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Increase in Hematocrit, Hemoglobin and Red Cell Mass in Normal Mice after Treatment with Cyclic AMP[1] (38543)

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A role for adenosine 3′,5′-monophosphate (cyclic AMP) in kidney erythropoietin (ESF) production has been suggested by many investigators (1–7). *In vivo* stimulatory effects of cyclic AMP or its derivatives on ^{59}Fe incorporation into red cells have been reported in polycythemic mice (1–6) and in plethoric mice (7). Studies with antisera to ESF have provided evidence that the erythropoietic effects of exogenous cyclic AMP are ESF dependent and probably are not due to stimulation of bone marrow erythropoiesis directly (6, 7). Finally, Peschle *et al.* (6) provided evidence for a renal cyclic AMP-mediated mechanism for ESF production with the finding that bilateral nephrectomy abolished the erythropoietic response to cyclic AMP. The ability of cyclic AMP to elevate plasma levels of ESF was also demonstrated in these experiments (6).

The present investigation is a further extension of our earlier studies (3, 5) to clarify the erythropoietic effects of cyclic AMP in which dibutyryl cyclic AMP was found to produce a significant increase in the hematocrit, hemoglobin concentration and red cell mass in normal mice.

Materials and Methods. Normal male CD-1 mice weighing 23–25 g were the experimental animals used in these studies. The mice were divided into three groups: (1) Normal mice; (2) dibutyryl cyclic AMP-treated mice; and (3) ESF-treated mice. Dibutyryl cyclic AMP was obtained from Sigma Chemical Company and was administered at a dosage of 8 mg, sc daily for a period of 14 days. ESF (human urinary ESF, 8 units/mg) was administered to the mice at a dosage of 0.6 units, sc daily for 14 days. After 2 wk of treatment, the mice were anesthetized with ether and exsanguinated via cardiac puncture. Microhematocrits, hemoglobin concentration and red cell mass of each animal were determined. Additionally, the final body weight of each mouse was recorded.

Red cell mass was determined with ^{51}chromium labelled red cells according to the method of Sterling and Gray (8). A separate group of ten donor mice was used to obtain red blood cells for labelling with ^{51}Cr (sodium chromate-200 mCi/mg, Amersham-Searle Corporation). The red cells from donor mice were incubated with 100 μCi of ^{51}Cr for one hour at 37°. Following this incubation, the labelled cells were washed twice with saline to remove excess chromium. The unlabelled donor mouse plasma was then mixed with the washed labelled red cells and 0.2 ml of this cell suspension was injected iv into the tail vein of each mouse. Thirty minutes later the animals were exsanguinated, a blood sample removed for radioactivity determination and the red cell mass of each mouse was calculated as previously described (8).

The remainder of the blood sample was used to determine the hematocrit (duplicate microhematocrits) and the hemoglobin concentration (cyanmethemoglobin method) on each test animal. The data are expressed as the mean ± standard error and the statistical significance of our experimental data was analyzed with the use of Dunnett's test for comparing several treatments with a single control (9).

Results. A summary of the effects of dibutyryl cyclic AMP and ESF on the hematocrit, hemoglobin concentration and red cell mass in normal mice is presented in Table I. Each value represents the mean ± standard error of five mice per test group. As depicted in Table I, the final body weight of all mice ranged between 28 and 29 g. There was no significant difference between the body weights of the control group and either of the

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TABLE I. EFFECTS OF DIBUTYRYL CYCLIC AMP AND ERYTHROPOIETIN (ESF) ON THE HEMATOCRIT, HEMOGLOBIN CONCENTRATION AND RED CELL MASS IN NORMAL MICE.[a]

Group	Final body weight (g)	Hematocrit (%)	Hemoglobin (g/100 ml)	Red cell mass (ml/100 g)
Control	29.0 ± 1.05	43.25 ± 0.85	12.59 ± 0.45	2.93 ± 0.20
Dibutyryl cyclic AMP (8 mg, sc daily)	28.4 ± 0.24	46.50 ± 0.64*	14.05 ± 0.26*	4.17 ± 0.08*
Erythropoietin[b] (0.6 units, sc daily)	29.0 ± 1.30	51.40 ± 1.54*	14.70 ± 0.35*	4.57 ± 0.39*

[a] The data are expressed as the mean ± standard error of five animals per group. Asterisks denote experimental values which are significantly ($P < 0.05$) different from that of the controls. The mean initial body weights of all mice were 23–25 g. Experimental groups were treated for two weeks with either dibutyryl cyclic AMP or ESF.

