to ureter ligation by one or the other procedure.

The uremic dogs were maintained for 7 to 15 days by peritoneal lavages according to Grollman, Turner and McLean's procedure (20). Lavages were started 72 hours after the second nephrectomy or ureter ligation.

Generally, after the second nephrectomy or ureter ligation the dogs refused food; no attempt was made to feed them. Two dogs were bled before the second nephrectomy; one was bled 200 cc. (1.5 per cent body weight) the day before nephrectomy; the other was subjected to three 200 cc. bleedings, during the week before the operation. Two dogs were severely bled (2.5 per cent body weight blood was drawn every day) immediately after removal of the second kidney. One dog was bled (1.5 per cent body weight blood withdrawn during four consecutive days) immediately after ureter ligation.

Bone marrow was drawn regularly by iliac crest puncture and stained by May-Grunewald-Giesma: erythroblasts were counted on 500 or 1,000 cells. Urea was measured by the urease technic (21). Potassium determinations were performed by flame photometer technic (22). Hematocrit and reticulocyte counts were determined every other day.

Iron metabolism studies were performed by the following methods. Plasma iron determinations were performed by the method of Hamilton, Gubler, Cartwright and Wintrobe (23). Blood volume was assumed to equal 8 per cent of body weight. Disappearance of Fe^{59} from the plasma, iron turnover and red cell uptake of Fe^{59}

were determined by the method of Huff and associates (24).

Plasma Fe^{59} disappearance and iron turnover were carried out 72 hours after second nephrectomy or ureter ligation; red cell incorporation of Fe^{59} was measured during the following days. Liver and spleen radioactivity was counted on tissue samples taken after death of the dog.

In an attempt to investigate marrow responsiveness to the erythropoietic factor in the uremic state,[1] one dog (H) was injected with a potent erythropoietic factor of human origin [2] before and after nephrectomy. This factor was prepared by collodion adsorption from the urine of an anemic patient (25). Injection of this active material into rats, 1 mg. daily during 14 days, resulted in a 40 per cent increase of the red cell volume (26). Iron measurements and marrow study were carried out during three periods: first period, normal dog under control conditions; second period, in the same dog, Fe^{59} was injected 24 hours after two successive daily I.V. administrations of 150 mg. of the erythropoietic factor; third period in the same dog, Fe^{59} was injected 24 hours after two successive daily I.V. administrations of 150 mg. of the erythropoietic factor which were begun 24 hours after the second nephrectomy.

It has not been possible to repeat this experiment which needed a very large amount of erythropoietic factor.

RESULTS

1. *Urea (Figures 1 and 2) and potassium measurements*

Urea and potassium plasma level was similar in both bilaterally nephrectomized dogs and ureter-ligated dogs. Three days after the second nephrectomy, which was the time of Fe^{59} injection, urea level was between 235 and 338 mg. per 100 ml. and potassium between 5.5 and 7.6 mEq. per L.; six days after nephrectomy, urea level was 240 to 532 mg. per 100 ml. and potassium was 6 to 8.7 mEq. per L. Three days after ureteral ligation, urea level was between 217 and 350 mg. per 100 ml. and potassium between 5.1 and 6.7 mEq. per L. Six days after ligation urea level was from 287 to 448 mg. per 100 ml. and potassium from 7.1 to 8.8 mEq. per L.

2. *Modifications of marrow erythroblasts*

After unilateral nephrectomy. Marrow of 13 dogs was investigated after unilateral nephrectomy and disclosed a decrease of normoblasts; after initial lowering, return to higher values was observed but counts mostly lower than preoperation were obtained in the following days. Before nephrectomy, the percentage of normoblasts varied from 14.4 to 41.9 (mean, 27.8) while 10 to 30 days after, it was 5.0 to 45.5 (mean, 19.2).

