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Comparison of the Reproductive Performance of Rats at High Altitude (3,800 m) and at Sea Level*

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INTRODUCTION

It is widely recognized that the fertility of most animals indigenous to sea level is reduced when they are moved to high altitudes (Altland, 1949; Mongé, 1960; Weihe, 1964). However, very little is known of the reproductive capabilities of rats derived from sea level stock but born at high altitude, i.e., first-generation natives. Previous reports on the reproductive performance of sea level native rats transported to high altitude as adults indicate that female animals may experience transitory anestrus (Chang and Fernandez-Cano, 1959; Weihe, 1964). However, when successfully mated, these animals give birth to first-generation offspring of normal size (Weihe, 1964; see also review by Dawes, 1968, p. 54). Ample evidence suggests that the postnatal growth of the first-generation rats at high altitude is significantly impaired (Chiodi and Sammartino, 1952; Krum, 1957; Moore and Price, 1948; Nelson, 1968; Timiras, 1964; Timiras and Woolley, 1966; Timiras, Krum and Pace, 1957). Reduced body weight and a reduction in the weight of most endocrine organs as well as cardiac hypertrophy and polycythemia have been reported in first- and second-generation rats at high altitude. Impaired fertility in these rats has been inferred; however, a search of the literature has failed to reveal any information concerning the reproductive performance of these animals. Consequently, a study of the reproductive capability of first-generation rats born at high altitude was conducted and comparisons were drawn with sea level control animals and sea level adult rats transported to high altitude. The estrous cycles of the three groups of animals were analyzed, and a comparison of their ability to maintain pregnancy was made including a study of the prenatal status of the fetuses from each group of animals.

MATERIALS AND METHODS

Three groups of female Long-Evans rats, approximately 120 days of age, were used in this study. The first group of 9 rats from the colony at Berkeley (elevation 76 m) served as sea level controls. The second group of 6 animals of Berkeley native stock was transported to Barcroft Laboratory of the White Mountain Research Station (3,800 m); the third group consisted of 11 first-generation native rats born at high altitude (3,800 m). At the onset of the experiment, the sea level controls and Berkeley native rats at 3,800 m weighed approximately 308 g, and the high altitude native rats weighed an average of 209 g. All animals were maintained under standard conditions of light, 14 hr of light and 10 hr of darkness, and temperature (20° to 25°C), and were fed AD LIBITUM a standard laboratory

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diet. The diet contained 67.5% wheat, 15% casein, 7.5% skim milk powder, 6.75% hydrogenated vegetable oil, 1% fish oil, 0.75% NaCl, 1.5% $CaCO_3$, and KI (added by spraying) to a level of 1 μg/g.

All of the female rats were bred with young adult males exposed to corresponding environmental conditions. Berkeley native stock rats (3,800 m) were bred after 17 days at high altitude to permit adjustment to the hypoxic environment. Day zero of pregnancy was considered to be the morning when spermatozoa were detected in the vagina. The following investigations were made.

FOOD CONSUMPTION DURING PREGNANCY. To examine the possibility that impairment of fertility at high altitude resulted from reduced appetite, daily food intake was measured from day zero of pregnancy until term in selected animals from each group. Each animal was caged separately and given access to a jar containing a known weight of food. Daily food intake was measured by weighing the food remaining in the jar as well as any residue in the cage. Body weights of the pregnant animals were taken on day zero and day 21 of pregnancy.

REPRODUCTIVE PERFORMANCE. Daily vaginal smears were taken on all groups of animals for 16 days prior to breeding, to record variations in the estrous cycle. On day 21 of pregnancy, the animals were autopsied and the number of fetuses per litter was recorded. The diameter of any resorption sites in the uteri were noted, and the number of corpora lutea in both ovaries was compared with the total number of fetuses and resorption sites.

FETAL MEASUREMENTS. The body weights and body lengths (nose - anus + anus - tail) of the fetuses were recorded. The placentas were cleaned of membranes and weighed. The fetal hearts were dissected free of surrounding tissues and weighed on a torsion balance. Fetal hematocrits were determined with the microhematocrit method.

STATISTICAL METHODS. The t test for non-paired data was applied to determine whether the first-generation high altitude rats differed statistically in their means from the other two groups of animals.

RESULTS

Average food consumption during pregnancy of the sea level control and Berkeley native stock rats at 3,800 m was similar when expressed as g/day (Table 1).

