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Effect of Anemic Hypoxia on Erythropoiesis of Normal and Uremic Dogs With or Without Kidneys[1,2]

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Previous studies have shown that the erythropoiesis in the dog is suppressed 3 days after bilateral nephrectomy (1). This suppression of erythropoietic function, observed at that time, is not related to uremic intoxication, as shown by maintenance of this function in uremic dogs with ureteral ligation (2). From these experiments, it could be assumed that the kidney of the dog elaborates an erythropoietic factor indispensable for normal daily production of red cells. However one might question about the ability of the uremic dogs with and without kidney to response to an acute need of red cells. In preliminary notes, it has been reported that after bilateral nephrectomy erythropoiesis was no longer stimulated by anemia (3, 4). The present study is designed to assess further the role of the kidney in the erythropoietic response to acute anemic hypoxia, after achieving uremia by nephrectomy and ureteral ligation, or implantation in the iliac vein. In addition, effects of sheep erythropoietin in the nephrectomised dogs are compared with the stimulating action of anemia.

MATERIAL AND METHODS

Thirty eight adult mongrel dogs of both sexes weighing between 12 and 25 kg were used. Eight dogs were submitted to bilateral nephrectomy, seven to unilateral nephrectomy, followed 15 to 30 days later by implantation of the remaining ureter in the iliac vein. Three dogs were subjected to bilateral ligation of ureters in one step, and six to unilateral nephrectomy followed at least 30 days later by ureteral ligation. This long delay was chosen in order to avoid experimentation during the acute phase of renal compensatory hypertrophy. After unilateral nephrectomy acute changes such as a wave of mitosis (5) and increase in circulation (6) occur in the remaining kidney which could influence experimental results. No difference was observed between the last two groups, these groups will be presented together. Nine normal and four sham-operated dogs were used as controls. For sham operation, a lumbar incision was performed and the kidney simply exposed for a short time. Surgical procedure and details concerning life maintenance of uremic dogs by peritoneal lavage have been previously described

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(7). All the dogs were submitted to bleedings (2% body weight) during two consecutive days starting immediately after surgery. Blood volume was maintained by infusion of Dextran 6 per cent. One dog received two I.V. injections of 720 cobalt units of sheep erythropoietin[1] after bilateral nephrectomy instead of being bled.

Erythropoiesis was investigated 3 days before any bleeding or surgery and 3, 5 and 7 days after the first bleeding or the first injection of erythropoietin. Three parameters were studied : the marrow normoblast percentage, the absolute numbers of reticulocytes and the plasma-iron turnover. Bone marrow was drawn by iliac-crest puncture and stained by May-Grunewald-Giemsa stain. Normoblasts were counted on 500 or 1000 cells. Reticulocytes were counted by the direct-smear method using brilliant cresyl blue. Determinations were made from 1000 cells. An index was chosen to express the results by a number proportional to the absolute amount of reticulocytes : red cell mass (ml) x reticulocytes (%). The plasma iron turnover was calculated according to the single dynamic pool model of Huff *et al.* (8). Five plasma samples were withdrawn for radio-assay every 20 minutes after radioiron injection and every 30 minutes when the radioiron plasma disappearance was expected to be slow. Plasma volume was measured by extrapo-

