Urinary Erythropoietin in Men Subjected to Acute Hypoxia.* (32114)

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The rate of erythropoiesis is controlled, in part, by erythropoietin (EP), the production of which depends mainly on tissue oxygen demand(1,2). Plasma levels of EP also reflect erythroid activity of the bone marrow through an erythroid tissue utilization(3,4).

When a permanent resident at sea level is brought up to high altitude, reticulocytes, erythroid precursors and plasma iron turnover rise abruptly during the first two weeks, to descend gradually and reach a "new steady state", which is 30% higher than that of residents at sea level(5). The same "high erythropoietic steady state" is found in natives born and living permanently at high altitudes(6).

Attempts to demonstrate EP in plasma or urine of permanent dwellers at high altitudes have yielded conflicting results. Carmena *et al*(6) infused plasma from people living at 15,000 feet into residents at high altitudes who had been brought down to sea level (in erythroid hypoplasia). They observed a small increase in reticulocytes and plasma iron turnover. They failed to demonstrate EP in the urine or plasma of volunteers living between 4,000 and 13,500 feet(7). Merino(8) collected plasmas between 13,000 and 15,000 feet and injected them into normal persons at sea level. He obtained a slight reticulocyte response. Scaro(9) described a small increase in erythropoiesis in fasted rats injected with plasmas of human beings living at 13,000-15,000 feet. Finally, Siri *et al*(10) demonstrated EP in plasma and urine of one man subjected to a simulated altitude of 16,400 feet but only on the first three days of hypoxia.

In this paper, we describe the erythropoietin content of the urine of 5 permanent residents at sea level, during several days of sojourn at 14,900 feet.

Materials and methods. Three women and two men, permanent residents at sea level, were brought to Morococha, Peru (14,900 feet). Total urines were collected daily and pooled, and processed by the technique of Hodgson *et al*(11). To evaluate the erythropoietic activity of the urinary extracts, we injected the material in fasted Wistar rats, intraperitoneally, on the 2nd and 3rd day of fasting, and 0.3 ml of normal rat plasma labeled with 0.3 μC of Fe^{59} was injected intravenously on the 4th day. Animals were sacrificed 3 hours later(12). The Hodgson formula(13) was used to express the fraction of plasma iron going to erythroid tissue per hour (F. I. Er.).

Results. There was an erythropoietic response in the volunteers as evidenced by reticulocytosis (Table I). The results of erythropoietin assays are presented in Table II. During the 2nd and 3rd day at high altitudes, we found the highest urinary erythropoietic activity; this decreased on the 4th and 5th day. A dose-response relationship was demonstrable on the 3rd day of hypoxia. It was estimated by comparison with a cobalt chloride dose-response curve that the urinary erythropoietin of 600 cc on the 3rd day was 2 units. This is a minimum estimate, since the efficiency of extraction was not established.

Discussion. The results suggest an early production of EP in response to acute hypoxia, and thereafter a rapid decline in urinary levels, as was observed by Siri *et al*(10) in one man subjected to simulated altitudes. The shape of the curve seems to be similar in different mammals, although the time of appearance and disappearance may differ. Stohlman(14), in rats, found the highest peak after 16-18 hours of continuous exposure to 23,000 feet, but at 48 hours levels dropped to

* Supported by Instituto de Biologia Andina, Lima, Peru and Consejo Nacional Investigaciones Cientificas y Tecnicas, Argentina.

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TABLE I. Reticulocytes Per Cent in 5 Volunteers Brought up to 14,900 Feet.

	Days at 14,900 feet				
Sea level	1	2	3	4	5
.8 ± .1	.7 ± .1	1.1 ± .2	1.4 ± .2	1.6 ± .2	1.7 ± .2

TABLE II. Fraction of Plasma Iron Going to Erythroid Tissue per Hr (F.I.E.R.). In parenthesis, no. of rats used. ± = Standard error.