TABLE 1. Comparison of pregnant Long-Evans rats at high altitude (3,800 m) and at sea level

Group	No. of rats	Onset wt. g	Weight gain day 0-21		Daily food intake per rat	
			Absolute (g)	Relative (g/100 g body wt)	g	g/100 g body wt.
Controls, Berkeley strain	4	295	98	33.2	18.8 ± 1.4	6.4 ± 0.4
High altitude, Berkeley strain	4	322	62	19.2	16.6 ± 1.1	5.2 ± 0.3*
High altitude, adapted strain (3,800 m)	4	209	75	35.9	15.1 ± 0.5*	6.8 ± 0.4

Values are means ± SE. *) Significantly different from controls ($p < 0.05$).

However, when these figures were corrected for body weight, the Berkeley native rats (3,800 m) consumed slightly less food per day than the sea level control group. The high altitude native rats ate somewhat less food per day than the sea level controls, although when expressed as g/100 g body weight, there was no difference between the two groups.

During pregnancy, body weight gain was greatest in the sea level control animals. However, when the weight gain during pregnancy was expressed as g/100 g body weight, the increase in weight was greatest in the pregnant high altitude native rats. It appears, therefore, that reduced appetite was not an important factor in the impaired fertility of the animals residing at high altitude.

Estrous cycles are compared in Fig. 1. The cycles of the Berkeley rats at 3,800 m followed the 4-5 day pattern typical of animals raised at sea level, whereas the cycles of the high altitude native rats were prolonged either in the diestrous or in the cornified phase of the cycle. The abnormalities in the estrous cycle of the high altitude native rats were associated with impaired fertility. Whereas pregnancy occurred in all sea level controls and Berkeley stock (3,800 m) mated, only four of the 11 high altitude native rats became pregnant.

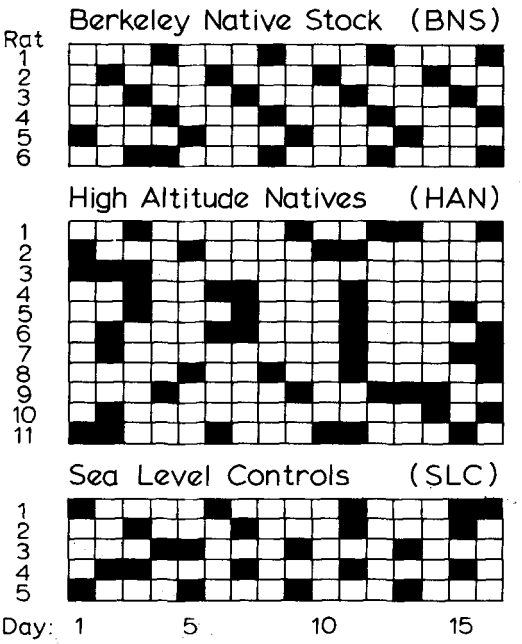

Fig. 1. Comparison of the estrous cycles of Long-Evans rats at high altitude and at sea level. Day of vaginal cornification = ■

As shown in Table 2, rats of each group had an average of one resorption site in the uterus on day 21 of pregnancy. The number of corpora lutea present in the ovaries of sea level control and Berkeley stock rats at 3,800 m was similar, whereas the ovaries of the high altitude native rats had significantly fewer corpora lutea ($p < 0.02$). The number of fetuses observed in utero in Berkeley stock at 3,800 m and high altitude native rats at 21 days was significantly less than that in sea level control animals ($p < 0.02$).

TABLE 2. Reproductive performance in Long-Evans rats at high altitude and at sea level

Group	No. of rats	Litter size	No. of resorption sites	No. of corpora lutea
Controls, Berkeley strain	4	11.0 ± 1.0*	1.0	13.0 ± 1.1
High altitude, Berkeley strain	3	5.3 ± 1.2	1.0	12.7 ± 1.2
High altitude, adapted strain (3,800 m)	4	7.5 ± 1.3	0.8	8.8 ± 0.8

*) Values are means ± SE. For p values, see text.

Fetal body weights were essentially similar in all groups (Table 3). Total body lengths of Berkeley stock fetuses at 3,800 m were significantly less than those of either high altitude natives or sea level control fetuses ($p < 0.001$). Heart weights of the fetuses were the same in all groups. Placentas from the Berkeley animals at 3,800 m were significantly heavier than those of controls ($p < 0.001$), and slightly heavier than those of the high altitude native animals ($p < 0.05$). The hematocrits of fetuses from Berkeley rats at high altitude were significantly lower than those of fetuses from either high altitude natives or sea level controls ($p < 0.001$).

TABLE 3. Comparison of 21-day fetuses at high altitude and at sea level

Parent group	No. of rats	Body weight, g	Body length, cm	Heart, mg	Placenta, g	Hematocrit
Controls, Berkeley strain	44	5.5 ± 0.20	6.4 ± 0.04	25.8 ± 1.7	0.52 ± 0.05	42.8 ± 0.5
High altitude, Berkeley strain	16	5.0 ± 0.60	5.8 ± 0.08	24.6 ± 1.6	0.83 ± 0.10	38.5 ± 1,0
High altitude, adapted strain (3,800 m)	30	5.0 ± 0.04	6.2 ± 0.04	26.7 ± 0.8	0.60 ± 0.02	42.7 ± 0.9

Values are means ± SE. For p values, see text.

