

Nephrectomy Abolishes the Increase in Small Acetylcholinesterase-Positive Immature Rat Megakaryocytes Induced by Acute Thrombocytopenia¹ (41715)

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Abstract. The effect of unilateral nephrectomy, bilateral nephrectomy, or ureter ligation on the proportion of small acetylcholinesterase-positive (SACHe+) cells was determined in the marrow of rats that were made acutely thrombocytopenic by an injection of platelet specific antiserum (APS). APS caused significant increases ($P < 0.025$ – $P < 0.005$) in the percentages of SACHe+ cells in the marrow of rats except in those rats that had been subjected to bilateral nephrectomy. Bilaterally nephrectomized rats not only failed to show increased numbers of SACHe+ cells after being made thrombocytopenic but other anephric rats that were not given APS had decreased percentages of SACHe+ cells ($P < 0.005$) when compared to untreated control rats. This finding indicates the importance of the kidney in the day-to-day production of a thrombocytopoiesis-stimulating factor (TSF) for the maintenance of SACHe+ cells. Unilateral nephrectomy and ligation of the ureters had little effect on altering the percentages of SACHe+ cells, suggesting that surgical stress and uremia do not interfere in the release and action of TSF. This study confirms previous work that the kidney is required for the production of TSF.

Previous investigations (1–4) have suggested that the kidney is a site of production of a thrombocytopoiesis-stimulating factor (TSF or thrombopoietin) and that human embryonic kidney cells in culture produce the TSF (5). In addition to studies in animals, other work showed that up to 50% of patients with chronic renal disease are thrombocytopenic (6). Further, thrombocytosis has been found in patients who have undergone renal transplantation (7). However, other workers (8) have concluded that the kidney is not necessary for TSF production. In the latter study, platelet counts were only slightly increased in nephrectomized rats after injection of platelet antiserum by day 3 (the time when rats usually die from uremia following nephrectomy). Since 4–5 days are required for platelet repopulation after an antisera injection, the rats did not live long enough to establish conclusively the role of kidneys in platelet production. This animal model is, therefore, unsuitable for such studies. However, an early cell in the megakaryocytic series of rodents,

i.e., the small acetylcholinesterase positive (SACHe+) cell, has been reported (9–11) to increase in the marrow at 6–8 hr after induction of acute thrombocytopenia. The SACHe+ cells appear to be controlled by the animal's TSF titer (9–11). Therefore, in the studies reported here, we tested the effects of unilateral and bilateral nephrectomy and ureter ligation on the production of SACHe+ cells in the marrow of thrombocytopenic rats.

Materials and Methods. Male Sprague-Dawley rats, used in these experiments, were given a standard laboratory diet (Purina Lab Chow) and water *ad libitum*. Groups of 6–10 rats each were either not treated or were subjected to unilateral nephrectomy, bilateral nephrectomy, or ureter ligation. Methoxyflurane anesthesia was given prior to surgery. A 2-cm incision was made along the lateral midline through the skin and a 1-cm incision was then made through the peritoneum. For unilateral nephrectomy, the left kidney was "tied-off" with 4-O silk and removed at the pedicle, insuring that all kidney material had been removed and that bleeding was minimal. The peritoneum was sutured with 4-O silk and the skin was sealed with stainless-steel wound clips. For bilateral nephrectomy, separate incisions were made for the removal of each kidney. The ureters of rats were obstructed by making a single midline incision and the ure-

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ters were ligated using 4-0 silk; the peritoneum and skin were sutured as above.

In each treatment group (6–10 rats each), approximately one-half of the rats were made thrombocytopenic by a single intraperitoneal (ip) injection of 0.25 ml of rabbit anti-rat platelet serum (APS; a gift from Dr. Carl Jackson of St. Jude Children's Research Hospital, Memphis, Tenn.). The anti-platelet serum had been absorbed with rat RBC as previously described (12). The antiserum was injected at 24 hr after surgery and, at 6 hr after the antiserum injection, the rats were bled to death from the abdominal aorta and their femurs removed. Hematocrit values and platelet counts were determined from aorta blood using standard techniques. Marrow taken from the femurs was mixed with a plasma expander (polyvinylpyrrolidone) and dispersed into a single cell suspension. Six bone marrow smears were prepared from each rat.

