Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia

KAI-UWE ECKARDT, URS BOUTELLIER, ARMIN KURTZ,
MICHAEL SCHOPEN, ERWIN A. KOLLER, AND CHRISTIAN BAUER
Department of Physiology, University of Zürich, CH-8057 Zurich, Switzerland

ECKARDT, KAI-UWE, URS BOUTELLIER, ARMIN KURTZ, MICHAEL SCHOPEN, ERWIN A. KOLLER, AND CHRISTIAN BAUER. *Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia.* J. Appl. Physiol. 66(4): 1785–1788, 1989.— This study was carried out to investigate the early changes in erythropoietin (EPO) formation in humans in response to hypoxia. Six volunteers were exposed to simulated altitudes of 3,000 and 4,000 m in a decompression chamber for 5.5 h. EPO was measured by radioimmunoassay in serum samples withdrawn every 30 min during altitude exposure and also in two subjects after termination of hypoxia (4,000 m). EPO levels during hypoxia were significantly elevated after 114 and 84 min (3,000 and 4,000 m), rising thereafter continuously for the period investigated. Mean values increased from 16.0 to 22.5 mU/ml (3,000 m) and from 16.7 to 28.0 mU/ml (4,000 m). This rise in EPO levels corresponds to 1.8-fold (3,000 m) and 3.0-fold (4,000 m) increases in the calculated production rate of the hormone. After termination of hypoxia, EPO levels continued to rise for ~1.5 h and after 3 h declined exponentially with an average half-life time of 5.2 h.

simulated altitude; half-life time; radioimmunoassay

THE ADJUSTMENT OF THE erythrocyte mass to the availability of O_2 in the mammalian organism is brought about by renal secretion of erythropoietin (EPO). However, the regulation of renal EPO production is not completely understood. Present knowledge is mainly based on experiments in laboratory animals. Direct data related to the regulation of EPO production in humans are confined to two observations. First, it has been shown that in anemias serum EPO levels are inversely correlated with the hematocrit (5, 7, 9), and, second, it was demonstrated that a sojourn at high altitude increases EPO concentrations in blood and urine (1, 3, 8, 12–14, 17). However, when subjects travel to high altitude (1, 3, 8, 12, 14), the interval required for ascent does not allow the precise study of early increase in EPO formation, because the onset of hypoxia is gradual and not clearly defined. During mountaineering expeditions (1, 12) additional factors, e.g., physical exercise, may also influence EPO formation in an unpredictable manner. Furthermore, with the exception of one study (12), in previous investigations in humans only bioassays for EPO that indicate a change in the serum EPO level at least threefold above the normal value were available.

The present study was therefore carried out to show a more definitive association between hypoxia and EPO formation in humans. We measured serum EPO levels with a sensitive radioimmunoassay in healthy volunteers exposed to simulated altitudes of 3,000 (9,840 ft) and 4,000 m (13,120 ft) under controlled conditions in a decompression chamber. The changes in serum EPO levels after acute onset and after termination of hypoxia, as well as the relationship between alveolar partial pressure of O_2 and EPO formation were determined.

SUBJECTS AND METHODS

Subjects. Six male volunteers (24–39 yr) participated in the study after being informed about the aim of the investigation and the experimental protocol. All participants were healthy and free from any hematologic, respiratory, or renal disorder.

Protocol. Two or three subjects were exposed at one time to simulated altitude in a decompression chamber for 5.5 h. Each subject was given one exposure to an altitude of 3,000 m (9,840 ft) and one exposure to an altitude of 4,000 m (13,120 ft). The period between altitude exposures of the same individual was at least 24 days. Atmospheric pressure was reduced from 720 Torr (base-line value, 430 m above sea level) to 520 and 460 Torr (3,000 and 4,000 m above sea level) within 30 min. The ascent was interrupted for 15 min at 600 Torr (2,000 m above sea level). During the sojourn in the hypobaric chamber subjects remained seated throughout the whole period of investigation. From each subject, 2 ml of venous blood were withdrawn via an indwelling cannula in the cubital vein 1 h before, every 30 min during, and immediately after the hypoxic exposure. Before each blood sampling alveolar O_2 and CO_2 tensions were determined and blood pressure and heart rate measured. In two subjects, blood samples were taken for an additional 6 h after the completion of the hypoxic exposure to 4,000 m.

