Effect of Hypoxia on Plasma Erythropoietin in the Rabbit.* (26383)

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It has been shown previously in the rat(1) that plasma erythropoietin (EPF) rises significantly in a short period (4-8 hours) in response to a hypoxic stimulus. This rise in EPF reaches a maximum at 24 hours and returns to normal at 48 hours even though the animal remains in a hypoxic atmosphere. Others have demonstrated the same general time response pattern(2). The present study was carried out to determine whether rabbits would respond similarly to a hypoxic stimulus.

Materials and methods. New Zealand white rabbits were kept at 10 vol % O_2 concentration by methods previously described (1). At various intervals, rabbits were removed from the chamber and immediately exsanguinated by bleeding *via* heart puncture. The heparinized plasma was prepared for assay by 2 methods; the heat deproteinization procedure of Borsook, *et al.*(3) and step 1 procedure of White, *et al.*(4). In both methods, the final material was concentrated 3 times when compared to the original volume of plasma. The plasma extracts were assayed in starved rats using the method of Fried *et al.*(5) and Mirand *et al.*(6).

Results. The results are summarized in Table I. Significant elevation of plasma EPF levels was evident at 8 hours. Peak levels were present at 24 and 48 hours. By 72 hours the level of EPF had returned to normal levels and remained there up to 120 hours. The "step 1" plasma extract was slightly more active than the heat deproteinized extract.

Discussion. These results demonstrate that the rabbit and the rat respond similarly to a hypoxic stimulus. The only demonstrable difference is that erythropoietin levels may remain elevated slightly longer in the rabbit than the rat(7).

Current interest in the chemistry of erythropoietin makes a quick, simple source of active material desirable. The above data point to plasma of the 24 hour hypoxic rabbit as such a source. Of real importance also is the question whether hypoxic EPF differs from post-hemorrhagic or anemic EPF. Previous studies in our laboratory(7,8,9) and elsewhere(10) have shown that EPF can be elicited in the plasma of hypoxic, nephrectomized rats. However, no assayable quantities of EPF appear in bled, nephrectomized rats(8,11,12). This suggests different sources of hypoxic and post-hemorrhagic

TABLE I. Erythropoietin Titer, Determined by 24 Hour Fe^{59} Uptake, of Starved Rats That Received Plasma from Rabbits Exposed to 10% O_2 for 0 to 120 Hours.

Plasma from hypoxic rabbits at hour	No. of rabbits	White step 1 plasma extract: 24 hr Fe^{59} uptake, %	White step 1 plasma extract: Hematocrit, %	Modified Borsook plasma extract: 24 hr Fe^{59} uptake, %	Modified Borsook plasma extract: Hematocrit, %
0	8	5.4 ± 1.1*(12)†	51.9	5.2 ± .6 (5)	51.3
4	8	5.4 ± 1.9 (12)	48.4	3.4 ± .9 (5)	48.2
8	8	13.5 ± 2.0 (12)	51.6	5.7 ± 1.2 (5)	50.2
24	8	19.4 ± 3.1 (11)	53.9	13.2 ± 1.6 (5)	50.8
48	8	18.2 ± 3.2 (12)	53.0	12.9 ± 2.3 (5)	51.4
72	4	4.6 ± 1.5 (5)	46.8	4.6 ± 1.5 (5)	47.3
96	4	4.7 ± .9 (5)	51.2	4.6 ± 1.7 (5)	51.0
120	4	8.6 ± 1.8 (5)	51.4	4.6 ± 1.8 (5)	53.0

Data represents the avg of several assays, all of which showed the same pattern of response.
* Stand. dev. † No. of normal starved rats used in assay for erythropoietin.

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EPF. Do they also differ chemically?

These data also emphasize another problem: namely the differing time response of hypoxic EPF and anemic EPF. The former shows a quick rise and a quick reversion to normal. The latter goes up quickly after a significant anemia is established and remains up for long periods of time. We have recently assayed plasma from severely iron-deficient rats that have been anemic for 3 months(13). High values of EPF were found without exception. Does this differing time response likewise imply a different source and/or a different identity of hypoxic and anemic EPF? Detailed studies of the chemical properties of EPF produced by these various stimuli are in progress in our laboratory.

Summary. Rabbits have been placed in a low O_2 atmosphere for varying periods of time and plasma erythropoietin content determined after set intervals of hypoxia. EPF was found to rise significantly at 8 hours, reach peak levels at 24 and 48 hours, and return to normal at 72 hours.

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1. Prentice, T. C., Mirand, E. A., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 231.
2. Stohlman, F., Jr., Brecher, G., *J. Lab. and Clin. Med.*, 1957, v49, 890.
3. Borsook, H., Graybiel, A., Keighley, G., Windsor, E., *Blood*, 1954, v9, 734.
4. White, W. F., Gurney, C. W., Goldwasser, E., Jacobson, L. O., Proc. of Laurentian Hormone Conf., 1959.
5. Fried, W., Plzak, L. F., Jacobson, L. O., Goldwasser, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 237.
6. Mirand, E. A., Prentice, T. C., *ibid.*, 1956, v93, 473.
7. ———, *ibid.*, 1957, v96, 49.
8. ———, *Proc. of 7th Internat. Congress of Internat. Soc. of Hematol.*, 1958, v2, 1.
9. Mirand, E. A., Prentice, T. C., Slaunwhite, W. R., *N. Y. Acad. Sci.*, 1959, v77, 677.
10. Gordon A. S., *Physiol. Rev.*, 1959, v39, 1.
11. Jacobson, L. O., Goldwasser, E., Fried W., Plzak, L., *Nature*, 1957, v179, 633.
12. ———, *Trans. Am. Assn. Physicians*, 1957, v70, 305.
13. Prentice, T. C., Mirand, E. A., unpublished data.

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A Microbioassay for Acetylcholine.* (26384)

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Many classical researches reported in literature are concerned with the action of acetylcholine (ach). In such work there has often been a need to determine low concentrations of ach in small volumes of fluid or perfusate. Direct chemical analyses by methods such as the one described by Hestrin(1) are ordinarily too insensitive for these applications and one must turn to a bioassay. Minz(2) has summarized the most frequently employed bioassay procedures and has pointed out the advantages and disadvantages of each. There is, for example, an approach in which one measures the contraction of the dorsal muscle of the leech, a preparation which will respond to ach at a concentration of 5×10^{-8} M. This preparation is suspended in a bath whose volume is usually about 10 ml and therefore if a 0.1 ml sample of solution is added to the bath, this sample, if it is to be successfully assayed, must contain ach at a minimal concentration of 5×10^{-6} M.

We wished to conduct certain experiments

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