After bilateral nephrectomy. A rapid marrow depletion of normoblasts was observed in 15 dogs (Figure 1). Forty-eight hours after the second nephrectomy, the nucleated red cell count had fallen from a previous 5 to 45.5 per cent to 0 to 3.8 per cent. At this stage most of the basophilic normoblasts had disappeared and after 72 hours cells of the orthochromatic type with pyknotic nucleus were almost the only cells remaining. Subsequently, all normoblasts disappeared. Even when two dogs were severely bled daily after the second nephrectomy to a hematocrit of 10 and 12 per cent, a similar disappearance of normoblasts from the marrow was observed. Moderate bleeding of two dogs prior to the second nephrectomy elicited the same response (Figure 1).

After ureteral ligation. As shown in Figure 2, 72 hours after ligation the normoblasts had decreased from 16.6 to 38.5 to 4.4 to 21.7 per cent, but never completely disappeared. Nine and 10 days after ligation, normoblasts in two dogs were still 13.8 and 9.4, respectively. After one dog was bled daily after ureteral ligation to hematocrit 16 per cent, the normoblast even increased from 16.6 to 19.8 per cent the sixth day after ligation, despite a high urea level (280 mg. per 100 ml.).

3. *Reticulocytes*

In the normal dog 0 to 0.6 per cent reticulocytes were counted. Five days after bilateral nephrectomy, no reticulocytes were observed while sometimes 0.6 per cent reticulocytes were still present nine days after ureter ligation.

4. *Iron measurements*

The marrow picture was confirmed by the iron turnover rate and Fe^{59} utilization determinations (Tables I and II).

Plasma iron level. Plasma iron level of nephrectomized dogs, (81 to 267 μg. per 100 ml., average, 151) was higher than in normal (83 to 183 μg.

[1] This part of the work was performed at the Donner Laboratory, University of California, under contracts with the Atomic Energy Commission.

[2] We are greatly indebted to Dr. Donald C. Van Dyke who kindly provided us with the erythropoietic factor.

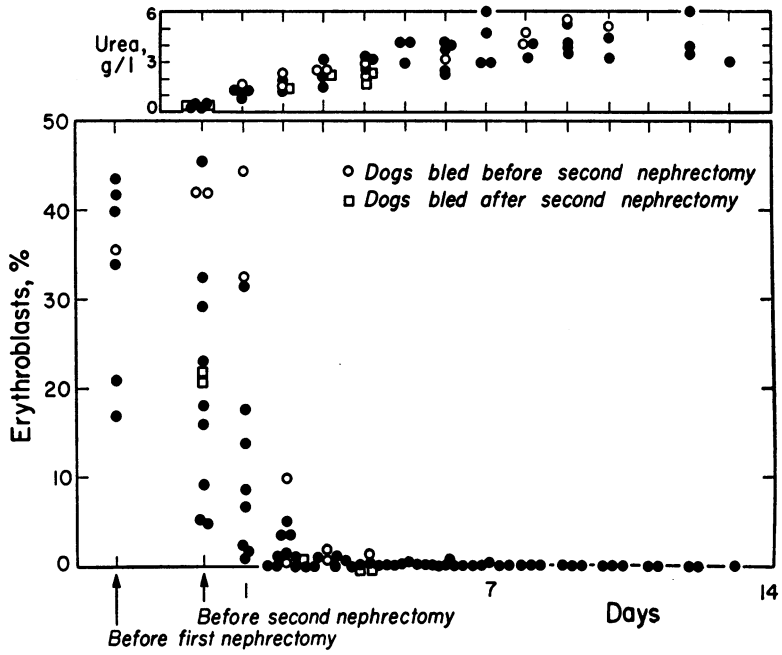

FIG. 1. DISAPPEARANCE OF ERYTHROBLASTS FROM THE MARROW AFTER BILATERAL NEPHRECTOMY

At the top of the graph are plotted the values for plasma urea before and after removal of the second kidney.

per 100 ml., average, 124) or in ureter-ligated dogs (58 to 136 μg. per 100 ml., average, 101).