Measurement of alveolar O_2 and CO_2 tensions. O_2 and CO_2 tensions (P_{O_2} and P_{CO_2}) were measured by mass spectrometry in the end-expiratory respiratory gas by means of an on-line computerized analysis system (2).

Measurement of EPO. EPO was estimated in serum of the blood samples by radioimmunoassay. The assay is based on a rabbit antiserum against pure recombinant human EPO and ^{125}I-labeled recombinant human EPO, used as tracer. Assay procedure was exactly as described (6), with the exception that the antiserum was further diluted (1:100,000 instead of 1:60,000) to increase sensitivity of the assay. In brief, 100 μl of antiserum diluted

in phosphate-buffered saline (PBS) and 20 μl of 30% bovine serum albumin in PBS were incubated with 100 μl of standard solutions (Second International Reference Preparation for EPO, World Health Organization) or 100 μl of serum samples for 24 h (4°C). Diluted ^{125}I-labeled EPO (100 μl, 8×10^{-11} mol/l) (Amersham International, Amersham, UK) was then added, and samples were incubated for another 24 h (4°C). Separation of bound vs. free ligand was carried out by means of a second antibody technique: 100 μl of goat anti-rabbit γ-globulin (Calbiochem Biochemistry AG, Lucerne, Switzerland) and 100 μl of rabbit γ-globulin (0.03 mg) were added for 4 h; samples were then centrifuged, the supernatant was aspirated, and the pellet was counted for ^{125}I radioactivity. Data were expressed as percent binding in the absence of unlabeled EPO (~35% of the radioactivity added), and calculations of unknowns were performed on the basis of a standard log dose-response curve derived by the spline function method (LKB Instruments Wallac Compu Gamma RIA program 1282–114). Samples were analyzed in duplicate, and all samples from individuals exposed simultaneously to hypoxia were analyzed in sequence with the use of the same assay to minimize the effect of interassay variation. The interassay coefficient of variation was 6.7% [(51 estimations of a sample containing 44.2 ± 3.0 (SD) mU EPO/ml)].

Estimation of production rates of EPO. Relative increases of the production rate of EPO were calculated from serum EPO values according to the equation $PR_t \div PR_0 = (\Delta EPO_t + \ln 2 \div t_{½} \times EPO_t) \div (\ln 2 \div t_{½} \times EPO_0)$ where EPO_0 and EPO_t are the basal serum level and the serum level at the end of hypoxic exposure and PR_0 and PR_t are the respective production rates of the hormone. The half-life time ($t_{½}$) of EPO was assumed to be 5.2 h, as estimated from the disappearance rate of EPO after termination of hypoxia.

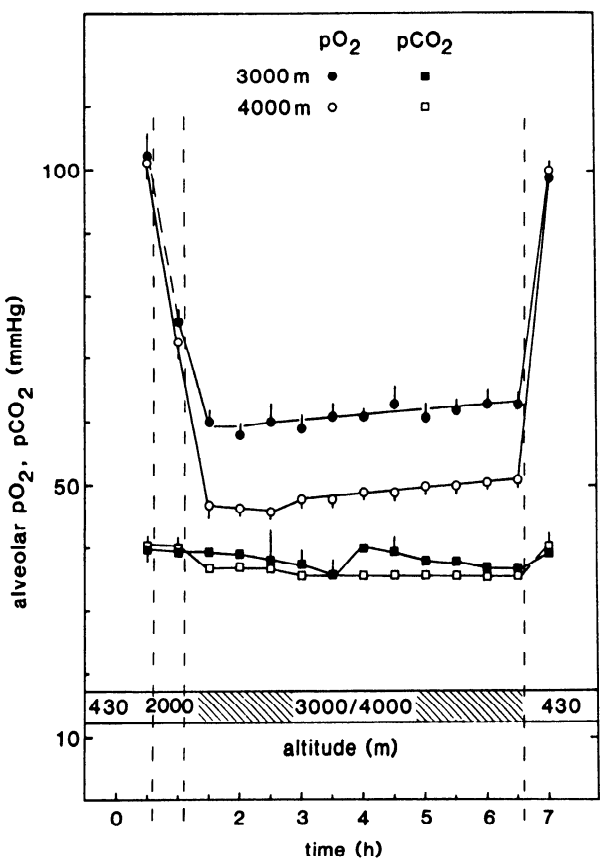

FIG. 1. Time course of alveolar P_{O_2} and alveolar P_{CO_2} (means ± SE, $n = 6$) under exposure to hypobaric hypoxia.

