Renoprival Hemolysis and Its Prevention by Renal Tissue

By E. E. Muirhead and Frances Jones

BILATERAL NEPHRECTOMY of the dog and rabbit is associated with a hemolytic state as demonstrated by a rapid decline in red blood cell volume in the absence of hemorrhage which, at the same time, is associated with elevation of serum bilirubin (mainly "indirect" type), stool urobilinogen and serum iron, and siderosis of liver, spleen and lymph nodes. While renoprival hemolysis progresses, the erythrocytes display a lowered reduced glutathione content, a reduction in glucose utilization and a decrement in inorganic phosphate flux. The erythrocytes appear to be removed from the circulation primarily by reticuloendothelial cells as indicated by erythrophagocytosis which is most evident in lymph nodes.

The present experiments were designed to study renoprival hemolysis and its prevention by renal tissue through the means of the radiochromium method for evaluation of the erythrocytic life span. In addition, certain general characteristics of the erythrocyte during renoprival hemolysis were appraised. The main question tested concerned itself with whether renal tissue per se was related to the survival of canine erythrocytes in vivo. Part of the material presented has appeared in abstract form.

METHODS

Adult mongrel dogs of either sex were used. Bilateral nephrectomy was performed at one sitting under ether anesthesia. The procedure, from induction of open-drop anesthesia to completion, required 10 to 20 minutes. Ureteral ligation was performed by isolating the ureter near the urinary bladder, cutting it between ligatures and burying the ligated cut ends. Uretocaval anastomosis was established by means of a polyethylene tube, as previously described. The ureter was ligated and connected to the inferior vena cava as one procedure and 4 days later, at the start of the experimental observations, the opposite kidney was removed under ether anesthesia. Before sacrifice the animal was placed under pentobarbital anesthesia, the inferior vena cava was isolated above and below the entrance of the polyethylene tube and opened. It was determined on each occasion that urine flowed without evident obstruction as an indication of a successful ureterocaval preparation. Explantation of renal tissue to peritoneum or lungs was conducted as described elsewhere. The renal tissue was explanted to the peritoneum or lungs and about 1 month later the remaining normal kidney was removed under ether anesthesia. Separate controls were established by explanting a segment of liver or the entire spleen to the peritoneum. The sham operated group was subjected to ether anesthesia and the operative manipulations precisely as the nephrectomized group except that following delivery of the kidneys and dissection of the renal pedicles the kidneys were replaced in their beds and the surgical wound was sutured.
The animals were fed a dietary formula containing 2 to 3 Gm. of casein per Kg. of body weight per day and about 25 calories per Kg. per day from an additional equal distribution of carbohydrate (glucose) and fat (dialyzed peanut oil). In addition, they received daily an intravenous infusion of saline amounting to 16 ml. per Kg. Peritoneal dialysis was conducted as previously described. The daily turnover of dialysis fluid varied between 2 and 4 liters.

The radiochromium tag of erythrocytes was conducted as follows. Ten to 20 ml. of blood was obtained under sterile conditions by syringe and 19-gauge needle, anticoagulated with acid-citrate-dextrose mixture, centrifuged and the plasma discarded. Saline containing 50–75 μc. of Cr₅¹ as Na₂Cr₂O₇ was added to make the cell suspension to original volume, mixed gently and incubated at 37 C. for 1 hour, gentle mixing being periodically interposed. The suspension was centrifuged (1000 rpm for 2 minutes) and the supernatant saline was discarded. The erythrocytes were washed three times with about 20 ml. of cold saline. The erythrocytes suspended in saline to about the original hemato-crit reading were injected intravenously.

All blood samples were collected in heparin-containing syringes and immediately transferred to test tubes calibrated at 4 ml. After hemolysis by freezing and thawing, counts were obtained in a scintillating well-type counter having a gamma ray sensitive sodium iodide-thallium crystal. The sample 24 hours after the injection was taken as the 100 per cent value to which subsequent counts were referred. In most instances all counts were determined at one time at the termination of the observations. The per cent survival of erythrocytes was considered as
\[
\frac{\text{cpm in 4 ml. sample (corrected for background)}}{\text{cpm in 24 hr. sample (corrected for background)}} \times 100.
\]

The per cent survival was plotted on semi-logarithmic paper and the time for 50 per cent of the activity to disappear (T₁/₂ Cr₅¹) was taken off the graph. The T₁/₂ Cr₅¹ value was used in comparing different groups by the t test.