Disappearance of Fe^{59} from plasma. Half-time plasma disappearance ($T_{1/2}$) of injected radioiron in bilaterally nephrectomized dogs was strikingly higher (201 to 435 minutes, mean, 299) than in normal dogs (39 to 153 minutes, mean, 89). After ureter ligation, normal values were obtained (58 to 171 minutes, mean, 96).

Iron turnover rate. Iron turnover was significantly lower in bilaterally nephrectomized dogs (0.09 to 0.49 mg. per Kg. per day, mean, 0.24) than in normal dogs where it averaged 0.64 mg. per Kg. per day (from 0.25 to 0.92 mg. per Kg. per day); $p < 0.01$. After ureter ligation iron turnover varied from 0.25 to 0.85 mg. per Kg. per day (mean, 0.48). The difference between nephrectomized and ureter-ligated dogs was significant ($p < 0.01$), but the difference between normal and ureter-ligated dogs was not significant.

Fe^{59} uptake in red cells. In normal dogs the maximal Fe^{59} incorporation varied from 58.7 to 80.9 (mean, 70.0). After bilateral nephrectomy Fe^{59} incorporation was practically abolished: from 0 to 12.5 per cent (mean, 5.3 per cent) (Table I). In Dog 2, despite important erythropoietic stimulation produced by bleedings before nephrectomy, Fe^{59} uptake (6.4 per cent) and iron turnover rate (0.20 mg. per Kg. per day) were also markedly reduced. In eight dogs with ureter ligation, incorporation in red cells three days after radioiron injection (that is, six days after the operation) averaged 46.8 per cent (16.3 to 76.9) as compared with 52 per cent (36 to 80.8 per cent) in normal controls. Five ureter-ligated dogs survived long enough to permit measurement of maximal uptake in red cells; this value averaged 61.3 per cent (35.8 to 80.8 per cent) (Table II). Only Dog 199 showed Fe^{59} red cell incorporation (35.8 per cent) lower than normal, although it was higher than nephrectomized values.

Storage of Fe^{59} in the liver. In two unilaterally nephrectomized dogs (No. 109 and No. 119) which had normal erythropoiesis, liver radioiron uptake was 16 and 24.6 per cent of the radioactivity injected. The same values were obtained in dogs with ligation of ureters (from 12.8 to 22.3 per cent, mean 16.1 per cent), whereas in bilater-