After the bone marrow smears had been allowed to air dry, the cells were stained using the direct coloring thiocholine method (13) for acetylcholinesterase. For staining, the marrow smears were incubated for 3 hr at room temperature in a solution consisting of acetylthiocholine iodide (30 mg), 0.1 *M* sodium phosphate, pH 6, (45 ml), 0.1 *M* sodium citrate (3 ml), 30 mM copper sulfate (6 ml), and 5 mM potassium ferrocyanide (6 ml). Following a 1-min rinse in 0.1 *M* sodium phosphate, postfixation was made in absolute methanol for 10 min and in 50% methanol for 30 sec. The cells were "counter stained" in Harris' hematoxylin, then "blued" for 2–3 min, and finally clarified in running tap water. The smears were examined and the proportion of small acetylcholinesterase-positive cells was determined microscopically. The SACHe+ cell population, as defined previously (14), are those cells that stain positively for AChE, are usually round, and are less than 13 μm in diameter as determined by an ocular reticule. For each rat, the total number of AChE-positive cells on six slides were scored (200–3200 cells/rat), the percentage of SACHe+ cells was calculated, and the average percentage was treated statistically as a single observation.

Student's *t* test was used to determine statistical significance.

Results. Figure 1 shows the results of measuring the packed cell volumes (PCV) and the platelet counts of normal rats and rats subjected to unilateral or bilateral nephrectomy or ureter ligation followed, in some rats, by APS treatment. As shown in panel A, the PCV of rats had a significant ($P < 0.005$) decrease following bilateral nephrectomy without an antiplatelet serum injection. This reduction in PCV probably reflected bleeding of the rats following the surgical procedure since the platelet counts were also slightly (but not significantly) decreased as compared to untreated control rats. However, bleeding cannot be the only mechanism for the reduction of PCV since thrombocytopenic-anephric rats had higher PCVs than did nephrectomized rats with normal platelet counts. All other treatments failed to significantly alter PCV of the animals. The platelet counts of the same rats are shown in panel B. In all cases, those rats given APS were severely thrombocytopenic at 6 hr following injection. As mentioned above, a slight, but not significant decrease in platelet counts was found in rats subjected to bilateral nephrectomy.

Figure 2 shows the percentages of SACHe+ cells of the same rats that were presented in Fig. 1. When compared to suitable controls, injection of APS led to significant increases ($P < 0.025$ – $P < 0.005$) in percentages of SACHe+ cells in the marrow of rats except in those rats subjected to bilateral nephrectomy. In addition, rats that were bilaterally nephrectomized but not given APS had decreased percentages of SACHe+ cells ($P < 0.005$) when compared to untreated control rats.

Discussion. The present study confirms previous work that the kidney is necessary for the production of TSF. As shown in this study, kidneys were required for increasing the number of SACHe+ cells after induction of thrombocytopenia. Since unilateral nephrectomy and ligation of the ureters had little effect on the numbers of SACHe+ cells, surgical stress, and uremia are probably not important in the release and action of TSF.

Apparently TSF release and/or action is required before elevated numbers of SACHe+ cells will appear in the rodent's marrow. An elevation in the percentage of SACHe+ cells