DISCUSSION

The results of this investigation indicate that reproductive capacity is impaired in rats at high altitude, although the degree of impairment appears to vary between animals born at this elevation and those initially exposed to the hypoxic environment as adults. Both the Berkeley rats at 3,800 m and the sea level controls had normal estrous cycles but apparently differed in their ability to maintain

pregnancy. For example, an excessive number of fresh corpora lutea was present in the ovaries of Berkeley stock rats (3,800 m) when viewed with respect to litter size, and this discrepancy may indicate failure of implantation or early embryonic death. There was no such disparity, however, in first-generation rats at high altitude; rather, the number of corpora lutea, though reduced in the rats of the high altitude native group, corresponded to the number of fetuses in the litter. The irregular estrous cycles observed in the high altitude native animals, the small number of corpora lutea, and the high incidence of infertile matings all suggest the possibility that pituitary gonadotrophic function of first-generation rats at high altitude is subnormal with respect to sea level control animals. It might be of considerable value to assess all of the gonadotrophic hormones and ovarian steroids systematically in first-generation rats at high altitude, and such studies are now in progress.

Marked cardiac hypertrophy and polycythemia have been observed in adult first- and second-generation rats at high altitude (Timiras, 1964; Timiras and Woolley, 1966). In the present study, however, the fetal hematocrits and cardiac measurements of the offspring of the high altitude native rats (i.e., second-generation at high altitude) show no such effects, suggesting that their cardio-vascular system is protected in utero from the hypoxic environment. A different mechanism may be compensating for the maternal hypoxic environment in the Berkeley native stock rats at 3,800 m, resulting in an increase in placental weight accompanied by reduced fetal hematocrits. The placental hypertrophy, with a concomitant increase in placental surface area, seen in Berkeley native stock animals at high altitude is similar to the changes found in the placentas of sheep transported to high altitude (Barron et al., 1964; Metcalfe, Novy, and Peterson, 1967). Interestingly, in oviparous species, such as the chicken, significant cardiac hypertrophy has been observed during embryonic development at high altitude (Atherton and Timiras, 1970). Although the placentas of the high altitude native rats are not significantly larger than sea level control placentas, the greatly elevated hematocrits of the high altitude native mothers (approximately 65-70%) apparently are sufficient to supply the fetus with an adequate amount of oxygen.

Besides the decreased hematocrits observed in Berkeley native stock fetuses (3,800 m), fetal body length was also somewhat reduced without any significant change in body weight. In contrast, the fetuses of first-generation rats at high altitude were almost identical in body size and cardio-vascular characteristics with sea level control fetuses. This would appear to indicate that the high altitude native animals were somewhat more successful than the Berkeley rats at high altitude in compensating for the metabolic changes required by the hypoxic environment.

The results of the present study indicate that reproductive capacity is, indeed, altered at high altitude, and, further, that such changes may be implicated in the subnormal postnatal growth and development of the progeny. They also suggest that, as in man (Lasker, 1969), the capacity of rats to adapt to high altitude is not limited to immediate physiologic adjustments but is passed on to succeeding generations to ensure their survival.

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REFERENCES

ALTLAND, P.D. (1949) : Effect of discontinuous exposure to 25,000 ft simulated altitude on growth and reproduction of the albino rat. J. exp. Zool., 110 : 1-18.

ATHERTON, R.W. and TIMIRAS, P.S. (1970): Erythropoietic and somatic development in chick embryos at high altitude (3,800 m). Amer.J.Physiol., 218 : 75-79.

BARRON, D.H., METCALFE, J., MESCHIA, G., HUCKABEE, W., HELLEGERS, A., and PRYSTOWSKY, H. (1964): Adaptation of pregnant ewes and their fetuses to high altitude. In: The Physiological Effects of High Altitude. W.H.Weihe (ed.), Macmillan, New York, 115-125.

CHANG, M.C. and FERNANDEZ-CANO, L. (1969): Effect of short changes of environmental temperature and low atmospheric pressure on the ovulation of rats. Amer.J.Physiol., 196 : 653-655.

CHIODI, H. and SAMMARTINO, R. (1952): Fatty degeneration of the liver caused by high altitude hypoxia in young albino rats. Acta Physiol.Lat.Amer., 2 : 228-237.

DAWES, G.S. (1968) : Foetal and Neonatal Physiology; a Comparative Study of the Changes at Birth. Year Book Medical Pub., Chicago.