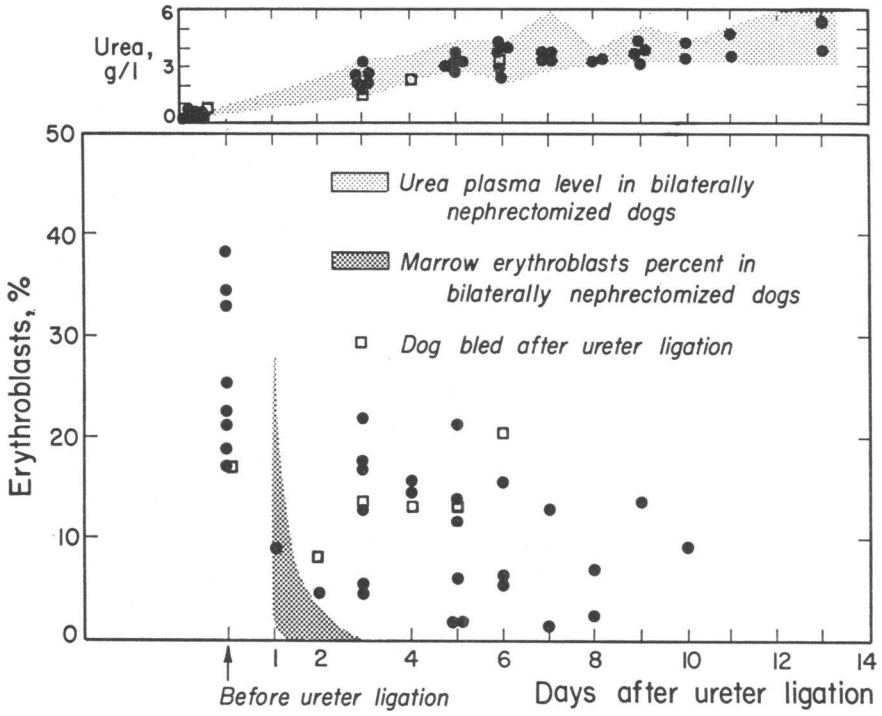

FIG. 2. ERYTHROBLAST COUNT IN THE MARROW BEFORE AND AFTER URETERAL LIGATION

At the top of the graph are plotted the values for urea plasma before and after ureteral ligation.

ally nephrectomized dogs in which erythropoiesis was suppressed, storage of Fe^{59} in the liver averaged 43.2 per cent (from 26.3 to 55 per cent).

Storage of Fe^{59} in the spleen. In unilaterally nephrectomized dogs as well as in ureter-ligated dogs, a very small amount of Fe^{59} was found in the spleen—from 0.5 to 2 per cent of radioactivity injected. The same magnitude of storage was obtained in bilaterally nephrectomized dogs except in one case (No. 11) which disclosed 13 per cent uptake in the spleen.

5. *Effect of unilateral ureteral ligation followed by contralateral nephrectomy*

One dog (No. 104, Table I) was submitted to ablation of the normal contralateral kidney 15 days after ureteral ligation. Erythropoiesis was similarly depressed as after bilateral nephrectomy: maximal Fe^{59} incorporation into red cells was 1.9 per cent, iron turnover was 0.27 mg. per Kg. per day and normoblast count was 1.2 per cent six days after nephrectomy.

6. *Effect of erythropoietic factor on erythropoiesis of one dog before and after bilateral nephrectomy*

Figure 3 shows the peak of reticulocytosis after two 150 mg. I.V. injections of human erythropoietic factor in a normal dog and the delayed increase of reticulocytes produced by the same amount of this active material after bilateral nephrectomy. The magnitude of the reticulocyte response after this operation cannot be interpreted because the dog died eight days after nephrectomy during the ascendent period of reticulocytosis. The same figure points out that the marrow was still relatively normal eight days after nephrectomy; at this time 9.5 per cent erythroblasts were counted compared with complete absence of erythroblasts observed in 15 bilaterally nephrectomized dogs after this delay (Figure 1). Plasma level of urea (312 mg. per 100 ml.) and potassium (7.5 mEq. per L.) was similar to other bilaterally nephrectomized dogs. Iron measurements are shown in Table III. Before nephrectomy, iron plasma level was unchanged before and

TABLE I

Iron measurement

Dog no.	Plasma Fe*	$T_{\frac{1}{2}}$	Plasma Fe turnover rate	Mean maximal Fe incorporation in red cells	Day of maximal Fe^{59} red cell incorporation	% Fe^{59} liver uptake	% Fe^{59} spleen uptake
	μg./100 ml.	*min.*	*mg./Kg./day*				
			A. Normal dogs				
A	108	82	0.52	58.7	8		
B	86	39	0.87	80.9	4		
C	111	66	0.74	67.5	7		
D	131	153	0.37	77.9	5		
E	83	120	0.25	63.2	7		
F	178	70	0.92	75.6	5		
G	183	91	0.84	70	7		
H	126	129	0.46	66	6		
I	112	62	0.77	70.5	5		
Mean	124	90	0.64	70	6		
			B. Unilaterally nephrectomized dogs				
109	167	66	1.17	70.5	5	24.6	1.5
119	117	102	0.57	57.8	4	16	0.5
196	81	47	0.59	83.4	11		
Mean	122	72	0.78	70.6	7	20.3	
			C. Bilaterally nephrectomized dogs				
6				2.9	5		
7	85	435	0.09	7.1	10	47.5	2
8	166	264	0.29	12.5	10	27.3	1.9
11†	81	201	0.20	6.4	8	49.5	13
17	211	245	0.34	0		55	1.3
18	267	241	0.49	0.6		51	0.9
19	106	285	0.21	1.3		32.6	0.6
27	125	375	0.12			40.7	0.6
28	170	348	0.19	11.8	4		
Mean	151	299	0.24	5.3	7.4	43.3	2.9
			D. Unilateral ureteral ligation followed by contralateral nephrectomy 15 days later				
104	128	229	0.27	1.9	5	23.4	0.4