Group-to-group transfusions in accordance with the grouping sera of Swisher and associates were not conducted since sufficient grouping sera were not available. Rather, a stringent major crossmatch between donor's erythrocytes and recipient's serum was performed on each occasion by means of the saline and antiglobulin (Coombs) technics. By the latter approach, plus the use of substantial numbers of individual observations, it was considered that incompatibility was circumvented in the over-all appraisal of results. The results of the controls, normal and sham, supported this interpretation.

Erythrocytes were used for transfusion between dogs when not only the saline cross-match was negative at room temperature but the antiglobulin crossmatch was negative grossly and microscopically. A few erythrocytic clumps seen microscopically were read as a + (w) reaction and the blood was not used in the transfusion. The antiglobulin serum was prepared in this laboratory and the antiglobulin procedure was performed in the test tube, both as previously described.

In the course of the experiments, 354 crossmatches were conducted between dogs. Of these, 33 were incompatible in saline at room temperature and 231 yielded varying degrees of incompatibility by the antiglobulin method, according to the approach used for reading the test. The following incompatible readings were recorded with the antiglobulin method: 4 as 4+; 18 as 3+; 29 as 2+; and 74 as + (w). Thus, approximately three-fourths of the donors were considered unsuitable for the purposes of these experiments. Likely, the + (w) reactions did not represent true incompatibilities. The desirability of excluded erythrocytes giving a 1+ and 2+ reaction in the crossmatch was indicated by life span measurements.

The life span of the incompatible erythrocytes was measured on occasions simultaneously by the Ashby differential agglutination technic and the radiochromium procedure. The erythrocytes from a D negative donor (Swisher notation) were transfused into a D positive recipient for the Ashby method. About 250 ml. of blood was removed from the recipient and an equal volume of donor's blood was transfused. The inagglutinable cell count was determined with the use of canine anti-D serum according to the technic.
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described by Kaplan and Zuelzer.\textsuperscript{13} The control inagglutinable cell count (anti-D serum plus D positive cells) was less than 35,000 per cu. mm. Prior to the transfusion an aliquot of the donor's erythrocytes (about 10 ml.) was tagged with Cr\textsuperscript{51}. For both methods the 24-hour value was considered as the 100 per cent value.

When incompatible erythrocytes yielding a 1+ to 2+ antiglobulin reaction in the cross-match were transfused into a normal recipient, the life span of the transfused erythrocytes was shortened in a similar fashion by both methods. Indeed, except for an early inexplicable drift in the Ashby results, the rates of disappearance of the erythrocytes from the circulation were the same for the two procedures and T\textsuperscript{1/2} was 8 days for the Cr\textsuperscript{51} approach and 11.5 days for the Ashby method. About 1 month later, following disappearance of the transfused erythrocytes, the same experiment was repeated using the same donor and recipient. On this occasion the results were almost the same with the two methods, T\textsuperscript{1/2} 9.5 days for Cr\textsuperscript{51} and 8.5 days for Ashby. The results not only supported the use of the Cr\textsuperscript{51} method for the measurement of a shortened life span of erythrocytes in the dog but they indicated the need to avoid the use of erythrocytes displaying a 1+ to 2+ antiglobulin reaction in the crossmatch for observation in the renoprival and other states.

The effectiveness of the Cr\textsuperscript{51} method was further indicated by measuring the life span of erythrocytes from nephrectomized dogs within the circulation of normal dogs by the Ashby and radiochromium methods simultaneously, using the same approach as described above (see Results).