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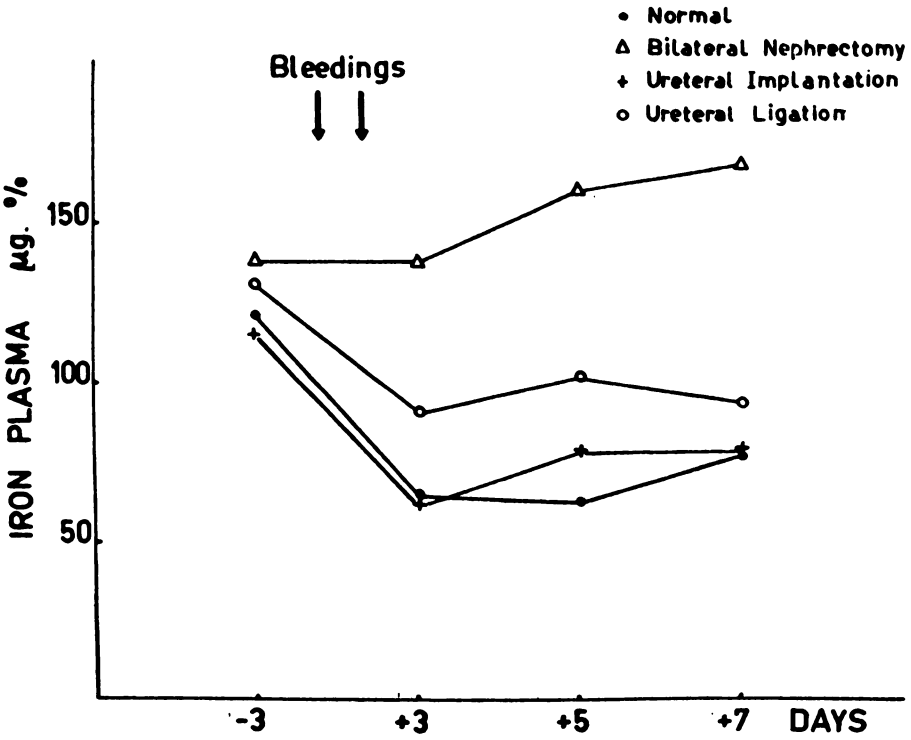

Fig. 1. Variations of iron plasma level measured 3 days before bleedings and surgery and 3, 5 and 7 days after in the different experimental groups.

lation of the Fe^{59} disappearance curve and by the Cr^{51} labeled cell method (9). Samples containing both radioiron and radiochromium were counted in a gamma ray spectrometer. Plasma iron concentration was determined by the method of Peters *et al.* (10) on two different samples collected during the iron turnover measurement and calculations were made of the average of both values which agreed within less than 10 per cent. Hematocrits were determined by a micromethod and urea plasma level by an Autoanalyser.

RESULTS

Iron Plasma Level

In normal dogs, the iron plasma level dropped from an average of 120 μg per cent to 63, 61 and 76 μg per cent respectively 3, 5 and 7 days after bleedings. In nephrectomised dogs, the plasma iron level increased to 158 and 166 μg per cent 5 and 7 days after bleedings. After ureteral implantation, the plasma iron dropped as in the control group, the fall was less striking after ureteral ligation (Fig. 1).

Blood Volume

Blood volume of normal dogs averaged 91.6 ml/kg. Values estimated by extrapolation of the Fe^{59} disappearance curve and with the Cr^{51} labeled cells agreed completely. The red cell mass measured in normal dogs averaged 46.6 ml/Kg and was not significantly different from the red cell mass estimated 20 to 150 days after unilateral nephrectomy : 44.9 ml/Kg.

Waldmann *et al.* have reported that the red cell mass of dogs was reduced 20 to 175 days after splenectomy (11). It was therefore interesting to see that removal of one kidney, which is a highly vascularized organ, was not followed by appreciable change in the red cell mass during the subsequent 20 to 150 days.

Radioiron Studies

—*Radioiron Disappearance Curve* The Fe^{59} T/2 averaged 65 minutes in the control group and dropped to 33, 24 and 28 minutes 3, 5 and 7 days after bleedings. In nephrectomised dogs, the T/2 increased from 56 minutes to 332, 395 and 486 minutes during the week following bleedings. In dogs with implanted ureter, after an initial drop on the 3rd day, the T/2 increased to 157 minutes. In the group with ureteral ligation the T/2 increased from 69 on the 3rd day to 97 minutes on the 7th day.

—*Plasma Iron Turnover* (Table I) In the control group, the iron turnover increased to 145, 185 and 239 per cent of the prebleeding values. In the nephrectomised dogs instead of increasing, the plasma iron turnover fell. In the group with implanted ureter, after an initial increase to 143 per cent of the normal value on the 3rd day, a drop to 110 and 64 per cent occurred. After ureteral ligation, a small increase was observed.

Normoblasts–Reticulocytes. (Table I)

Marrow normoblasts percentages increased in the control group. In nephrectomised dogs, a drastic reduction of the normoblasts percentage was noted: 0.3 per cent 7 days after bleedings. A moderate decrease was observed after ureteral implantation. After ureteral ligation and bleedings, normolasts percentage remained at the control value on the 3rd and 5th day and dropped to 12.9 per cent on the 7th day.

A direct relationship was found between variations of the normoblasts percentage in the marrow and the plasma iron turnover and the absolute numbers of reticulocytes (Fig. 2).