* Iron plasma levels were measured on plasma samples taken 30 to 120 minutes after Fe^{59} injection.
† This dog was submitted to three 200 cc. bleedings the week before nephrectomy.

TABLE II

Iron measurements in ureter-ligated dogs

Dog no.	Plasma Fe	$T_{\frac{1}{2}}$	Plasma Fe turnover rate	% Fe incorporation after second day	% Fe incorporation after third day	Mean maximal Fe^{59} incorporation in red cells	Day of maximal Fe^{59} red cell incorporation	% Fe^{59} liver uptake	% Fe^{59} spleen uptake
	μg./100 ml.	*min.*	*mg./Kg./day*						
90	58	75	0.37		30.7	*		15.5	1
156	110	171	0.25			56	4	22.3	1.6
169	70	56	0.47	56.4	*			12.8	1.2
174	106	106	0.43			80.8	4	16.1	1.7
185	90	89	0.44		41.4	*		13	0.8
193	131	64	0.85			71.8	5	16	1.6
198	108	110	0.45			62	4	15.7	0.5
199	136	99	0.55			35.8	5	17.6	0.8
Mean	101	96	0.48			61.3	4.5	16.1	1.2

* Dog died.

TABLE III

Iron measurements in one dog in the normal state, after injection of erythropoietic factor and after bilateral nephrectomy plus injection of erythropoietic factor

	Plasma Fe level	$T_{\frac{1}{2}}$	Fe turnover	% Fe uptake in red cells after 2 days	Maximal % Fe^{59} uptake in red cells	Day of maximal Fe^{59} red cell uptake
	μg./100 ml.	*min.*	*mg./Kg./day*			
Normal dog	126	129	0.46	29	64	6
After injection 300 mg. erythropoietic factor	122	48	1.28	83	101	5
After bilateral nephrectomy and injection 300 mg. erythropoietic factor	35	44	0.40	20	83	5
Average values of 9 bilaterally nephrectomized dogs.	151	299	0.24		5.3	7.4

after erythropoietic factor injection. Iron turnover increased from 0.48 mg. per Kg. per day to 1.28 mg. per Kg. per day, and maximal Fe^{59} red cell uptake, which was 64 per cent before, reached 101 per cent after injection. Furthermore, the speed of the Fe^{59} red cell uptake was strikingly increased; 24 hours after Fe^{59} injection, 32 per cent of the radioactivity appeared in the red cells, while only 13.5 per cent was counted after this delay in the normal dog without administration of the erythropoietic factor.

Injection of the erythropoietic factor in the dog after bilateral nephrectomy is associated with a fall in plasma iron to 35 μg. per 100 ml. Iron turnover was of the same magnitude as in the normal dog without erythropoietic factor injection (0.40 mg. per Kg. per day) and maximal Fe^{59} red cell uptake averaged 83 per cent. Strictly speaking, this last result is not comparable with the previous red cell uptake because of reduction of the iron labile pool.