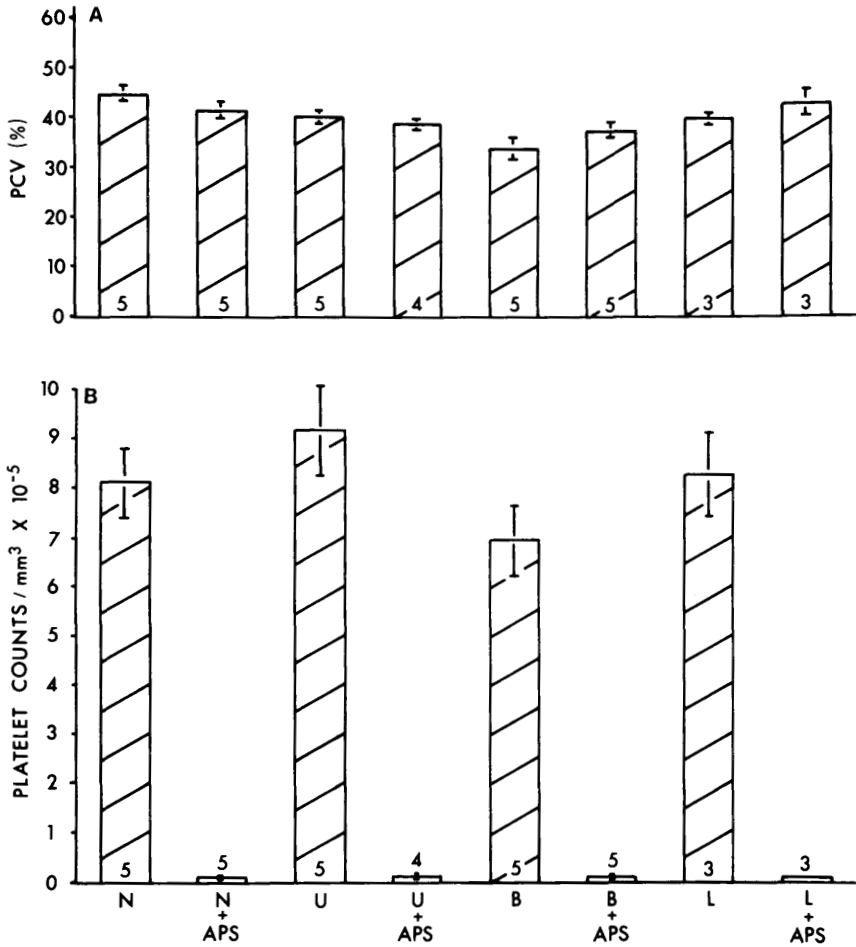


FIG. 1. The effects of no treatment (N), unilateral nephrectomy (U), bilateral nephrectomy (B) or ureter ligation (L) with and without injection of platelet specific antiserum (APS) on packed cell volumes (PCV), panel A, and platelet counts, panel B, of rats. The vertical lines represent the standard errors and the numbers on the bars represent the number of rats per treatment.

in the marrow of animals rendered acutely thrombocytopenic has been described (9, 11). Jackson (9) showed a twofold increase in the number of SACHe+ cells in rats at 6 hr after receiving an injection of antiplatelet serum. This work was confirmed in mice (11); SACHe+ cells were increased at 6 hr after platelet specific antisera injection by threefold over normal rabbit serum-injected controls. In addition, Long and Henry (10) showed that platelet hypertransfusion can significantly decrease the number of SACHe+ cells in mice. These observations provide evidence that these cells are early cells in the megakaryocytic series

and that their numbers are controlled by the animal's platelet count and, presumably, TSF titers.

TSF from kidney cell culture medium (11, 14) and plasma from thrombocytopenic mice (14) was shown previously to increase the proportion of SACHe+ cells in the marrow of mice. The increase was of the same magnitude and occurred at the same time as was observed in mice made severely thrombocytopenic. It should also be mentioned that in one study (14) TSF caused a positive dose-response relationship between the percentage of SACHe+ cells observed and the dose of TSF. Therefore,

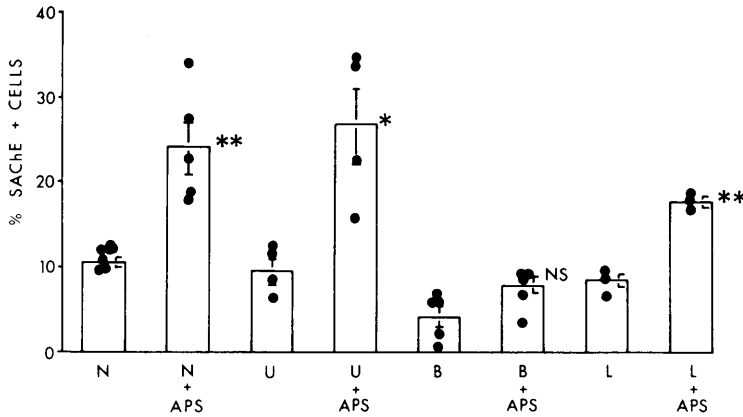


FIG. 2. The effects of the same treatments as indicated in legend to Fig. 1 on the percentage small acetylcholinesterase-positive (SACHe+) cells in marrow of rats. Each point (●) is the average percentage SACHe+ cells from six slides of about 1500 megakaryocytes from each rat. The bars represent the average for each treatment group and the vertical lines indicate the standard errors. NS, not significantly different from bilaterally nephrectomized rats; * $P < 0.025$; ** $P < 0.005$ indicate significant changes from suitable control rats that were not injected with APS.

these studies illustrate that the numbers of SACHe+ cells are controlled by the level and action of TSF.

Other studies have shown that hepatectomy also leads to thrombocytopenia and decreases megakaryocyte ploidy (15); the liver, therefore, has been considered to be a possible site of TSF production. However, liver cells in culture do not produce the TSF factor (16, 17). Further, hepatectomy may lead to severe nutritional problems that can affect hematopoiesis. Early work had indicated that the spleen was a possible location for TSF production (18), but splenectomized animals do not become thrombocytopenic. In contrast, the present study shows that the kidney is necessary for producing the factor(s) that regulates the proportion of the SACHe+ cells.