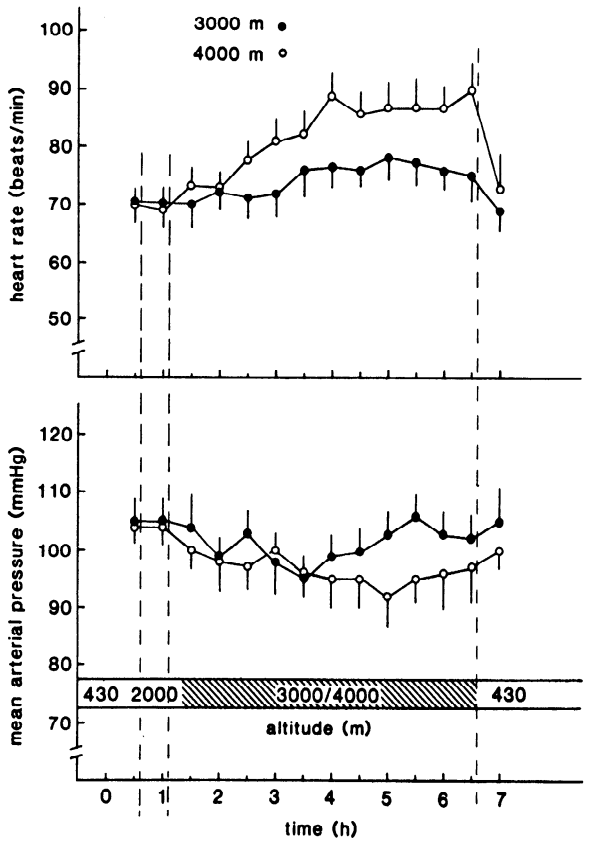

FIG. 2. Changes in heart rate and mean arterial blood pressure [(systolic pressure + 2 × diastolic pressure) ÷ 3] under exposure to hypobaric hypoxia (means ± SE, $n = 6$).

Statistics. Levels of significance were calculated using Student's paired t test. $P < 0.05$ was considered statistically significant.

RESULTS

Figure 1 shows the time course of the mean alveolar P_{O_2} ($P_{A_{O_2}}$) and alveolar P_{CO_2} ($P_{A_{CO_2}}$) tensions for the six subjects during exposure to simulated altitudes of 3,000 and 4,000 m. The mean $P_{A_{O_2}}$ after arrival at final altitude was 60.9 Torr at 3,000 m and 48.6 Torr at 4,000 m. The mean $P_{A_{CO_2}}$ was 38.0 Torr at 3,000 m and 33.6 Torr at 4,000 m ($P < 0.001$ vs. base-line values).

Heart rate and mean arterial blood pressure [(systolic pressure + 2 × diastolic pressure) ÷ 3] during hypoxic exposure are illustrated in Fig. 2. At both altitudes mean arterial blood pressure declined after onset of hypoxia. However, at the end of hypoxic exposure it had returned to values not significantly different from base line ($P > 0.05$). Heart rate increased at both altitudes with increasing hypoxia and remained elevated during the sojourn [mean heart rate between 3 and 5.5 h after arrival at final altitude 6.2 (3,000 m) and 17.8 beats/min (4,000 m)