The red cell count was determined with diluting pipette and counting chamber as standardized by the National Bureau of Standards. The hematocrit was determined by Wintrobe's method\textsuperscript{14} to which a correction for trapped plasma was added.\textsuperscript{15} The hemoglobin concentration was determined by the alkaline hematin method.\textsuperscript{16} The reticulocyte count was obtained by the brilliant cresyl blue method.\textsuperscript{17} The mechanical fragility of erythrocytes was determined by the Shen, Castle and Fleming method\textsuperscript{18} as previously described,\textsuperscript{12} utilizing fresh blood and sterile defibrinated blood after 24 hours incubation at 37 C. The platelet count was determined by the direct method using Reese-Ecker fluid for dilution.\textsuperscript{19} The white blood cell count was obtained by the usual dilution and counting chamber. The osmotic fragility of erythrocytes was obtained by the method of Suess et al.\textsuperscript{20} utilizing both fresh blood and defibrinated blood incubated for 24 hours at 37 C. under sterile conditions. The plasma urea nitrogen concentration was determined by an urease method.\textsuperscript{21}

The erythrocytic life span studies will be described under eight groups, including a normal control group.

RESULTS
I. Peripheral Blood Following Bilateral Nephrectomy

Table 1 relates certain features of the peripheral blood before and 4 days following bilateral nephrectomy in the absence of dialyzing procedures.

a. Characteristics of the erythrocytes: As depicted in table 1, a slight decrement in the mean value for the red cell count, hematocrit reading and hemoglobin concentration transpired, but the changes for the group were not significant. There was no significant change in the reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Thus, the erythrocytes remained normochromic and normocytic. The direct antiglobulin (Coombs) test remained negative in all but one example in which there was no change from the control (both control and test values gave slightly positive reaction).

The osmotic fragility of fresh erythrocytes and erythrocytes incubated for 24 hours under sterile conditions was not different from the control. The mechanical fragility test, as conducted on fresh and incubated erythrocytes,
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Table 1

<table>
<thead>
<tr>
<th></th>
<th>RBC 10^6/cu.mm.</th>
<th>Hemat. %</th>
<th>Hb Gm. %</th>
<th>Retic. %</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.37 ± .37</td>
<td>45.3 ± 2.3</td>
<td>13.2 ± 1.0</td>
<td>0.63 ± .16</td>
<td>83.2 ± 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>±S.E.</td>
<td>4.76 ± .34</td>
<td>39.6 ± 3.3</td>
<td>13.3 ± 1.7</td>
<td>0.46 ± .07</td>
<td>83.3 ± 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>.06</td>
<td>~15</td>
<td>~4</td>
<td>~.35</td>
<td>&gt;.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.—Plasma Urea Concentration (mg./100 ml.)

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Control Mean ± SE</th>
<th>Fourth Day Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renoprival</td>
<td>12</td>
<td>61 ± 4.6</td>
<td>396 ± 42</td>
</tr>
<tr>
<td>Ureterocaval</td>
<td>11</td>
<td>79 ± 4.9</td>
<td>408 ± 21</td>
</tr>
<tr>
<td>Renal cortex explantation</td>
<td>5</td>
<td>51 ± 4</td>
<td>311 ± 10</td>
</tr>
<tr>
<td>Whole kidney explantation</td>
<td>5</td>
<td>48 ± 3.7</td>
<td>210 ± 19</td>
</tr>
<tr>
<td>Renal medulla explantation</td>
<td>10</td>
<td>50 ± 1.6</td>
<td>254 ± 15</td>
</tr>
</tbody>
</table>

These data relate the general degree of azotemia for various groups not submitted to dialysis. The mean value for plasma urea concentration plus the standard error of the mean are given. The azotemia was substantial by the 4th day in all groups depicted while the erythrocytic life span varied between groups. Azotemia appeared lower in groups having whole kidney and medulla explants.

indicated an increase in resistance to mechanical trauma for fresh cells but no change from the control value after incubation.

b. Other blood examinations: The peripheral white blood cell count was moderately elevated due primarily to neutrophilia. There was no significant change in the peripheral platelet count.

By the 4th day following the nephrectomies, azotemia was outstanding, as indicated in table 2. Table 2 also relates the degree of azotemia of other groups subjected to measurement of erythrocytic survival.