STATISTICAL ANALYSIS

All the results obtained from nephrectomised dogs at any time after bleedings were significantly different from that of other groups (p between 0.05 and 0.001 for the different parameters compared by Fisher test). No difference was observed on the 3rd day between normal dogs and dogs with ureteral ligation but on the 5th and 7th day the erythropoiesis was significantly lower in the last group than in the normal group (p between 0.05 and 0.001 for the different pa-

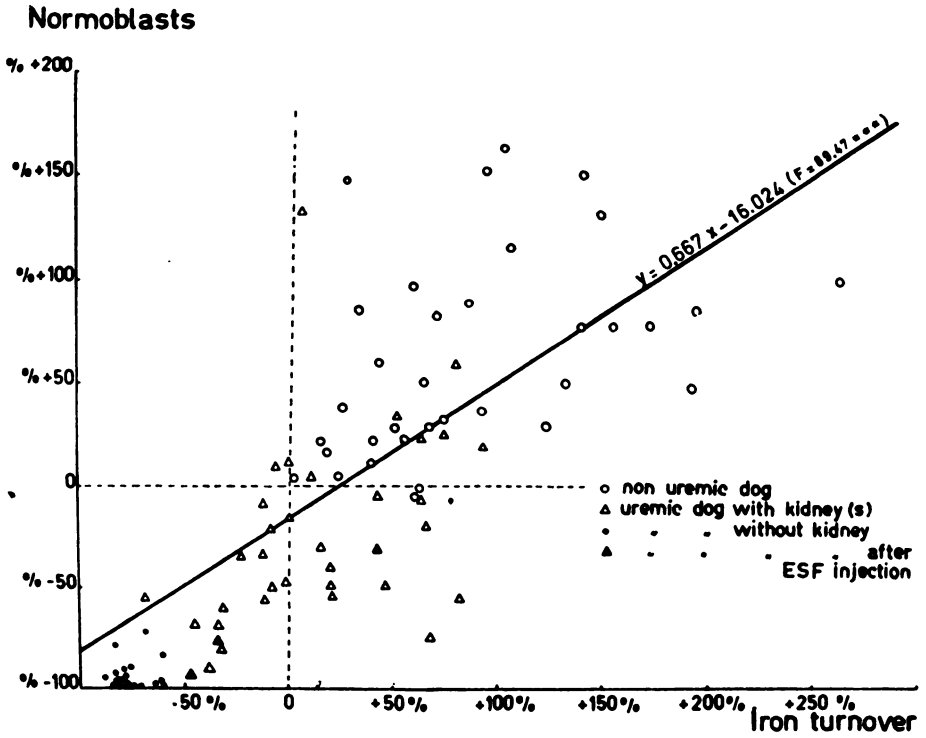

Fig. 2. Relationship between plasma iron turnover and normoblasts percentage expressed in percent above or below the control level. The regression coefficient is statistically different from zero : $p<0.001$.