DISCUSSION

The data presented indicate that bilateral nephrectomy of the dog suppresses erythropoiesis. After bilateral nephrectomy normoblasts disappeared from the marrow. The initial disappearance of basophilic and polychromatic forms suggests normal erythron maturation, but arrest of stem cell differentiation into normoblasts. Removal of both kidneys abolishes the capacity of the dog to produce red cells regardless of the severity of the anemic stimulus. This is illustrated by the aplastic marrow of two severely bled nephrectomized dogs. In anemic anuric patients, a similar marrow picture is observed (6, 13). It must be emphasized that disappearance of normoblasts from the marrow after bilateral nephrectomy was not observed by other investigators working on the rabbit (8, 27). Since the normoblast count is

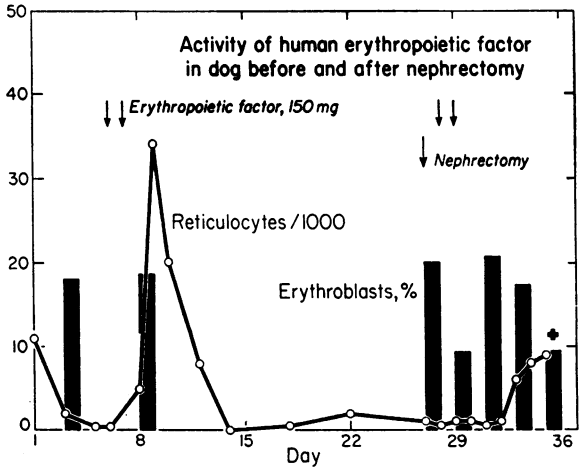

FIG. 3. EFFECT OF HUMAN URINARY ERYTHROPOIETIC FACTOR ON RETICULOCYTES AND BONE MARROW ERYTHROBLASTS IN A DOG BEFORE AND AFTER BILATERAL NEPHRECTOMY [3]

[3] From "Kinetics of Cellular Proliferation," F. Stohlman, Jr., Ed. New York, Grune and Stratton, 1959.

a relative one, the striking difference between the dog and rabbit could be explained by a greater degree of granulopoiesis after nephrectomy in the dog. However, the complete disappearance of normoblasts and the fact that the basophilic and polychromatic forms disappear even sooner indicate that this phenomenon is not due to dilution by granulopoiesis. This dilution mechanism, however, could explain the transient reduction in normoblasts after unilateral nephrectomy where basophilic and polychromatic normoblasts are still present. After ureteral ligation, these forms are also maintained and reduction of nucleated red cells could also be explained by increased leukopoiesis. However, the lowered normoblast count after ligation might also be secondary to progressive renal damage involving reduced erythropoietic secretion. It is shown (Dog no. 104, Table I) that if one ureter is ligated and the opposite normal kidney removed 15 days later, the same picture is obtained as after bilateral nephrectomy. Finally, some toxic effect of waste products on marrow could not be excluded.

Ferrokinetic results agree with morphologic observations of the bone marrow. The observed low iron turnover, the low Fe^{59} red cell incorporation, the increased Fe^{59} storage in the liver after bilateral nephrectomy are all characteristic of marrow hypoplasia. Neither surgical wound infection nor malnutrition could explain the ferrokinetic results. Freireich, Miller, Emerson and Ross (28) have shown that local infection in the dog does not interfere with Fe^{59} red cell incorporation. Nephrectomized dogs refused food and might be subjected to a negative nitrogen balance after peritoneal dialysis as pointed out by Muirhead, Jones, Stirman and Lesch (29) but Fe^{59} was injected before start of peritoneal dialysis so that certain ferrokinetic measurements could be made before significant nitrogen depletion occurred. It is interesting to note that ferrokinetic studies performed on ureter-ligated dogs are almost normal regardless of the same conditions of malnutrition and the same peritoneal lavage schedule.

The small discrepancy in the labile iron pool between normal and bilaterally nephrectomized dogs cannot explain the low Fe^{59} uptake. Furthermore, it can be seen that even nephrectomized dogs (Nos. 11 and 19, Table I) with normal plasma iron levels, have a low iron uptake (6.4 and 1.3 per cent). Fe^{59} measurements of the spleen showed that there was no red cell sequestration. Only Dog 11 which had been bled before investigation disclosed spleen Fe^{59} uptake of some importance. Only about 50 per cent of the radioactivity injected in the nephrectomized dog can be accounted for in the red cells, liver and spleen. Almost all radioactivity disappears from the plasma within 24 hours and only insignificant amounts of Fe^{59} are detected with irrigating fluid. It seems not unlikely that some Fe^{59} might be located in marrow reticuloendothelial system.

