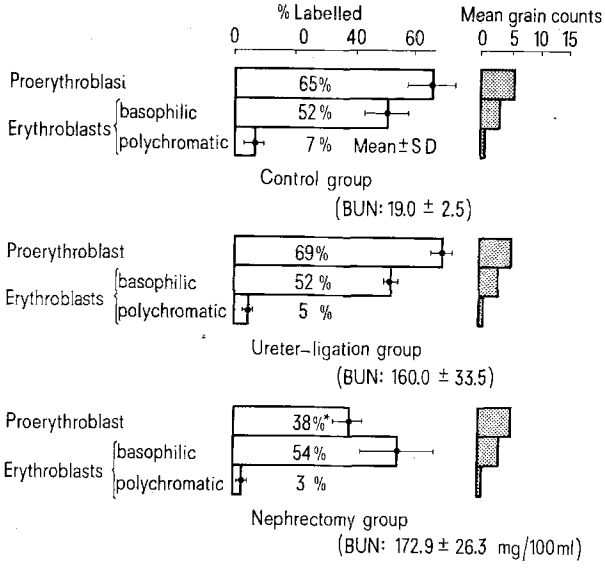
Erythropoiesis in Acute Azotemic Rats With and Without Kidney Tissue

The erythropoietic function in normal and acutely azotemic rats was studied with particular reference to the following questions: 1. what is the first disturbance of erythropoiesis in acute uremia ? and 2. is this disturbance attributable to the retention of nitrogenous waste (as measured by the serum urea concentration), or is it related to the absence of functioning renal tissue ?

Materials and methods. Male albino rats (Wistar-King A strain) were used throughout the experiments. 3 groups were established: in the first group both kidneys were removed in 1 stage procedure (under ether anesthesia, flank incisions), in the second group both ureters were ligated at the ureteropelvic junction through bilateral flank incisions, and the third group, control group was subjected sham operation. The animals were fed water and commercial chaw ad libitum.

For the measurement of heme synthetic activity, ^{59}Fe incorporation into heme fraction was determined. 20 h after surgery, the rats were injected with 2.0 μCi of ^{59}Fe i.p. and were killed 240 min later under ether anesthesia. Blood obtained by cardiac puncture at this time was used for ^{59}Fe red cell incorporation, reticulocyte count, hematocrit and serum urea nitrogen determination. The radioactivity of ^{59}Fe in heme fraction extracted from the femur marrow by the modified TEALE[1] method was measured by the well-type scintillation counter and the heme content was determined colorimetrically with Drabkin reagent.

For the measurement of DNA and RNA synthesis, 23 h after surgery ^{3}H-thymidine (40 μCi/100 g body weight) or ^{3}H-uridine (100 μCi/100 g body weight) was injected as a single i.m. dose in the thigh, and the rats were killed 60 min later. The nucleic acids were extracted by the modified SCHMIDT and THANNHAUSER[2] technique. The final nucleic acid hydrolyzate obtained by this method was volumetrically pipetted into metallic planchet and dried under infrared lamp. The tritium content of these samples was measured by low-background GM counter. Incorporation of ^{3}H-thymidine, or -uridine into DNA or RNA fraction was expressed as cpm per mg of DNA or RNA.



Effect of acute uremia on ^{3}H-thymidine uptake by the erythroid cell series. * Significant Difference from the control group ($p < 0.05$).

Autoradiography of the erythroid cells in bone marrow was carried out by dipping method with microradioautography emulsion (Sakura NR-M_2) on bone marrow smear from the femur after ethanol fixation and Giemsa staining.

Results. A moderately severe azotemia was present in all nephrectomized and bilateral ureter-ligated rats at 24 h with average serum urea nitrogen values ranging from 160 to 220 mg/100 ml.

There were no significant alterations in bone marrow uptake of ^{3}H-uridine, ^{59}Fe incorporation into heme fraction, or peripheral reticulocyte count and ^{59}Fe red cell incorporation between all experimental animal groups.

A supression of bone marrow uptake of ^{3}H-thymidine was noted in nephrectomized rats, but this was not statistically significant. However, autoradiographic study of the bone marrow showed a significant supression of ^{3}H-thymidine labelling index of the proerythroblast in nephrectomized rats. Other individual cell groups of erythroblasts revealed no significant changes between the control, ureter-ligated and nephrectomized animal groups (Figure).

Discussion and conclusion. These experiments show that azotemia present in the rat 24 h after bilateral nephrectomy is associated with a significant supression of ^{3}H-thymidine labelling in proerythroblasts of the bone marrow, whereas more mature erythroblasts show no significant supression. In the equally azotemic bilateral ureter ligated rats, the ^{3}H-thymidine labelling indices of erythroblasts were not affected. Thus nephrectomy and ureter ligation do not similarly affect the proliferation of each erythroid cell series. The supression of proliferative activity of the most immature erythroblast, proerythroblast, appears to be the first alteration observed after the removal of renal tissue.

The deficiency of erythropoietin seems to be of significant importance in alteration of erythroid cell series in this experiment, since it is known that the kidney is the prime site for the regulation of erythropoietin production[3], and erythropoietin, which has a half-life of 3 to 5 h in circulation[4–6], acts on a cell (erythropoietin responsive cell) to initiate differentiation into erythroblasts and then full maturation of the erythroblasts occurs without the need for additional erythropoietin[2].

These observations may also provide an explanation for the decrease of erythroblasts in acute renal failure, where the anemia lags in time behind all other chemical and clinical signs of uremia.

Zusammenfassung. Verglichen mit akut urämischen, nicht nephrektomierten Ratten wird festgestellt, das der Einbau von Thymidin in Proerythroblasten sich in akut urämischen, nephrektomierten Ratten signifikant vermindert.

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[1] W. J. TEALE, Biochim. biophys. Acta *35*, 543 (1959).
[2] W. C. SCHNEIDER, J. biol. Chem. *164*, 747 (1946).
[3] S. B. KRANTZ and L. O. JACOBSON, *Erythropoietin and the Regulation of Erythropoiesis* (University of Chicago Press, Chicago 1970).
[4] J. F. CONTRERA, J. F. CAMISCOLI, A. H. WEINTRAUB and A. S. GORDON, Blood *25*, 809 (1965).
[5] F. STOHLMAN JR., Ann. N. Y. Acad. Sci. *77*, 710 (1957).
[6] F. STOHLMAN JR. and G. BRECHER, Proc. Soc. exp. Biol. Med. *100*, 40 (1959).