Although deGabriele and Penington (8) concluded that the kidney was not necessary for the production of TSF, other more recent data point to the kidney as a possible source of the hormone (1-4). In agreement with our present work, Krizsa (1) concluded that the kidney was necessary for the appearance of a "post-hemorrhagic thrombopoietic factor." TSF was not present in the serum of donor rats after nephrectomy but was present in the serum of rats after ureter ligation or with intact kidneys; furthermore, TSF could be produced in rats after other organs (such as the spleen,

adrenal, or pituitary glands) had been removed. In a follow-up study, Krizsa *et al.* (2) showed that increased TSF production was associated with increased incorporation of leucine in the kidneys of mice made thrombocytopenic by an injection of platelet specific antisera. These data provide further evidence of a role for the kidney in the production of TSF.

In previous work from our laboratory (4), the kidney was found to be necessary for the appearance in plasma of TSF following the production of acute thrombocytopenia. Extracts of plasma from normal rats, rats injected with rabbit anti-rat platelet serum, nephrectomized rats, and nephrectomized rats injected with antiserum were each tested in the immunothrombocytopenic mouse assay for the presence of TSF. Only an extract of plasma from unoperated rats made thrombocytopenic by an injection of antiserum gave positive results for TSF. The data of the present report confirm our previous studies and support the contention that, in rodents, the kidney is necessary for TSF production.

Klener *et al.* (3) showed that vinblastin treatment caused a significant increase in the serum TSF levels in animals. The TSF release was abolished by bilateral nephrectomy but not by bilateral ligation of the ureters. In agreement with our present results, these au-

thors believed that the kidney is a site of production of TSF in rodents.

From their work, deGabriele and Penington (8) concluded that the regulation of platelet production is not by the kidney but by a system entirely independent of that governing erythropoiesis. They compared the effects of platelet antiserum in nephrectomized rats with uninjected nephrectomized-control rats. The nephrectomized-control rats showed about the same increase in platelet counts during the maximal 4 days of survival as did the APS-treated rats; only one rat survived to Day 4. Also, only one of the rats injected with platelet specific antiserum following nephrectomy survived to Day 3; its platelet count was 393,000/mm³, which is about one-half the value of nephrectomized control rats and only about 75% of the normal rats injected with platelet specific antiserum for this same time period. If platelet counting is used to measure TSF production, then these rats do not live long enough after nephrectomy to determine the kidney's role in platelet production following thrombocytopenia. Our present study does not support the conclusions of deGabriele and Penington (8) since bilateral nephrectomy abolished the animal's ability to produce the TSF so necessary to an increase in SACHe+ cells (11).

Our anephric rats had fewer SACHe+ cells than did the normal rats. This finding supports the contention that a product from kidneys (TSF) is necessary for the day-to-day maintenance of platelet counts. Moreover, uremia did not appear to interfere with platelet production of the rats, since those with ligated ureters had normal platelet counts. These data are in agreement with the results of Larrain and Langdell (19) who reported that ureter ligation in dogs caused no change in platelet counts within a 3- to 6-day study.

In addition to work with rodents, Górski and co-workers (7) suggest that the kidney is a source of thrombopoietin in man. These workers reported a case of post-transplant thrombocytosis and, in a retrospective study, they found that 47% of 75 renal allograft recipients had had an episode of thrombocythemia. It is also interesting that platelet counts decrease shortly before an episode of acute renal graft rejection (20) and that

thrombocytopenia is frequently found during rejection. The thrombocytopenia has been postulated to result from a local deposition of platelets in the graft. However, it is possible that, during rejection, a decrease in renal thrombopoietin production may lead to thrombocytopenia. These studies in patients appear to agree with the results of the present work showing that the kidney has a role in the production of factors that control thrombopoiesis.

The hypothesis that the kidney is a site of thrombopoietin production received additional support when we (21) reported the detection of thrombopoietin in the medium of human embryonic kidney cells in culture. The factor has many similarities to TSF from thrombocytopenic animals (5); TSF from kidney cell culture medium stimulates thrombopoiesis and megakaryocytopoiesis in mice and has immunologic similarities and physical and chemical relationships to TSF from thrombocytopenic animals. Our data, therefore, support the findings of other studies that show the requirement of the kidney for TSF production.

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