POLYMORPHONUCLEAR LEUCOCYTOSIS AND LYMPHOPENIA IN ACUTE RENAL FAILURE AND METABOLIC ACIDOSIS IN THE RAT

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SUMMARY

1. Peripheral blood leucocytes were studied before, and 48 h after acute induction in the rat of either uraemia (by bilateral ureteric ligation or bilateral nephrectomy) or of metabolic acidosis in the absence of uraemia (by ingestion of NH₄Cl).

2. A significant, fourfold increase in polymorphonuclear leucocyte count was seen after bilateral ureteric ligation, but no increase occurred in sham-operated animals nor after bilateral nephrectomy, despite equivalent uraemia and acidosis. A lesser degree of polymorphonuclear leucocytes was also seen after unilateral ureteric ligation. A significant but also lesser degree of polymorphonuclear leucocytosis was seen after metabolic acidosis in the absence of uraemia. Correction of acidosis in another group of animals with bilateral ureteric ligation did not prevent polymorphonuclear leucocytosis.

3. Significant lymphopenia, of approximately equal degree, was seen after bilateral nephrectomy, and after metabolic acidosis, but did not occur in control groups.

4. These findings support the hypothesis that polymorphonuclear leucocytosis in acute uraemia or ureteric obstruction may be due to a direct renal mechanism, such as release of a leucopoietin, whereas lymphopenia may be due to metabolic changes associated with uraemia or metabolic acidosis.

Key words: acute renal failure, metabolic acidosis, polymorphonuclear leucocytosis, lymphopenia.

Leucocytosis is common in acute tubular necrosis (Jensson, 1958; Riis & Stuugaard, 1959a; Merrill, 1962; Montgomerie, Kalmanson & Guze, 1968; Hamburger, 1968) and in acute diabetic acidosis (Wintrobe, 1967) in man even in the absence of infection. The present study aimed to observe the changes in numbers of circulating leucocytes in acute uraemia in the rat, and to determine whether these are due to the metabolic effects of uraemia or to direct metabolic changes.
renal mechanisms. Foster & Mirand (1970) had already observed a polymorphonuclear leucocytosis in mice after ureteric ligation.

This work was presented in part at the Southern Section American Federation for Clinical Research meeting (1971) and published in abstract form (Luke & Simon, 1971).

METHODS

Experimental plan

Blood polymorph and lymphocyte counts were determined before, and after (1) production of acute uraemia owing to either bilateral ligation of ureters (Group 1) or bilateral nephrectomy (Group 2); Group 3 consisted of sham-operated control animals; (2) acute uraemia induced as in Group 1 but with correction of acidosis with intraperitoneal NaHCO₃ (Group 4); (3) unilateral ligation of the ureter (Group 5); and (4) acute acidosis induced by ingestion of NH₄Cl (Group A); Group B consisted of control animals.

Sprague–Dawley rats weighing 150–250 g were given no food for 16 h before the experiments but were allowed free access to water. During the 48 h post-operative period rats were kept in individual metabolic cages at constant temperature and received no food or water. At the start of the experiment and at its conclusion (48 h), blood was obtained from the tail. After anaesthesia with intraperitoneal pentobarbital (5 mg/100 g rat) the tail was warmed in warm (35–40°C) water and the tip (approximately 1 cm) cut off with sharp scissors. The 48 h blood for leucocyte counting was obtained by cutting the tail 1 cm proximal to the first amputation. The first one or two drops of blood were discarded and two separate pipettes were filled from separate but consecutive drops of blood for leucocyte counts. Total leucocytes were counted in a haemocytometer counting chamber. A blood smear was stained with Wright's stain for differential leucocyte counting. Haematocrit was also determined on tail blood at 0 and 48 h by use of heparinized capillary tubes. Heparinized aortic blood was then taken at 48 h for determination of arterial pH by the micro-Astrup apparatus (Radiometer, Copenhagen); blood urea nitrogen (BUN) was measured by the method of Crocker (1967).