II. Lifespan of Erythrocytes in Normal Dogs Using the Radiochromium method

a. Autotransfusion (fig. 1): In 12 experiments the Cr51-tagged erythrocytes were reinjected into the same normal animal. The disappearance of radioactivity was curvilinear, similar to results in man,22,23 and touched baseline at 90 to 115 days (with one exception). The T½ Cr51 varied between 16 and 34 days (average 26 days).

b. Homotransfusion: In eight experiments the Cr51-tagged erythrocytes from a normal donor were injected into a normal recipient. The curve of disappearance of radioactivity was similar to that of the autotransfused group, touching baseline at 98 to 108 days (with one exception). The T½ Cr51 for this group varied between 22 and 40 days (average 34 days).

The over-all average T½ Cr51 for the normal group was 29 days (n = 20).

c. Sham operated group plus autotransfusion: In four examples of this type,
Fig. 1.—The life span of canine erythrocytes in the normal state as obtained with the radiochromium method is shown. On the left are the values plotted on regular coordinate paper. On the right are plots on semilogarithmic paper from which the $T_{1/2}$ Cr values were obtained. The results were derived following autotransfusion into 12 normal dogs.

$T_{1/2}$ Cr varied between 29 and 43 days and averaged 36 days. Thus, the operation itself did not disturb the life span of the erythrocytes of the dog. These results were similar to those for the rabbit when other parameters were used in appraising in vivo hemolysis.2

III. Simultaneous Ashby and Radiochromium Life Span Measurements Following Bilateral Nephrectomy

On four occasions approximately 250 ml. of blood taken from dogs 4 days after nephrectomy were transfused into normal recipients by exchanging the blood volume for volume. As in the preliminary incompatible transfusion, D negative renoprival donors and D positive normal recipients were used. An aliquot of donor’s erythrocytes was tagged with Cr. The erythrocyte life span of all four examples was markedly shortened. $T_{1/2}$ Cr averaged 9 and 11 days respectively. The difference between the two methods was not significant ($p \sim 0.6$). In two examples the results with the Ashby method revealed return of the inagglutinable cell count to baseline before the red cell radioactivity had disappeared.

The results indicated a shortening of the red cell life span following renal ablation, and supported the use of the Cr method under the circumstances of the experiment.
IV. Lifespan of Erythrocytes from Renoprival Donors by Cr\textsuperscript{51} Method When Transfused into Normal Recipients

a. Small volume of erythrocytes transfused: On 25 separate occasions, 5 to 10 ml. of erythrocytes taken 4 days following renal ablation were tagged with Cr\textsuperscript{51} and transfused into normal recipients. A family of curves derived by this approach. In 13 examples the life span was markedly shortened, and radioactivity disappeared from the blood within 10 to 25 days. In an additional seven examples the life span was shortened to a lesser extent, with disappearance of radioactivity in 28 to 70 days. In five examples the life span approached the lower limits of normal. T\textsubscript{1/2} Cr\textsuperscript{51} ranged from 4 to 24 days, the average value being 12.7 days. The results for the entire group are significantly different from the normal and sham operated controls (p < .001).

b. Larger volume of erythrocytes used as in IVa: The experiment of IVa was repeated on six occasions with the modification that a larger volume of erythrocytes, 100–125 ml., was tagged with Cr\textsuperscript{51} before transfusion into normal recipients. T\textsubscript{1/2} Cr\textsuperscript{51} varied between 9 and 22 days and averaged 15.5 days. The results were different from normal (p < .001). The larger volume of erythrocytes did not limit a rapid removal of the cells from the circulation.

c. Erythrocytic life span 1, 2, 3 and 4 days following renal ablation: Four separate experiments were conducted. The erythrocytes (5–10 ml.) following renal ablation were tagged and transfused into normal recipients after (1) 3 and 4 days, (2) 2 and 4 days, (3) 1 and 4 days, and (4) after 1, 2, 3 and 4 days in one case. In all instances there was a markedly shortened survival, T\textsubscript{1/2} Cr\textsuperscript{51} averaging 7 days. Thus, the disturbance of the erythrocyte causing a shortened life span following nephrectomy appeared as pronounced after 24 hours as after 96 hours following the nephrectomies.

d. The life span of the erythrocytes was shown to be shortened 9 and 16 hours after the renal ablation. T\textsubscript{1/2} Cr\textsuperscript{51} in these two experiments amounted to 10.5 and 12 days.