Retention of toxic products due to absence of renal secretory function is obviously not the cause of arrested erythropoiesis after bilateral nephrectomy. Dogs with ureteral ligation maintained erythropoietic function despite the same state of azotemia and hyperkalemia. Of particular interest is the observation that after administration of erythropoietic factor a bilaterally nephrectomized uremic dog can maintain a normal erythroblastic marrow picture. It seems thus that the bone marrow is able to respond to erythropoietic hormone in spite of uremia or absence of the kidneys. These results strongly suggest that the kidney produces an erythropoietic factor. In the rat, Jacobson and associates (16) and Goldwasser, Fried and Jacobson (30) have pointed out the lack of erythropoietic factor response to hypoxia after nephrectomy, but other workers did not obtain the same results (31). On the other hand, Erslev (27), working on the rabbit, concluded that the kidney did not play a role in erythropoietic function. Other investigations point to the role of the kidney in erythropoietic factor production. Van Lessen, Stefanini and Smith (32) have shown in the dog that partial ligation of only one renal vein induced polycythemia. These findings could explain the association of renal carcinoma and polycythemia observed in some patients (33–35). More recently Osnes (36) observed in mice that irradiation of both kidneys reduced the erythropoietic factor responses which normally follow bleedings. Recent demonstration of rapid disappearance of the erythropoietic factor from plasma of anemic dogs after bilateral nephrectomy added strong evidence to support the role of the kidney in erythropoietic factor production (37). The regulatory role of the kidney in erythropoiesis could be linked to the very specialized renal circulation.

It is known that the oxygen saturation of blood in the renal vein is very high (averaging 80 to 90 per cent) and that normal renal arteriovenous oxygen difference is about 2.4 ml. per 100 ml. blood (38, 39) as compared to the mixed venous and arterial blood oxygen difference of 3.4 to 6.1 ml. per 100 ml. blood. According to the "cell separation" theory of Pappenheimer and Kinter (40), "The peritubular circulation is supplied with a cell-poor component of blood and the oxygen saturation in the blood leaving the actively metabolizing tissue of the kidney may be far lower than that in the renal vein." Thus normally most of the tubules should be at the borderline of hypoxia. Such a system could be very sensitive to blood oxygenation if, when the arterial hematocrit decreases, cell separation might increase, making the tubules more hypoxic. It could be possible that some oxygen-sensitive tubular endocrine system [juxtaglomerular apparatus, for instance, as suggested by Osnes (36)] might respond to a lack of oxygen by increased erythropoietic factor production.

SUMMARY

The relationship between the kidney and erythropoiesis was investigated. Forty-four mongrel dogs were used of which 15 dogs were subjected to bilateral nephrectomy and nine to ureter ligation. Controls consisted of nine normal dogs and three unilaterally nephrectomized dogs. Uremic dogs were maintained for 7 to 15 days by peritoneal lavage.

Removal of both kidneys was followed by a rapid depletion of erythroblasts from the marrow; often none could be found after 72 hours. Iron measurements showed a low iron turnover and reduced Fe^{59} incorporation in red cells of nephrectomized dogs.

Although bilateral ureteral ligation resulted in the same elevation of blood urea nitrogen and the same degree of starvation as in the nephrectomized group, erythropoiesis was essentially normal. The erythroblast population of the marrow was maintained. Although the plasma iron turnover was reduced, it was significantly higher than in the nephrectomized group. Fe^{59} incorporation into red cells was of the same magnitude as in the control group.

Maintenance of marrow erythroblasts was observed after injection of erythropoietic factor into one bilaterally nephrectomized dog.

It is concluded from these data that the cessation of erythropoiesis after nephrectomy cannot be accounted for by urea intoxication and that the kidney is probably the source of an erythropoietic factor.

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