If any duplicate leucocyte counts differed by more than 10% the results from that animal were discarded; this led to the exclusion of only two rats from the study. In the period between immediate post-operative recovery and 48 h there was only one death.

Acidosis was produced by using NH₄Cl (150 mmol/l) in 5% dextrose as the drinking solution; control rats ingested 5% dextrose only. No food was given to either group.

Both bilateral nephrectomy and bilateral ureteric ligation were carried out through two dorsal incisions. The ligatures were of silk. Before nephrectomy a double tie was placed round the renal artery and vein, and also the ureter. The adrenals were carefully dissected away from the upper pole of both kidneys in all operated groups (1–5). Sham operation consisted of mobilization and preparation of both kidneys for ligation of the renal hilum and ureter, and the adrenal dissection. Acidosis was corrected by intraperitoneal injection of 0·75 mmol of NaHCO₃ (5 ml of NaHCO₃, 150 mmol/l) at 20 h and again at 40 h after surgery.

No gross evidence of urinary-tract infection was seen in the obstructed kidneys. Histology was studied in four of the obstructed kidneys in Group 1.

Statistically significant changes between 0 and 48 h within each experimental group are defined as occurring when $P < 0·05$ in the paired t-test. The change in leucocyte counts between
Leucocytosis in acute renal failure

0 and 48 h is compared by analysis of variance among experimental groups 1–5 and by the t-test between Groups A and B.

RESULTS

Effect of acute uraemia (Table 1)

The degree of acidosis and uraemia produced in Groups 1 and 2 was very similar (Table 1). Control rats (Group 3) showed no change in polymorph count. A fourfold increase in polymorph count, which was highly significant, occurred in Group 1 (bilateral ureteric ligation) but there was no significant increase in Group 2 after bilateral nephrectomy (Table 1). Correction of acidosis (Group 4) did not prevent polymorph leucocytosis after bilateral ligation. Although there was no statistically significant increase in polymorph counts after unilateral ureteric ligation (Group 5), this was due to a fall in polymorph count at 48 h in one rat, which had the highest initial (0 h) leucocyte count of all the rats studied (4402 cells/mm³).

| Table 1. Effect of uraemia on polymorph and lymphocyte counts
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Group 1*</td>
<td>Group 2</td>
<td>Group 3</td>
<td>Group 4</td>
<td>Group 5</td>
</tr>
<tr>
<td><strong>Polymorph count (cells/mm³)</strong></td>
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<tr>
<td>0 h</td>
<td>1863 ± 320†</td>
<td>2161 ± 292</td>
<td>1571 ± 299</td>
<td>1586 ± 299</td>
</tr>
<tr>
<td>48 h</td>
<td>7005 ± 988</td>
<td>3040 ± 517</td>
<td>1531 ± 212</td>
<td>10306 ± 996</td>
</tr>
<tr>
<td>Δ (48 h-0 h)</td>
<td>+5142 ± 970‡</td>
<td>+879 ± 704</td>
<td>-40 ± 299</td>
<td>+8720 ± 1855‡</td>
</tr>
<tr>
<td>t-test on Δ within group</td>
<td>P&lt;0·001</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P&lt;0·001</td>
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<tr>
<td><strong>Lymphocyte count (cells/mm³)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>10981 ± 1040</td>
<td>9970 ± 1011</td>
<td>10094 ± 884</td>
<td>11156 ± 995</td>
</tr>
<tr>
<td>48 h</td>
<td>6377 ± 1144</td>
<td>6013 ± 1128</td>
<td>10068 ± 1593</td>
<td>5935 ± 721</td>
</tr>
<tr>
<td>Δ (48 h-0 h)</td>
<td>-4604 ± 611‡</td>
<td>-3956 ± 633‡</td>
<td>-26 ± 943</td>
<td>-5241 ± 374‡</td>
</tr>
<tr>
<td>t-test on Δ within group</td>
<td>P&lt;0·001</td>
<td>P&lt;0·001</td>
<td>N.S.</td>
<td>P&lt;0·001</td>
</tr>
<tr>
<td><strong>BUN (mg/100 ml)</strong></td>
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<td></td>
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<tr>
<td>0 h</td>
<td>299 ± 17‡</td>
<td>293 ± 24‡</td>
<td>32 ± 2·6</td>
<td>228 ± 8·3‡</td>
</tr>
<tr>
<td>48 h</td>
<td>92 ± 8‡</td>
<td>102 ± 14‡</td>
<td>55 ± 3·1</td>
<td>48 ± 2·2</td>
</tr>
<tr>
<td>Δ (48 h-0 h)</td>
<td>-3·8 ± 1·9</td>
<td>-11·9 ± 2·4‡</td>
<td>-2·5 ± 3·0</td>
<td>-1·8 ± 1·5</td>
</tr>
</tbody>
</table>