V. Lifespan of Erythrocyte from Renoprival Source as Measured within the Renoprival and Normal Environments

Four days after bilateral nephrectomy the life span of the erythrocytes was determined within normal recipients (T\textsubscript{1/2} Cr\textsuperscript{51}, 5 days). After the disappearance of radioactivity, the life span of the same normal recipient’s erythrocytes was determined to be normal by autotransfusion, demonstrating the absence of an intrinsic hemolytic mechanism within the recipients. Then the same recipients were nephrectomized after the erythrocytes had been tagged with radiochromium. These cells were followed for 4 days within their own environment. The lifespan was found shortened (T\textsubscript{1/2} near 5 days). After 4 days these erythrocytes were also followed in a normal recipient and the same shortened survival was noted. Figure 2 relates the results of the two experiments conducted in this fashion.

These observations support further a defect within the erythrocyte itself following renal ablation of the dog—in other words, an intracorpuscular defect as a consequence of the renoprival state.
VI. Lifespan of Erythrocytes 4 days after Ureteral Ligation as Measured in Normal Recipients

Of the 10 experiments in this group, in four the radioactivity touched baseline within 12 to 28 days, in four the radioactivity disappeared in about 30 to 55 days and in two it disappeared in 82 to 92 days. \( T^{1/2} \) Cr\(^{51} \) for this group varied between 4 and 21 days and averaged 12.4 days. The results were different from normal (\( p < .001 \)) but not different from the renoprival group (\( p \approx .65 \)). The kidney with acute hydronephrosis did not protect against in vivo hemolysis.

VII. Lifespan of Erythrocytes 4 Days Following Ureterocaval Anastomosis as Measured in Normal Recipients

a. Without dialysis: Four examples of this group were carried for 4 days without dialysis, i.e., in the same manner as the renoprival group. Radioactivity of the blood disappeared in the recipient within 50 to 95 days (fig. 3). \( T^{1/2} \) Cr\(^{51} \) varied between 18 and 23 days and averaged 20.5 days.

b. With dialysis: Ten animals of this group were subjected to daily peritoneal dialysis. Radioactivity disappeared in the recipient between 28 and 102 days. \( T^{1/2} \) Cr\(^{51} \) varied between 14 and 23 days and averaged 18.6 days. The results were not significantly different from those of the undialyzed group.

For the entire group, \( T^{1/2} \) Cr\(^{51} \) averaged 19 days. The results were different...
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Fig. 3.—The radiochromium life span of erythrocytes derived 4 days after ureterocaval anastomosis and measured in normal recipients is shown. The life span is significantly greater than following either renal ablation or ureteral ligation.

from normal (p < .001) as well as from the renoprival and ureteral ligation groups (p < .005, < .001).

The presence of intact renal tissue without an external excretory outlet afforded significant protection against in vivo hemolysis. The protection was not complete even when daily dialysis was added to the regimen.

VIII. Explanation of One Renal Medulla to Lungs, 1 Month Later Removal of Remaining Kidney, 4 Days Later Measurement of Life Span of Erythrocytes in Normal Recipient

There were five of these experiments. Blood radioactivity touched baseline in 42 to over 100 days (fig. 4). T½ Cr51 varied between 12.5 and 26 days and averaged 20 days. The results were different from normal (p < .01) and the renoprival group (p < .001), but no different from the ureterocaval group (p ~ .6).

These observations support additionally the view that intact renal tissue without an external excretory outlet, and particularly renal medulla, protects against in vivo hemolysis. As in the case of the ureterocaval experiment, the protection was not complete.

IX. Renal Autoexplantation and Measurement of Erythrocytic Survival by Autotransfusion

The renal autoexplantation was performed and 20 to 30 days later the opposite kidney was removed. At the same time about 10 ml. of the animal's
Fig. 4.—The renal medulla of one kidney was autoexplanted to the peritoneum and about 1 month later the opposite kidney was removed. Four days later the erythrocytes were tagged and followed in normal recipients. The results are the same as following ureterocaval anastomosis.