TABLE I

EFFECTS OF BLEEDINGS ON THE ERYTHROPOIESIS PARAMETERS IN THE DIFFERENT EXPERIMENTAL GROUPS

Days after bleedings	*Number of dogs*	*Hematocrit* %	*Red cell mass* ml/Kg	*Iron Turnover* mg/kg/day	%	*Reticulocytes Index* values	%	*Normoblasts* % of total cells	%	*Urea* mg %
				NORMAL DOGS						
−3	9	50.6 ± 6.1*	47.6 ± 8.6	0.90 ± 0.28	100	397 ± 380	100	20.4 ± 6.0	100	21.4 ± 9.9
+3	9	32.9 ± 4.3	31.8 ± 5.6	1.27 ± 0.40	145	946 ± 572	335	27.4 ± 7.1	142	—
+5	9	32.8 ± 4.2	34.5 ± 8.1	1.60 ± 0.37	185	1883 ± 1352	735	32.9 ± 8.3	176	—
+7	9	33.2 ± 4.1	35.1 ± 6.3	2.02 ± 0.64	239	2445 ± 2088	836	32.5 ± 4.9	173	20.3 ± 8.6
				SHAMS						
−3	4	51.3 ± 2.8	39.2 ± 9.7	0.76 ± 0.19	100	661 ± 27	100	21.8 ± 3.5	100	24.0 ± 5.6
+3	4	37.0 ± 3.1	28.8 ± 4.7	1.10 ± 0.58	160	1581 ± 504	240	36.5 ± 8.7	183	—
+5	4	36.8 ± 3.2	28.2 ± 3.1	1.55 ± 0.60	202	1529 ± 543	230	36.1 ± 16.8	177	—
+7	4	38.3 ± 2.5	31.5 ± 6.2	1.67 ± 0.87	218	1966 ± 571	296	39.6 ± 3.7	199	23.1 ± 5.0
				BILATERAL NEPHRECTOMY						
−3	8	46.6 ± 4.9	36.2 ± 5.9	1.09 ± 0.31	100	184 ± 86	100	20.1 ± 5.4	100	31.0 ± 6.3
+3	8	26.4 ± 6.4	26.4 ± 7.5	0.26 ± 0.11	24	44 ± 33	22	2.1 ± 1.5	12	325 ± 56.4
+5	8	24.6 ± 6.2	20.1 ± 7.3	0.27 ± 0.14	26	6 ± 5	3.5	0.4 ± 0.4	2	397 ± 91
+7	5	23.2 ± 3.8	19.4 ± 5.3	0.24 ± 0.06	20	0.8 ± 0.8	0.4	0.3 ± 0.3	2	488 ± 110

TABLE I—*continued*

EFFECTS OF BLEEDINGS ON THE ERYTHROPOIESIS PARAMETERS IN THE DIFFERENT EXPERIMENTAL GROUPS

Days after bleedings	*Number of dogs*	*Hematocrit* %	*Red cell mass* ml/Kg	*Iron Turnover* mg/kg/day	%	*Reticulocytes Index* values	%	*Normoblasts* % of total cells	%	*Urea* mg %
				URETERAL IMPLANTATION						
−3	7	50.0 ± 4.5	47.6 ± 9.3	0.70 ± 0.18	100	233 ± 143	100	20.0 ± 5.0	100	30.0 ± 8.7
+3	7	27.6 ± 2.9	27.5 ± 5.9	1.01 ± 0.34	143	378 ± 39	162	11.7 ± 3.9	62	285 ± 53
+5	5	23.4 ± 3.9	23.9 ± 7.3	0.74 ± 0.15	110	204 ± 204	85	12.1 ± 7.7	63	416 ± 42
+7	5	23.0 ± 2.7	24.1 ± 7.0	0.43 ± 0.17	64	66 ± 54	31	7.8 ± 1.4	46	533 ± 142
				URETERAL LIGATION						
−3	9	49.8 ± 6.4	46.6 ± 12.2	0.80 ± 0.20	100	264 ± 214	100	21.8 ± 8.1	100	19.1 ± 6.0
+3	9	29.2 ± 4.0	27.6 ± 4.8	0.95 ± 0.51	117	559 ± 451	249	22.1 ± 13.7	102	286 ± 71
+5	7	26.2 ± 4.8	26.1 ± 5.6	0.94 ± 0.35	122	655 ± 594	246	20.8 ± 11.6	93	374 ± 77
+7	5	23.0 ± 5.1	21.0 ± 4.9	0.88 ± 0.38	126	595 ± 527	229	12.9 ± 0.7	67	412 ± 84
				BILATERAL NEPHRECTOMY AND ERYTHROPOIETIN INJECTION						
−3	1	54.0	46.9	0.75	100	675	100	30.3	100	27
+3	1	45.0	42.4	1.06	142	1092	162	21.5	70	295
+5	1	45.5	40.4	0.48	65	1391	206	2.1	23	232
+7	1	35.0	31.4	0.39	53	439	65	2.1	7	420

*standard deviation.

rameters compared by Fisher test). There was no significant difference between uremic dogs whether uremia was induced by ureteral implantation or ligation.

Effect of Sheep Erythropoietin on the Erythropoiesis of one Nephrectomised Dog. (Table 1)

Three days after injection of 1,440 cobalt units of erythropoietin in the nephrectomised dog, the iron turnover increased to 140 per cent of the basal level as in the normal group following bleeding and fell later to 70 per cent. Instead of observing an acute depletion of normoblasts in the marrow, a slow reduction occurred. Three days after bilateral nephrectomy, the normoblasts percentage was still 21.5 per cent; thereafter a drop to 2.1 per cent was noted on the 7th day. The reticulocytes index increased moderately from 675 to 1,391.