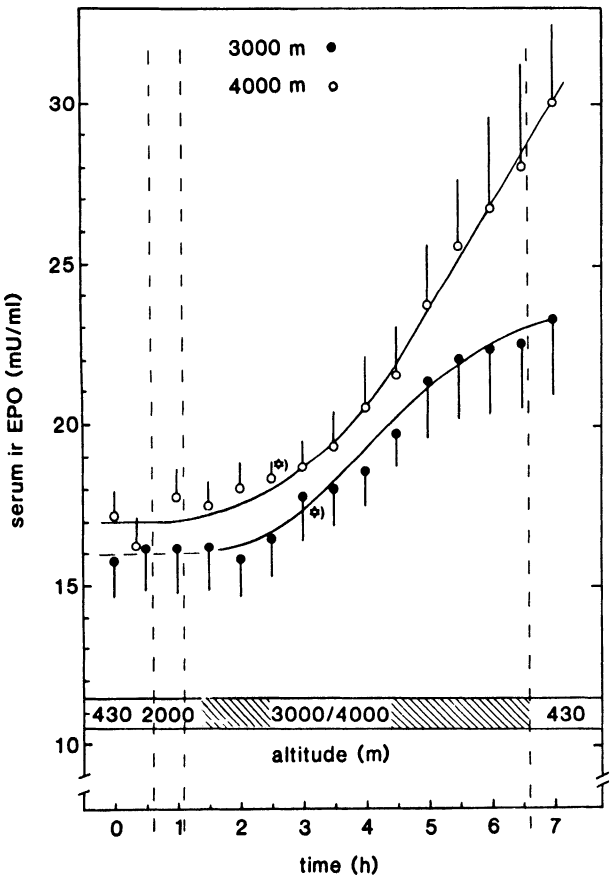

FIG. 3. Time course of serum erythropoietin (EPO) levels as measured by radioimmunoassay in response to hypobaric hypoxia (means ± SE, $n = 6$). * $P < 0.05$.

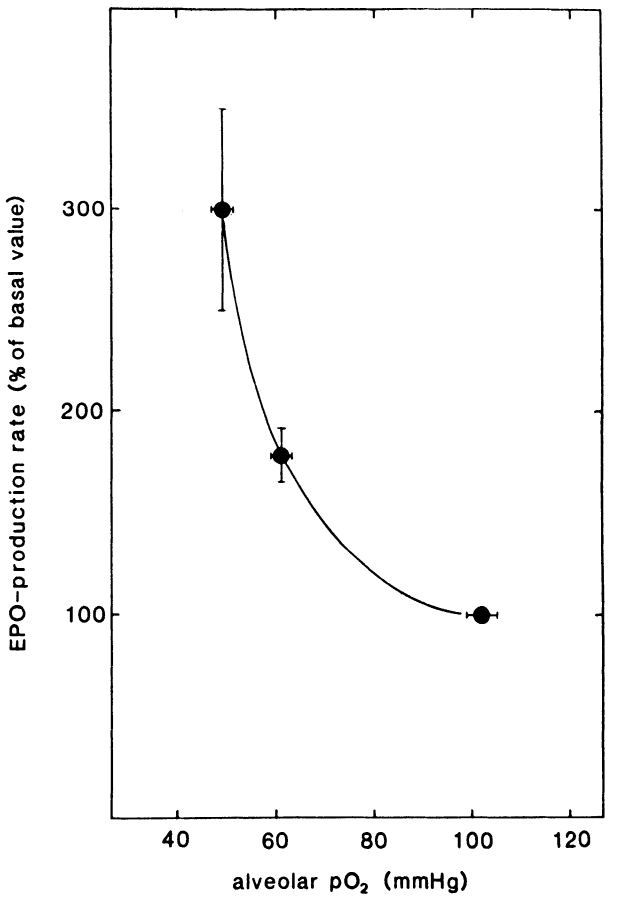

FIG. 5. Relationship between alveolar Po_2 and relative changes in the production rate of erythropoietin (EPO) (means ± SE, $n = 6$). Production rates were calculated from increases in serum EPO values, assuming a mean half-life time for EPO of 5.2 h.

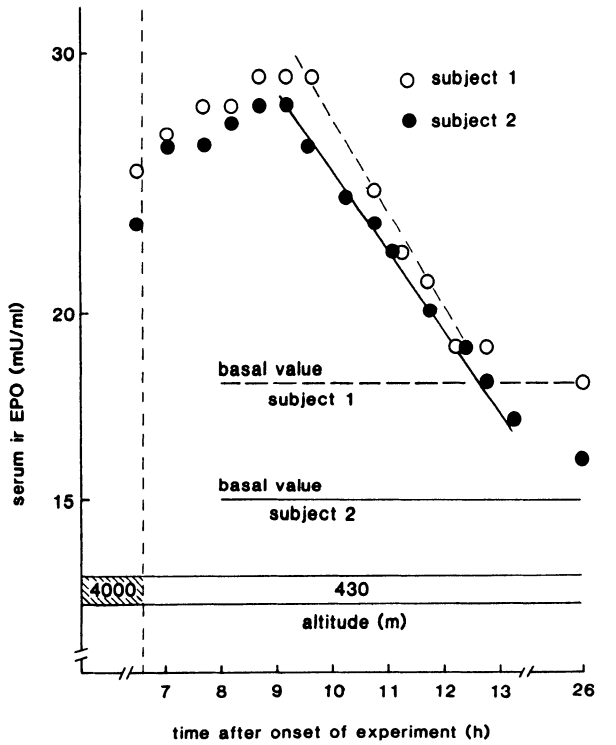

FIG. 4. Serum erythropoietin (EPO) values (log scale) after termination of hypobaric hypoxia (4,000 m) in 2 subjects.

above base-line value; $P < 0.05$ and $P < 0.01$, respectively].