Equivalent of urine injected in each rat. (ml)	Days at 14,900 feet				
	1	2	3	4	5
180	8.6 ± 1.2 (5)	31.6 ± 6.4 (6)	15.8 ± 3.1 (6)	19.9 ± 7.5 (6)	10.8 ± 3.8 (5)
300	9.3 ± 3.0 (6)	31.7 ± 5.3 (5)	22.1 ± 4.3 (5)		16.2 ± 2.0 (6)
600	12.9 ± 2.6 (4)		34.1 ± 9.4 (5)	30.8 ± 4.8 (5)	14.1 ± 3.5 (6)
Normal urine (residents at sea level)					
60 c.c.	6.9 ± .9 (6)				
600 c.c.	7.6 ± 1.3 (5)				
1 ml Saline	5.5 ± 1.4 (6)				

almost normal values. Prentice and Mirand (15) obtained the same type of curve with rats exposed to 10% O_2. Erslev(16) observed a substantial increase in serum EP of rabbits exposed to 10% O_2 after 20-48 hours. In the same animal, Prentice and Mirand(17) described an elevation of EP in plasma at 8 hours, with the peak between 24 and 48 hours, and a return to normal levels by 72 hours. In guinea pigs, exposed to 10% O_2, the highest plasma erythropoietic activity was reported to be at 24 hours, with minimal activity present after 48-72 hours(18). Finally, in mice exposed between 18,000 and 20,000 feet, the peak was found at 8-16 hours(19, 20).

We think that the rapid rate of increase in EP levels in different mammals is the response to an acute hypoxic stimulus, and its early disappearance could be explained by utilization of the hormone by an active erythroid bone marrow, with the establishment of a new steady state.

Summary. The urinary erythropoietin content of residents at sea level, were measured during a 5-day sojourn at 14,900 feet. We found an early EP rise with the peak on the 2nd-3rd day of exposure. Thereafter, activity declined. The rapid increase in activity is considered to reflect the response to a severe hypoxic stimulus, and the subsequent decrease, utilization of the hormone by an active erythroid bone marrow.

The authors are grateful to Doctors Cesar Reynafarje, Baltazar Reynafarje, Jose Ramos and Tulio Velazquez for their cooperation in making this work possible.

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Received February 24, 1967. P.S.E.B.M., 1967, v125.

Isolation of St. Louis Encephalitis Virus from a Naturally-Infected Gray Fox *Urocyon cinereoargenteus.** (32115)

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St. Louis encephalitis (SLE) virus has been repeatedly isolated from wild and domestic birds and from mosquitoes which feed on them; hence, both birds and mosquitoes are implicated in its natural maintenance. There is serologic evidence that feral and domestic mammals are infected (1-10), but recovery of this virus from naturally-infected mammals other than man had not been described prior to 1966 when Sulkin and co-workers reported the isolation of SLE virus from bats(11). This paper reports the isolation of SLE virus from the brain of a California gray fox, *Urocyon cinereoargenteus,* which was shot July 27, 1957 in Amador City, Amador County, California. The animal was obviously ill, but did not attack or run; it was suspected to have rabies and the head was sent to this laboratory for examination.

Materials and methods. A 10% suspension of the fox brain tissue in 0.75% bovine albumin in phosphate-buffered saline (BABS) was prepared by grinding the tissue in a mortar, and the suspension was clarified by centrifugation at 3,000 rpm for 20 minutes. The supernatant fluid was removed and penicillin and streptomycin were added to give a final concentration of 1,000 and 5,000 units per ml; the mixture was allowed to stand at room temperature for 30 minutes and was then inoculated intracerebrally (i.c.) in 0.03 ml amounts into 8 adult (4 week old) Swiss albino mice. The suspension was bacteriologically sterile in glucose and thioglycollate broth cultures. For subsequent passages, the brains of sick or dead mice were harvested, made into 10% suspensions as above and inoculated i.c. into groups of 8 adult mice or i.c. and intraperitoneally (i.p.) in 0.01 and 0.03 ml amounts, respectively, into litters of 6 suckling (1- to 4-day-old) mice. Mice were observed for 28 days before being discarded as negative. All brain suspensions were bacteriologically sterile.

Slip smears of brain tissue from the fox and from sick or dead passage mice were stained by Seller's method and examined for Negri bodies. Portions of the brains or brain suspensions were stored at —70°C in flame-sealed glass ampules.

Virus was identified by neutralization tests in suckling mice. Serial 10-fold dilutions of infected mouse brain in BABS were mixed with equal volumes of specific immune or normal rabbit or hamster serum. All sera were inactivated at 56°C for 30 minutes. Serum-virus mixtures were incubated 1 hour at 37°C and then inoculated into mice i.p. in 0.03 ml volumes, using 1 mouse litter (6 mice) per dilution. Mice were observed for 14 days, deaths were recorded and the LD_{50}

* This study was supported by grant AI-01475 from Nat. Inst. of Allergy & Infect. Dis., USPHS, Dept. of HEW.