Measurement of Erythroid Mitotic Activity in Bone Marrow by Use of Colchicine.* (18840)

WILSON C. GRANT. (Introduced by Walter S. Root.)

From the Department of Physiology, College of Physicians and Surgeons, Columbia University.

In order to establish the effectiveness of an erythropoietic stimulus, one conventionally measures the concentration or total volume of erythrocytes, the percentage of reticulocytes, or degree of bone marrow hyperplasia. The values obtained indicate the end products of erythropoietic stimulation but do not yield more than inferential information concerning the dynamic erythropoietic process. Since it is generally agreed that new red blood cells arise ultimately from mitotic division of the erythroid elements of the marrow, it is probable that early evidence of erythropoietic stimulation would be found in marrow mitotic activity. Unfortunately, very few mitotic figures are found even in stimulated bone marrow because of their transient nature. The mitotic arresting property of colchicine has been well known since 1934(1). The arrest of marrow cells, in addition to other active cells, has been described(2-5). In an attempt to measure empirically the functional (mitotic) state of erythroid cells, the marrow of normal and erythropoietically stimulated rats after treatment with colchicine was examined.

Methods. Adult, albino rats of both sexes weighing from 135 to 220 g were used in this study. Colchicine (S. B. Penick and Co.) in aqueous solution was injected subcutaneously in doses from 0.01 to 0.20 mg per 100 g of body weight. Injections were made between 9:00 and 11:00 a.m. to avoid any irregularities produced by the often suggested diurnal variations in mitotic activity. From 1 to 8 hours after the injections, animals were sacrificed with chloroform, partially exsanguinated and the femurs removed. Marrow from the proximal end of the femur was suspended in homologous serum, smeared on slides, fixed in Helley's Fluid and stained with Hemotoxylin-Eosin-Azur II(6).

Results. Many easily recognized mitotic figures were evident in the cells of both erythroid and granulocyte series of marrow treated at dosage levels greater than 0.01 mg per 100 g body weight and duration greater than 1 hour. Although ample evidence was available of intense mitotic activity in all members of the granulocyte series, only the erythroid cells were considered in this study. To quantitate the evidence of mitotic division, 1000 or more nucleated marrow cells of all types were counted and the number of erythroid cells in mitosis was expressed in terms of percentage. The great bulk of dividing erythroid cells were early normoblasts and late erythroblasts and were found in prophase. The basophilic or early erythroblasts blocked in metaphase represented a relatively small number.

Concentration and duration. To measure the influence of dosage level, a total of 32 rats were given from 0.01 to 0.20 mg per 100 g of body weight. Five hours elapsed in this group between the time of injection and sacrifice. It will be noted in Fig. 1 that 0.01 mg produced no change in the mitotic percentage from that of the water injected control. The maximal response was obtained with 0.10 and 0.15 mg and a decrease became apparent at 0.20 mg. The influence of the time between injection and sacrifice was examined and the results are presented in Fig. 2. A total of 55 rats were given 0.01, 0.05 and 0.10 mg per

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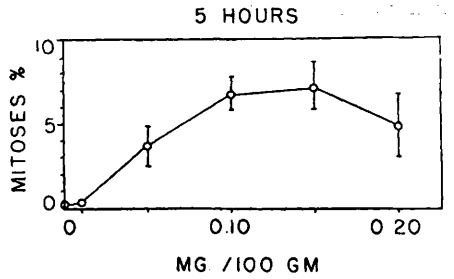

FIG. 1. Influence of increasing dosage of colchicine on erythroid mitotic percentage of rat marrow. Five hr elapsed between injection and marrow sampling.

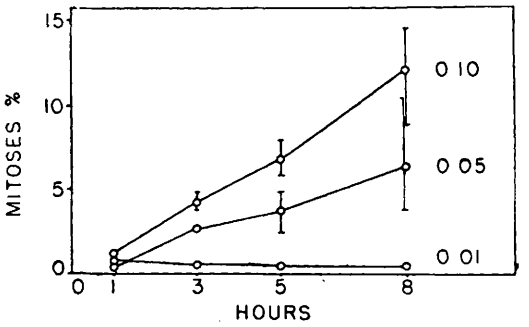

FIG. 2. Influence of increasing time between injection of colchicine and marrow sampling on erythroid mitotic percentage dosage levels expressed in mg per 100 g body wt.

100 g and allowed 1, 3, 5 and 8 hours duration. At the lowest dose, no elevation of the mitotic percentage occurred above that of the water injected control regardless of duration. At both 0.05 and 0.10 mg levels an increase in mitotic percentage occurred with duration to 8 hours. In a few additional measurements made at the 0.10 mg dosage level after 24 hours duration, the mitotic percentage was reduced to that of the water injected control. It may be argued that these changes in mitotic percentage of erythroid cells are a result of either an absolute or relative increase in *total* erythroid elements. To this end, the percentage of total nucleated marrow cells which were erythroid was determined in doses of 0 to 0.20 mg with 5 hours duration. No appreciable change in the erythroid percentage representation was evident in any instance.