The time course of serum EPO levels in response to the two different hypoxic stimuli is depicted in Fig. 3. The first significant increase above the mean of the two base-line values was found at 3,000 m after 114 min ($P < 0.05$) and at 4,000 m after 84 min ($P < 0.05$). Thereafter, EPO levels increased continuously for the period investigated. At 4,000 m this increase was linear to the end of the hypoxic exposure, whereas at 3,000 m, the slope of the serum values tended to flatten after ~4 h.

After the termination of hypoxia (4,000 m) EPO levels were estimated for up to 6 h in two of the subjects. The values are shown in Fig. 4. EPO levels continued to rise for ~1.5 h after normoxia was restored and started to decline after ~3 h. In both subjects EPO values fitted single exponential regression curves during the declining phase ($\log y = -0.064x + 2.079$, $r = -0.98$ for *subject 1* and $\log y = 0.054x + 1.941$, $r = -1.00$ for *subject 2*). Half-life times of disappearance resulting from these slopes were 4.7 h in *subject 1* and 5.6 h in *subject 2*.

DISCUSSION

The present study was carried out to investigate EPO formation in humans in the early phase of hypoxic exposure. Elevated EPO concentrations have been described in urine (3, 8) and blood (1, 12–14, 17) within 24

h after the onset of hypoxia. The earliest increases were reported by Siri et al. (17) and Miller et al. (13), who, after exposing human volunteers to simulated altitudes of 4,500 or 5,000 m, detected EPO in serum after 12 h by means of a bioassay. With a sensitive radioimmunoassay we were able to identify the first significant increase of serum EPO levels after ~1.5 h and a subsequent linear rise in EPO concentrations (Fig. 3). This time course of EPO values in humans is in accord with previous findings in rodents (4, 15, 16). In particular, the time lag between onset of hypoxia and elevation of serum EPO values is very similar in humans and rodents. This interval suggests that no stores of EPO are available for rapid release into the circulation. In rats, this has been supported by the finding that significant amounts of EPO were only extractable from kidney tissue during hypoxia and not in normoxic animals (10, 11). On the basis of the similar temporal relationships it may be inferred that information obtainable only in animals during the delay period also applies to humans. So far mRNA for EPO has been demonstrated in rat kidneys after 1 h of hypoxia (16), and it has also been demonstrated in rats that EPO levels in kidney tissue precede the rise in serum levels for ~30 min (4, 16).

The time required for transducing the stimulating signal for EPO production into a rise of serum EPO values is further reflected by the ongoing increase in EPO levels after termination of hypoxia (Fig. 4). From the slope of the subsequent decline in EPO values, a half-life time of disappearance of ~5.2 h was estimated from the observations in two subjects. This period is longer than that previously reported in mice (3.25 h) (1).

Assuming an EPO half-life time of 5.2 h for all subjects allows an estimation of the individual increase of renal production rates of EPO from the increase of serum EPO values. This quantity more directly reflects the extent of stimulation of EPO formation than serum values alone. At 3,000 m, renal production rates for EPO increased 1.8-fold and at 4,000 m they increased 3.0-fold. The relationship between the production rate of the hormone and $P_{A_{O_2}}$ as an indicator for the O_2 supply of the body is depicted in Fig. 5. It appears from this relationship that the regulation of EPO formation is rather conservative when O_2 supply is only slightly reduced and, on the other hand, EPO production is strikingly enhanced when O_2 tensions, potentially harmful for vital functions of the organism are approached.

We thank R. Della Bruna, H. Marti, and H. Niederberger for participating in the investigation, and we thank W. Gehret for the art work.

This study was supported by the Swiss National Science Foundation Grant 3.023-084 and the Hartmann Müller Stiftung für Medizinische Forschung.

K.-U. Eckardt acknowledges a fellowship from the German Research Foundation.

Part of the results were presented in abstract form at the Annual Meeting of the Swiss Society of Haematology, Basel, May 1988 (*Schweiz. Med. Wochenschr. Suppl.* 23: 66, 1988).