* Group 1 bilateral ligation (n = 9); Group 2 bilateral nephrectomy (n = 10); Group 3 control (n = 8); Group 4 bilateral ligation and bicarbonate (n = 6); Group 5 unilateral ligation (n = 9).
† Mean ± SEM.
‡ P<0·01 as compared to Group 3 (control) by analysis of variance.
§ See results for further analysis of the findings.

The count rose in the remaining eight animals; in these eight rats the mean change (±SEM) was +817 ± 258 cells/mm³ (P<0·02). Applying the signed rank-test to Group 5 (n = 9), the rise in polymorph count is also significant (P<0·01).

Significant and marked lymphopenia was seen in all experimental groups but was not noted as a response to sham operation (Group 3).

Histology of the obstructed kidneys showed dilatation of pelvis, collecting ducts, tubules and Bowman’s space without evidence of bacterial invasion or inflammatory response. All
cultures of pelvic urines taken at 48 h from an additional series of twelve rats, handled as in Group 1 of the present series, showed no bacterial growth.

Changes in mean haematocrit during the 48 h of the study are also shown in Table 1. After bilateral nephrectomy (Group 2) there was a significantly greater fall in haematocrit than in controls or in rats after bilateral ureteric ligation.

<table>
<thead>
<tr>
<th>TABLE 2. Effect of acidosis on polymorph and lymphocyte counts</th>
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<tr>
<td><strong>Group A</strong></td>
</tr>
<tr>
<td><strong>Polymorph count (cells/mm³)</strong></td>
</tr>
<tr>
<td>0 h</td>
</tr>
<tr>
<td>48 h</td>
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<tr>
<td>Δ (48 h—0 h)</td>
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<tr>
<td><strong>Lymphocyte count (cells/mm³)</strong></td>
</tr>
<tr>
<td>0 h</td>
</tr>
<tr>
<td>48 h</td>
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<tr>
<td>Δ (48 h—0 h)</td>
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<tr>
<td><strong>BUN (mg/100 ml)</strong></td>
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<tr>
<td><strong>Arterial [H⁺] (n mol/l)</strong></td>
</tr>
</tbody>
</table>

N.S., not significant.

* Ingesting NH₄Cl, n = 9.
† Control, n = 7.
‡ Mean ± SEM.
§ t-test on change between 0 h and 48 h within group.

Weight loss in the 48 h period was 38 ± 4 g (mean ± SEM) in control animals, 22 ± 2 g after bilateral nephrectomy, and 19 ± 4 g after bilateral ureteral ligation. These weights in all cases exclude kidney weights, which were determined at the time of their removal.

**Effect of acidosis in the absence of uraemia** (Table 2)

Significant acidosis, as compared with control animals, was produced by the ingestion of NH₄Cl. The polymorph count rose, and the lymphocyte count fell, in the acidotic rats; both changes were statistically significant (Table 2). Although the drop in lymphocyte count was of similar magnitude to that seen in the acute uraemia experiments, the rise in polymorph count, by about 60% from the start of the experiment, is much less than that seen after bilateral ureteric ligation.