KELLEY, F.C. (1967) : Neonatal mortality in rats born at altitude. Ph.D.Thesis, University of California, Berkeley.

KRUM, A.A. (1957) : Reproduction and growth of laboratory rats and mice at altitude. Ph.D.Thesis, University of California, Berkeley.

LASKER, G.W. (1969) : Human biological adaptability. Science, 166 : 1480-1486.

METCALFE, J., NOVY, M.J. and PETERSON, E.N. (1967): Reproduction at high altitudes. In: Comparative Aspects of Reproductive Failure. K.Benirschke (ed.), Springer-Verlag, New York, 447-457.

MONGÉ, C.M. (1960) : Aclimatación en los Andes. Extractos de investigaciones sobre Biologia de Altitud. Fac.de Med., Lima.

MOORE, C.R. and PRICE, D.J. (1948): A study at high altitude of reproduction, growth, sexual maturity and organ weights. J.exp.Zool., 108 : 171-216.

NELSON, M.L. (1968) : Thyroid function and hypophysial content of growth hormone and thyrotropin in rats raised at high altitude. Ph.D.Thesis, University of California, Berkeley.

TIMIRAS, P.S. (1964) : Comparison of growth and development of the rat at high altitude and at sea level. In: The Physiological Effects of High Altitude. W.H.Weihe (ed.), Macmillan, New York, 21-30.

TIMIRAS, P.S., KRUM, A.A. and PACE, N. (1957): Body and organ weights of rats during acclimatization to an altitude of 12,470 feet. Amer.J.Physiol., 191 : 598-604.

TIMIRAS, P.S. and WOOLLEY, D.E. (1966): Functional and morphological development of brain and other organs of rats at high altitude. Fed.Proc., 25 : 1312-1320.

WEIHE, W.H. (1964) : Some examples of endocrine and metabolic functions in rats during acclimatization to high altitude. In: The Physiological Effects of High Altitude. W.H.Weihe (ed.), Macmillan, New York, 33-42.

ABSTRACT. - Estrous cycles and reproductive performance were compared among rats born at high altitude, rats of Berkeley stock transported to 3,800 m, and sea level controls. Disturbances of reproductive function observed in experimental groups were not due to reduced food intake. Estrous cycles of Berkeley stock at 3,800 m were normal, whereas cycles of high altitude native rats were irregular and fertility was impaired. Litter size was reduced in both groups at high altitude with fewer implantation sites than numbers of corpora lutea observed in the Berkeley stock rats at 3,800 m. In the high altitude native animals, numbers of corpora lutea correlated with implantation sites. Placentas from Berkeley stock rats (3,800 m) were heavier than those from the other groups, and fetal hematocrits from these animals were reduced.

ZUSAMMENFASSUNG. - Der Oestruszyklus und die Fortpflanzungsleistung wurde bei drei Gruppen Ratten verglichen: (a) Kontrollen in Berkeley (Seehöhe); (b) Tiere der Berkeley-Kolonie in 3.800 m Höhe und (c) Tiere einer seit mehreren Generationen in 3.800 m gehaltenen Kolonie. Der Oestruszyklus der neu in die Höhe gebrachten Tiere war normal, während er bei den Tieren der höhenadaptierten Kolonie unregelmässig und die Fertilität geschwächt war. Die Wurfgrösse war bei beiden Höhengruppen kleiner. Bei den höhenadaptierten Tieren entsprach die Anzahl der Implantationsstellen der Zahl der corpora lutea, dagegen war bei den frisch in die Höhe verbrachten Tieren die Anzahl Implantationsstellen kleiner als die der corpora lutea. Die Placentagewichte waren bei den Höhentieren beider Gruppen kleiner und die foetalen Hämatokrits herabgesetzt.

RESUME. - On a comparé le cycle menstruel et la propension à la reproduction de trois groupes de rats placés dans les conditions suivantes: (a) un groupe de contrôle à Berkeley (niveau de la mer); (b) un groupe de la colonie de Berkeley transporté à 3.800 m d'altitude et (c) des bêtes vivant depuis plusieurs générations à 3.800 m d'altitude. Le cycle menstruel des bêtes nouvellement transportées en altitude est resté normal alors que celui des animaux acclimatés était irrégulier et leur fertilité diminuée. Dans les deux groupes d'altitude, le nombre des jeunes par portée était réduit. Pour les bêtes acclimatées à l'altitude, le nombre des implantations correspondait à celui des corpora lutea. On note par contre moins d'implantations que de corpora lutea chez les rats nouvellement placés en altitude. Le poids des placentas était inférieur pour les deux groupes d'altitude à celui du groupe de plaine et le taux d'hémoglobine réduit chez les foetus.