Address for reprint requests: K.-U. Eckardt, Physiologisches Institut, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

Received 29 June 1988; accepted in final form 2 December 1988.

REFERENCES

1. ABBRECHT, P. H., AND J. K. LITTELL. Plasma erythropoietin in men and mice during acclimatization to different altitudes. *J. Appl. Physiol.* 32: 54–58, 1972.
2. BOUTELLIER, U., T. KÜNDIG, U. GOMEZ, P. PIETSCH, AND E. A. KOLLER. Respiratory phase detection and delay determination for breath-by-breath analysis. *J. Appl. Physiol.* 62: 837–843, 1987.
3. CARMENA, A. O., N. GARCIA DE TESTA, AND F. L. FRIAS. Urinary erythropoietin in men subjected to acute hypoxia. *Proc. Soc. Exp. Biol. Med.* 125: 441–443, 1967.
4. CLEMONS, G. K., D. DEMANNICOR, S. L. FITZSIMMONS, AND J. F. GARCIA. Immunoreactive erythropoietin studies in hypoxic rats and the role of the salivary glands. *Exp. Hematol. NY* 15: 18–23, 1987.
5. COTES, P. M. Immunoreactive erythropoietin in serum. I. Evidence for the validity of the assay method and the physiological relevance of estimates. *Br. J. Haematol.* 50: 427–438, 1982.
6. ECKARDT, K. -U., A. KURTZ, P. HIRTH, P. SCIGALLA, L. WIECZOREK, AND C. BAUER. Evaluation of the stability of human erythropoietin in samples for radioimmunoassay. *Klin. Wochenschr.* 66: 241–245, 1988.
7. ERSLEV, A. J., J. WILSON, AND J. CARO. Erythropoietin titers in anemic, nonuremic patients. *J. Lab. Clin. Med.* 109: 429–433, 1987.
8. FAURA, J., J. RAMOS, C. REYNAFARJE, E. ENGLISH, P. FINNE, AND C. A. FINCH. Effect of altitude on erythropoiesis. *Blood* 33: 668–676, 1969.
9. GARCIA, J. F., S. N. EBBE, L. HOLLANDER, H. O. CUTTING, M. E. MILLER, AND E. P. CRONKITE. Radioimmunoassay of erythropoietin: circulating levels in normal and polycythemic human beings. *J. Lab. Clin. Med.* 99: 624–635, 1982.
10. JELKMANN, W., AND C. BAUER. Demonstration of high levels of erythropoietin in rat kidneys following hypoxic hypoxia. *Pfluegers Arch.* 392: 34–39, 1981.
11. KATSUOKA, Y., B. BECKMAN, W. J. GEORGE, AND J. W. FISHER. Increased levels of erythropoietin in kidney extracts of rats treated with cobalt and hypoxia. *Am. J. Physiol.* 244 (*Renal Fluid Electrolyte Physiol.* 13): F129–F133, 1983.
12. MILLEDGE, J. S., AND P. M. COTES. Serum erythropoietin in humans at high altitude and its relation to plasma renin. *J. Appl. Physiol.* 59: 360–364, 1985.
13. MILLER, M. E., M. RØRTH, H. H. PARVING, D. HOWARD, I. REDDINGTON, C. C. R. VALERI, AND F. STOHLMAN. pH effect on erythropoietin response to hypoxia. *N. Engl. J. Med.* 288: 706–710, 1973.
14. REYNARFARJE, C., J. RAMOS, J. FAURA, AND D. VILLAVICENCIO. Humoral control of erythropoietic activity in man during and after altitude exposure. *Proc. Soc. Exp. Biol. Med.* 116: 649–650, 1964.
15. SCHOOLEY, J. C., AND L. J. MAHLMANN. Evidence for the de novo synthesis of erythropoietin in hypoxic rats. *Blood* 40: 662–670, 1972.
16. SCHUSTER, S. J., J. H. WILSON, A. J. ERSLEV, AND J. CARO. Physiologic regulation and tissue localization of renal erythropoietin messenger RNA. *Blood* 70: 316–381, 1987.
17. SIRI, W. E., D. C. VAN DYKE, H. S. WINCHELL, M. POLLYCOVE, H. G. PARKER, AND A. S. CLEVELAND. Early erythropoietin, blood, and physiological responses to severe hypoxia in man. *J. Appl. Physiol.* 21: 73–80, 1966.