DISCUSSION

These experiments indicate that bilateral nephrectomy in the dog suppresses the erythropoietic response which normally occurs during anemic hypoxia. After nephrectomy, in spite of severe bleedings, normoblasts and reticulocytes disappeared nearly completely. The iron turnover fell in nephrectomised bled dogs to the same extent as in unbled nephrectomised animals (2, 3). No decrease in iron plasma level was observed in the renoprival group with depressed erythropoiesis in spite of bleeding. This agrees with the hypothesis that the drop of the iron plasma level which follows acute hemorrhage is due to increased utilisation of iron for hemoglobin synthesis (12). Other workers have reported that nephrectomy impaired the erythropoietic response to anemia. Some of their results are at variance with the present data. Erslev has shown that the plasma iron turnover of nephrectomised rabbits was increased by an anemic stimulus. However, this increase was not so marked as that observed in normal animals submitted to the same stimulus (13). In the nephrectomised rats, Reissman *et al.* observed a lack of erythropoietic response to anemia but reticulocytes remained at a normal level (14). These discrepancies may have been due to species variations and suggest that in rats and rabbits, the erythropoietic function is partially independent of the presence of the kidney. That uremic intoxication was not the major factor implicated in the absence of erythropoietic response to bleedings appears supported by erythropoiesis measurement of the nephrectomised group when compared with similar measurement of the ureteral ligation and implantation groups. In dogs made uremic by ligation or implantation of the ureter in the iliac vein, the erythropoietic response although not normal, was strikingly different from that observed after bilateral nephrectomy (p betweeen 0.05 and 0.001 for the different parameters compared by Fisher test) in spite of the same level of uremia.

Previous experiment showed that the daily production of red cells is impaired after nephrectomy (2). The present data let suppose that there is not a different mechanism independent from the kidney which is responsible for stimulating production of red cells when they are urgently need. It seems that the basic mechanism of erythropoiesis regulation which depends from the balance be-

tween oxygen demand and supply in the tissues (15) is suppressed by nephrectomy. The data are in agreement with the hypothesis that the kidneys are involved in erythropoietin production (2, 16) and with previous studies which showed that the erythropoietic factor of the plasma of anemic dogs dropped to an unmeasurable level 24 hours after nephrectomy (17).

The impaired erythropoietic response observed in dogs with ureteral implantation or ligation could be due to a reduced production of erythropoietin by functionally altered kidney or to the possible depressing action of uremia on the marrow and/or on the kidney itself as the source of production of the hormone. Pathological examination showed discrete abnormalities in kidneys of dogs with ureteral implantation or ligation.[1] However, the function of these kidneys can hardly be considered as normal. After ureteral ligation, it has been shown that both glomerular filtration and renal blood flow are reduced (18, 19). After ureteral implantation, the ureter was always found dilated at autopsy and it is likely that the kidneys in these animals were working under an increased osmotic load due to the high urea plasma level.

It has already been reported that erythropoiesis of the nephrectomised dog is stimulated by erythropoietin injection of human origin (2). After injection of 1,440 cobalt units of sheep erythropoietin, the erythropoietic response of one nephrectomised dog was similar to that obtained after bleeding dogs with ureteral implantation. These data add to the evidence that uremic intoxication, at least of short duration, plays no role in the suppression of erythropoiesis which follows bilateral nephrectomy.

SUMMARY

The effect of acute anemic hypoxia on erythropoiesis has been compared in normal and uremic dogs. Uremia was achieved by nephrectomy or ureteral ligation or implantation of the ureter in the iliac vein. Erythropoiesis was measured 3 days before and 3, 5 and 7 days after bleedings and surgery by plasma iron turnover, absolute reticulocyte counts and marrow normoblast percentages. Bilateral nephrectomy abolished the erythropoietic response which normally occurs after acute anemic hypoxia. In uremic dogs still having one or both kidneys after a transient increase, a decrease in erythropoiesis was observed. These results suggest that in the dog, there is no emergency mechanism stimulating erythropoiesis independent of the kidney. No definite conclusions can be drawn concerning the role of uremia in the lack of normal response to bleedings of uremic dogs still having kidneys. Sheep erythropoietin is able to stimulate erythropoiesis in the bilaterally nephrectomised dog.

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