Erythrocytes were tagged with radiochromium and reinjected as an autotransfusion. The 24-hour radioactive count was considered as the 100 per cent value. Counts were taken after 2, 3 and 4 days and computed as per cent of the 1-day value. The 4th day value was taken for statistical comparison of the various groups with the normal (control) group. Figure 5 summarizes the results.

a. Normal control: The 4th day count varied between 78 and 96 per cent of the 1st-day count. The average value was 88.7 per cent for the 14 experiments.

b. Renoprival state: In 21 experiments the 4-day count varied between 24 and 92 per cent and averaged 64.6 per cent. These results differed from the normal group.

c. Whole kidney explanted to peritoneum: In seven experiments the whole kidney was explanted to the peritoneum. The 4th-day value varied between 81 and 95 per cent and averaged 86.4 per cent. These results were not different from the normal (p < .05).

d. Renal medulla explanted to either peritoneum or lungs: For 10 experiments the 4th-day value varied between 79 and 93 per cent. The average was 85.6 per cent. The results were not different from the normal (p > .2).

e. Renal cortex explanted to peritoneum: When the renal cortex was explanted, 4th-day values in 11 such experiments varied between 62 and 84 per
Fig. 5.—The figure consists of a semilogarithmic plot of the per cent disappearance of radioactivity of the blood after the erythrocytes were tagged with radiochromium and autotransfused. The first 24-hour counts were considered as the 100 per cent value. The average 4th-day value is given below. The p value represents the difference from the normal group. Whole kidney and renal medulla explantation gave results similar to the normal. The other groups were associated with various degrees of shortened survival of erythrocytes.

<table>
<thead>
<tr>
<th></th>
<th>Whole kidney explant</th>
<th>Medulla explant</th>
<th>Cortex explant</th>
<th>Spleen, liver explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>A$_{v}$</td>
<td>88.7</td>
<td>86.4</td>
<td>85.6</td>
<td>73.5</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>7</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>p</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

The results with these groups complement the results described under section VIII and add support to the view that renal tissue without an excretory outlet protects against in vivo hemolysis. The renal medulla appears to be the main source of such protective function.

**Comment**

The results of the present study indicate a prominent reduction of the erythrocyte life span following bilateral nephrectomy of the dog. Former studies, conducted under similar conditions, demonstrated a reduction of the red blood cell mass associated with elevation of the serum bilirubin and serum iron concentrations, increased excretion of urobilinogen in the stool, siderosis of liver, spleen and lymph nodes and erythrophagocytosis, especially within lymph nodes. Thus, the shortening of the erythrocytic life span follow-
ing renal ablation appears to be due primarily to destruction of erythrocytes rather than to their sequestration away from the general circulation. The term renoprival hemolysis appears appropriate for this condition.

Renoprival hemolysis was associated with normochromic and normocytic erythrocytes which displayed a normal osmotic fragility and negative direct antiglobulin test. The mechanical fragility of fresh erythrocytes was decreased by the method used but unaltered when incubated cells were used in the test. The plasma and serum did not display gross evidence for hemoglobinemia. These observations suggest the possibility of the cellular uptake of the erythrocytes by reticuloendothelial cells via a mechanism unrelated to overt coating of the cells with globulin. The latter possibility implies a surface change of the erythrocyte, but one which does not alter its shape.

The erythrocytic defect associated with renoprival hemolysis was shown to be of the intracorpuscular type, as the shortened lifespan was demonstrated in the normal state as well as in the renoprival one. The erythrocytic defect seemed to develop very rapidly, apparently within 24 hours or less. Although retention of waste products was proceeding during this time, advanced uremia had not had time to evolve.