**DISCUSSION**

High granulocyte counts are common in both acute and chronic renal failure in man (Jensson, 1958; Riis & Stuugaard, 1959a, b), and are associated with bone-marrow hyperplasia even in
Leucocytosis in acute renal failure 401

the absence of infection (Hamburger, 1968). These studies demonstrate that, in the rat, a considerable polymorph leucocytosis occurs after bilateral ureteric ligation but not after bilateral nephrectomy, although the degree of uraemia and acidosis seen in these two groups is virtually identical. Correction of acidosis to control levels in the rats with bilateral ureteric ligation does not prevent polymorph leucocytosis. Metabolic acidosis in the absence of uraemia, produced by ingestion of NH₄Cl, is associated with a definite increase in granulocytes in the blood but the change is of considerably lesser magnitude.

These experiments suggest that a renal mechanism, rather than the metabolic changes of uraemia per se, underlies the polymorph leucocytosis of acute renal failure. There is gross, histological and bacteriological evidence that this renal mechanism was not pyelonephritis. Polymorph leucocytosis and lymphopenia are seen after increased adrenocortical hormone secretion (Forsham, 1962), and there is evidence that acute renal failure, in man at least, may be associated with increased secretion of adrenocortical hormones (Montgomerie et al., 1968). However, lymphopenia occurred, without polymorph leucocytosis, in the nephrectomized rats, suggesting that, for the granulocyte change at least, a direct renal mechanism exists.

Since metabolic acidosis can increase adrenal cortical hormone secretion in the rat (Sartorius, Calhoon & Pitts, 1953) this may be the mechanism of the lesser degree of polymorph leucocytosis seen in acute metabolic acidosis without uraemia. However, acute metabolic acidosis also induces major changes in renal metabolism.

Dehydration with consequent plasma hyperosmolality, which has been claimed to be a factor in inducing leucocytosis (Tullis, 1948), is unlikely to be responsible for the differences in polymorph leucocytosis in these experiments, since weight loss was not different after bilateral nephrectomy or bilateral ureteric ligation.

Polymorph leucocytosis can be due to a shift of cells from the marginal to the circulatory blood compartment, diminished egress from the vascular compartment, or increased inflow of cells from the bone marrow into the blood compartment (secondary to increased marrow release and/or increased marrow production) or to combinations of these mechanisms (Boggs, 1967). It is unlikely that leucocytosis of this degree in the rat, 48 h after start of the study, could be due to a shift of cells from marginal sites (Lapin, Lobue, Gordon, Zanjani & Schulz, 1969) but all of the other mechanisms are possible. The present experiments cannot distinguish between these possibilities. Nevertheless, a leucopoietin and/or leucocyte-releasing factor has been found in high concentration in extracts of ox kidney (Bierman, 1964) and high concentrations of a 'colony-stimulating factor' which stimulates bone-marrow cells in vitro have been demonstrated in the serum of mice with bilateral or unilateral ureteric ligation and a selective polymorphonuclear leucocytosis (Foster & Mirand, 1970). It may be that a leucopoietin may be released in acute renal damage.

Lymphopenia in man is common in both chronic and acute renal failure (Jensson, 1958; Riis & Staugaard, 1959a, b) and there are quantitative abnormalities in lymphocyte function (Montgomerie et al., 1968). In these experiments lymphopenia appears to be a more non-specific response than polymorph leucocytosis since it was found, to an approximately similar degree, in all the experimental groups studied. Recent studies have confirmed lymphocyte depletion in acute uraemia in various experimental animals, including the rat, and have suggested that the lymphopenia is attributable to a toxic substance in uraemic plasma, rather than to a stress-induced increase in cortisol secretion (Slavin & Gallagher, 1970).

The higher haematocrit at 48 h in the rats with bilateral ligation as compared with neph-

rectomized rats may be related to the maintenance of erythropoietin levels after bilateral ureteric obstruction in contrast with its virtual disappearance after bilateral nephrectomy (Mirand & Murphy, 1969). The haematocrit differences cannot be attributed to differences in the degree of dehydration since weight loss in the 48 h study period was similar in the two groups. The differences also seem too large to be accounted for by altered fluid distribution between extracellular and intracellular compartments which might result from maintained renin secretion in the group with bilateral ureteric ligation.

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REFERENCES


