Stimulation of Erythropoiesis in Plethoric Mice by Prostaglandins and Its Inhibition by Antierythropoietin[1] (35931)

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Prostaglandins, particularly of the E series, are potent vasodilators of most vascular beds, and some attention has been devoted to their possible role in the control of renal blood flow (1–3). Considerable evidence indicates that the primary regulation of plasma erythropoietin resides in the kidney (4). It appeared possible that prostaglandins might redistribute renal blood flow in plethoric mice, and thus stimulate erythropoiesis. The present studies were undertaken to determine whether erythropoiesis was stimulated by prostaglandins in the rodent, and whether the stimulation was erythropoietin dependent.

Materials and Methods. Female LAF_1/Jax mice weighing about 25 g were used. Mice were made plethoric by exposure to increasing amounts of carbon monoxide as described by Fogh (5). The mice were used 7 days after removal from the chamber when erythropoiesis was maximally suppressed.

Prostaglandins (PG) E_1, E_2, and $F_{2\alpha}$ were obtained from Dr. John E. Pike of the Upjohn Company. They were dissolved in 1 ml of ethyl alcohol and diluted with saline to 100 ml to give a final concentration of 100 μg/ml. Mice were injected sc with 1 μg/g of body weight. Some mice received the PGE_1, and PGE_2 on two consecutive days.

Antierythropoietin immune serum obtained from rabbits immunized with human urinary erythropoietin was injected iv in a volume of 0.1 ml immediately before each PGE_1 or PGE_2 injection. One milliliter of this particular immune serum can neutralize the biological activity of 25 IRP units of human erythropoietin or about 2.5 units of sheep or mouse erythropoietin. The method of preparation and properties of antierythropoietin have been summarized elsewhere (6). Control groups received similar injections of normal rabbit serum.

Groups of plethoric mice were exposed to a simulated altitude of 22,000 ft (321 Torr) for 6 hr; separate groups were injected with PGE_1, PGE_2, or $PGF_{2\alpha}$ immediately before the hypoxic exposure, and one group received saline.

Fifty-six hours after the first prostaglandin injection the plethoric mice were injected iv with 0.5 μCi of ^{59}Fe as the citrate. All mice were bled 72 hr after the ^{59}Fe injection, and the radioactivity in 0.5 ml of blood was measured. The results are expressed as the percentage of the injected ^{59}Fe in the calculated blood volume. The blood volume of these plethoric mice was assumed to be 7% of the body weight. The average hematocrit of all groups was 64.1 $\pm$ 1.1% at the end of the assay. Each experimental group contained 6–8 assay mice.

Results. The data shown in Table I indicate that a single sc injection of PGE_1 and PGE_2, at a dose of 1 μg/g of body weight, significantly stimulates erythropoiesis in plethoric mice, as measured by ^{59}Fe incorporation. $PGF_{2\alpha}$ at the same dosage did not stimuate erythropoiesis.

The data in Table I also show that prostaglandins of the E series significantly ($p<.001$) potentiate the erythropoietic response of plethoric mice exposed to a 6 hr hypoxic exposure, increasing the ^{59}Fe incorporation about twofold. Significant potentiation was not observed with $PGF_{2\alpha}$ ($p<.1$).

The data presented in Table II indicate that the erythropoietic response elicited by PGE_1 and PGE_2 injected sc on two consecu-

[1] This work was done under the auspices of the United States Atomic Energy Commission.

TABLE I. Erythropoietic Response of Plethoric LAF_1 Mice to Prostaglandins with or without a Brief Hypoxic Exposure.

	72 hr ^{59}Fe incorporation
Saline	0.60 ± 0.02[a]
PGE_1	2.77 ± 0.41
PGE_2	3.84 ± 0.57
$PGF_{2\alpha}$	0.73 ± 0.05
Hypoxia,[b] 6 hr	12.7 ± 2.4
+ PGE_1	23.6 ± 1.4
+ PGE_2	26.7 ± 0.77
+ $PGF_{2\alpha}$	19.5 ± 3.1

[a] Standard error of the mean.

[b] Simulated altitude of 22,000 ft (321 Torr). Prostaglandins given once sc, 1 μg/g of body weight.

tive days is completely abolished when antierythropoietin is injected before each PG injection.

Discussion. These experiments clearly indicate that prostaglandins of the E series significantly stimulate erythropoiesis in the plethoric mouse as measured by the 72 hr ^{59}Fe incorporation into red blood cells. It is equally clear that this stimulation is erythropoietin dependent, since it does not occur in antierythropoietin-injected plethoric mice.

It is tempting to speculate that, among the wide variety of physiological and pharmacological actions of prostaglandins (1–3), their role as vasodilators of vascular beds is involved in the observed erythropoietin-dependent stimulation of erythropoiesis. Lee (7) has shown that the renal blood flow of dogs is increased after PGE_1 and PGE_2, but not $PGF_{2\alpha}$. Possibly similar changes occur in the mice used in these experiments after PGE_1 and PGE_2 injection, causing a redistribution of renal blood flow with a decreased O_2 tension in some appropriate tissue, resulting ultimately in an increased erythropoietin production. The failure of $PGF_{2\alpha}$ to stimulate erythropoiesis may relate to its failure to alter renal blood flow. However, only further experiments can clarify the mechanism of prostaglandin stimulation of erythropoiesis.

The ubiquitous distribution of prostaglandins in almost all mammalian tissues considered, along with the results of the present experiments, suggests that some caution is required before concluding that the erythropoietic stimulation observed after injection of various tissue extracts into plethoric mice is the direct result of the presence of erythropoietin in the extract.

TABLE II. Erythropoietic Response of Plethoric LAF_1 Mice Receiving Prostaglandins and Antierythropoietin.

	72 hr ^{59}Fe incorporation
Saline	0.91 ± 0.10[a]
PGE_1 + normal rabbit serum	5.71 ± 0.36
PGE_1 + rabbit antierythropoietin	0.62 ± 0.06
PGE_2 + normal rabbit serum	6.80 ± 1.1
PGE_2 + rabbit antierythropoietin	0.66 ± 0.07

[a] Standard error of the mean. Prostaglandins given sc on two consecutive days 1 μg/g of body weight; 0.1 ml of immune sera after each PG injection.

Summary. Prostaglandins of the E series stimulated erythropoiesis in the plethoric mouse, whereas prostaglandin $F_{2\alpha}$ was inactive. The erythropoietic stimulation was erythropoietin dependent, since it did not occur in antierythropoietin-injected mice.

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Received June 3, 1971. P.S.E.B.M., 1971, Vol. 138.