That retention of waste products, in the classical sense of uremia, was not the major factor implicated in the erythrocytic defect appears supported by life span measurements of the renoprival group when compared with similar measurements of the ureteral ligation and ureterocaval groups. Acute ureteral ligation was associated with a lowering of the erythrocytic life span to the same degree as following renal ablation, while ureterocaval anastomosis was not attended by as marked shortening of the life span as noted with ureteroligation and nephrectomy. The difference between the results of the former two groups (nephrectomy, ureteral ligation) and those of the latter group (ureterocaval) was highly significant. Yet, both ureteral ligation and ureterocaval anastomosis tend to give rise to a similar level of uremia (control BUN 10, 4 day 210 mg./100 ml. in one example of ureteroligation). The results of these two groups suggest that the state of the renal tissue was a factor in the evolution and prevention of the in vivo hemolysis. Acute ureteral obstruction is followed by hydronephrosis, reduction of renal blood flow and necrosis of renal tissue, especially the medulla. Conversely, hydronephrosis does not follow ureterocaval anastomosis and the entire renal parenchyma is not only intact but undergoes considerable hyperplasia and hypertrophy.

The addition of peritoneal dialysis with a balanced salt solution to the ureterocaval preparation did not alter the state of the erythrocytic life span as compared to a similar nondialyzed group. These results were surprising and suggested the presence of variables in the in vivo hemolysis which were not entirely controlled by the renal tissue and by the partial removal of waste products. Whether these variables were solely of in vivo origin or whether they were related to the technical handling of the erythrocytes outside of the body prior to infusion was not ascertained during these studies. Although the technical handling of erythrocytes from normal animals did not appear to alter their life span within the normal environment, this aspect of the results seems to require additional attention.
Autoexplanted renal medulla afforded the same degree of protection against the hemolysis as the ureterocaval maneuver when the life span studies were conducted in normal recipients. These results implied positive support for the renal medulla as a major source of the function of the kidney which protected against in vivo hemolysis and correlated reciprocally with the lack of protection observed following ureteral ligation, a condition known to injure mainly the renal medulla.

Additional support for a medullary origin of the protective function against hemolysis was obtained by autoexplantation followed by a short-term erythrocytic survival study using the infusion of tagged autogenous erythrocytes. Transplanted whole kidney and renal medulla gave results similar to those of normal controls while renal ablation without explantation and renal ablation plus autoexplantation of renal cortex, spleen or liver yielded results which differed from the control and indicated a lowered survival of erythrocytes in the circulation.

The nature of the function of the kidney which appears to protect against in vivo hemolysis remains obscure. Since the function is primarily nonexcretory in type, the issue of a renal principle acting either directly on the erythrocytes at the tissue level or indirectly via a humoral factor is raised.

**SUMMARY**

The life span of erythrocytes is prominently shortened following bilateral nephrectomy of the dog. This is due to an intracorpuscular defect which appears to develop rapidly. While this defect develops, the erythrocytes remain normochromic and normocytic, display a normal osmotic fragility and are not coated with globulin. Intact renal tissue, as exemplified by the ureterocaval preparation, improves significantly the life span of the erythrocyte. Autoexplanted renal cortex, liver and spleen and the acutely hydronephrotic kidney do not protect against the shortened life span of the erythrocyte. The renal function which appears to protect against in vivo hemolysis seems to be primarily of nonexcretory type and mainly of medullary origin.

The results suggest the presence of a renal principle operating either directly at renal tissue level or indirectly by a humoral factor in preventing in vivo hemolysis.

**Summario in Interlingua**

Le duration vital de erythrocytos es prominentemente accurtate in le can post nephrectomia bilateral. Isto resulta de un defecto intracorpuscular que pare disveloppar se rapidemente. In le curso del disveloppamento de iste defecto, le erythrocytos remane normochromic e normocytic, illos exhibi un normal fragilitate osmotic, e illos porta un revestimento de globulina. Intacte tissu renal—exemplificate per le preparato uterocaval—meliora significative mente le duration vital del erythrocytos. Auto-explantate cortice renal, hepate, e splen, e tissu ab renes acutemente hydronephrotic non protege contra le accurtamento del duration vital de erythrocytos. Le function renal que pare proteger contra hemolyse in vivo pare esser primarimente del typo nonexcretori e principalmente de origine medullari.
Le resultados sugieren que el presentía de un principio renal que actúa (1) directamente al nivel del tejido renal o (2) indirectamente vía un factor humorales en prevenir la hemólisis in vivo.

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REFERENCES


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