Erythropoietic stimuli. If the arrest of erythroid mitoses as demonstrated, measures empirically physiological activity and not merely a pharmacological artifact, the application of a proven erythropoietic stimulus should provoke an increase in the mitotic percentage of marrow above that of the unstimulated rat. All animals were given the standard dose of 0.05 mg colchicine per 100 g body weight and 5 hours elapsed between injection and sacrifice. The erythroid mitotic percentage of 14 normal rats given the standard dose was 2.5 to 4.8%, averaging 3.6%. Fifteen rats were bled by cardiac puncture 2 cc/100 g body weight. One to 3 days after the hemorrhage, the standard dose of colchicine was administered and the mitotic percentage after 5 hours was found to range from 3.8 to 12.0% with an average of 6.9%. These rats had lowered hematocrit values as a result of the bleeding but had not at that time shown a marked reticulocyte response. Six rats were placed for 6 hours per day for 2 to 3 days (12 to 18 hours) in a low pressure chamber in which the pressure was maintained at 300 mm Hg (23,-500 feet). On the day following the last chamber exposure, colchicine was administered. The mitotic percentage ranged from 5.6 to 8.5% with an average of 7.0%. The mitotic percentage values of the bled rats and of those subjected to low barometric pressure were significantly different from those of normal rats as determined by Fisher's "t" test. These results are presented in Fig. 3.

Discussion. The increased erythroid mitotic percentages found in the marrows of rats after

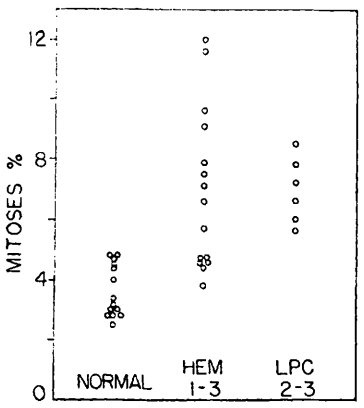

FIG. 3. Comparison of erythroid mitotic percentages of normal rats, rats 1 to 3 days after hemorrhage (HEM.) and those subjected to 300 mm Hg barometric pressure (LPC) intermittently 2 to 3 days (12 to 18 hr).

anoxic anoxia or hemorrhage indicate that physiological activity is being measured by the use of colchicine blockade. Increased numbers of mitoses were evident before a real increase in either the hematocrit or reticulocyte percentage was detected in the circulating blood.

The ability of the bone marrow to produce erythrocytes depends on the size and activity of the *functional* marrow volume. The total volume of erythroid marrow cells may be estimated by histological examination. Determination of the rate of mitotic division by the colchicine technic provides an empirical measure of the functional activity of the marrow.

The mechanism by which colchicine arrests mitotic division is unknown. It is not believed that the number of mitotic figures is increased by the alkaloid. Brues(7) found no evidence of stimulation of mitosis by colchicine in regenerating rat liver nor did Mellgren (8) in the developing adrenal gland.

The work reported involved the sacrifice of the rat in order to obtain adequate marrow samples. Preliminary observations indicate that in animals as large as the rabbit, marrow samples obtained by the needle puncture following colchicine administration give essentially the same results as those observed in the rat. Such a modification provides a technic capable of wider application.

Summary. 1. The influence of colchicine on arrest of erythroid mitoses of intact rat marrow was measured quantitatively. 2. A comparison was made after colchicine injection of the erythroid mitotic percentage of normal rats and of those of erythropoietically stimulated animals. Fourteen normal rats showed a range of 2.5 to 4.8%, average 3.6%. Fifteen rats, 1 to 3 days after moderate hemorrhage, gave 3.8 to 12.0%, average 6.9%. Six rats exposed for 6 hours per day for 2 to 3 days (12 to 18 hours) to a barometric pressure of 300 mm Hg yielded 5.6 to 8.5%, average 7.0%. Mitotic percentage increase above normal was evident in the stimulated animals before an established reticulocyte response was observed.

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Effect of Therapeutic Doses of Ephedrine on Renal Clearances in Normal Man.* (18841)

MORTON H. MAXWELL,† PABLO MORALES,‡ AND CHARLES H. CROWDER, JR.§
(Introduced by H. W. Smith.)

From the Department of Physiology, New York University College of Medicine, New York.

On the basis of unpublished observations of Chasis, Goldring, and Smith(1), Smith states that ephedrine has no or a negligible effect on the renal circulation; he reproduces only one experiment in which 35 mg of ephedrine injected intravenously caused a small and probably insignificant decrease in renal plasma flow (diodrast clearance) and filtration rate (inulin clearance), and concludes that the mode of action of adrenaline and

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† Present address, Department of Medicine, New York Hospital, Cornell University Medical School, New York.

‡ Fulbright Fellow from the Philippines.

§ Life Insurance Medical Research Fund Student Fellow.

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