[b] Human urinary erythropoietin.

experimental groups (dibutyryl cyclic AMP and ESF), indicating that the differences in hematologic parameters were not due to variations in the normal blood volumes of the experimental groups.

The mean control hematocrit was 43.25 ± 0.85%. Treatment with dibutyryl cyclic AMP for two weeks produced a significant ($P < .05$) increase in the hematocrit up to 46.50 ± 0.64%, while the administration of ESF increased the hematocrit to 51.40 ± 1.54%. A parallel increase in hemoglobin concentrations was observed with the two treatments. The mean control hemoglobin concentration was 12.59 ± 0.45 g/100 ml. Treatment with dibutyryl cyclic AMP or ESF significantly ($P < .05$) increased hemoglobin concentrations to a mean of 14.05 ± 0.26 and 14.70 ± 0.35 g/100 ml, respectively.

The changes in red cell mass are also shown in Table I. The normalized red cell mass (ml/100 g) is depicted. The data indicate that dibutyryl cyclic AMP significantly ($P < 0.05$) elevated the red cell mass 42.3% over that of the controls, and ESF treatment produced a significant increase in this parameter to over 56% of that of the controls.

Discussion. Several investigators (1–7) have provided evidence that cyclic AMP stimulates erythropoiesis. This effect is a consequence of an increase in kidney ESF production. The purpose of the present investigation was to determine if the erythropoietic effects of cyclic AMP such as enhanced ^{59}Fe incorporation are also associated with other hematological changes commonly seen following a prolonged increase in plasma erythropoietin levels, such as elevations in the hematocrit, hemoglobin concentration and red cell mass (10).

The normal hematological values for the control mice in our studies (Table I) are in excellent agreement with previously reported data for this species (11). Also shown in Table I is the enhanced erythropoietic response of normal mice to dibutyryl cyclic AMP treatment. The hematocrit and hemoglobin concentrations of the dibutyryl cyclic AMP-treated group were significantly increased to approximately 10% above the control group, while the red cell mass was elevated to 42.3% above the control. The daily dosage of dibutyryl cyclic AMP used in these experiments (8 mg, sc) was equivalent to 0.6–0.8 unit ESF as determined by a previous dose-response curve to dibutyryl cyclic AMP in the polycythemic mouse bioassay (5). The animals injected with dibutyryl cyclic AMP tolerated well the chronic treatment with this cyclic nucleotide, and their terminal body weights were not significantly different from those of the control group (Table I).

The erythropoietic response of the mice injected with 0.6 unit ESF for 2 wk was also enhanced when compared to that of control

mice. ESF-treated animals exhibited hematocrits and hemoglobin concentrations which were increased approximately 20% above the controls, while the red cell mass was elevated to 56% above the controls. These data are very similar to the findings which have been reported by other investigators who studied the effects of chronic ESF treatment in rats (12, 13). In the present study as well as previous investigations (12, 13), the percentage increase in red cell mass of ESF-treated animals was greater than the percentage increase in hematocrit and hemoglobin concentration of this group. Similar findings were obtained in the dibutyryl cyclic AMP mice (Table I). The red cell mass is probably a better indicator of increased erythropoiesis, since it is not influenced by alterations in plasma volume as are the hematocrit and hemoglobin concentration (14).

The hematologic changes presented in the present experiments in mice correlate well with earlier reports in human subjects following treatment with ESF (15, 16). The data in Table I indicating a positive erythropoietic effect of dibutyryl cyclic AMP suggests that agents which stimulate an increase in the production of renal cyclic AMP may be of therapeutic use in certain anemias. Cobalt has certainly been used clinically with some success to increase ESF production (17). A cyclic AMP-link in cobalt-mediated ESF production has already been described (5, 18). Other agents which stimulate the production of renal cyclic AMP may also prove beneficial in increasing kidney ESF production.

Summary. Chronic treatment of normal mice with either dibutyryl cyclic AMP or erythropoietin produced elevations in the hematocrit, hemoglobin concentration and red cell mass when compared to these same hematological parameters in untreated mice. Dibutyryl cyclic AMP increased red cell mass by 46% while ESF treatment resulted in a 56% increase in red cell mass. These studies confirm earlier reports of the effects of cyclic AMP in increasing radioactive iron incorporation into red cells and further indicate that this change is associated with an absolute increase in red cell mass. Agents which increase renal cyclic AMP concentrations probably stimulate erythropoiesis as a consequence of increased kidney production of erythropoietin.

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