28. Scheig, R. L., Isselbacher, K. J., J. Clin. Invest., 1963, v42, 975.

29. Maling, H. M., Wakabayashi, M., Horning, M. G., Advances in Enzyme Regulation, 1963, v1, 247.

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Independent Influence of Chronic Hypoxia and Sexual Development Upon Circulating Erythrocyte Concentration in Male Chickens.* (31332)

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Two conditions are rather systematically associated with increases in circulating red cell concentrations in homeotherms—"maleness" and hypoxia. At sexual maturity, the hematocrit of male mammals becomes greater than that of females(1), and similar changes also occur in the domestic fowl(2). It also has been demonstrated that exogenous androgens have an erythropoietic stimulus in mature mammalian females and male castrates, but not necessarily in sexually mature males (3). The polycythemia of animals living at high altitudes was reported by Viault in 1890, and this has been the subject of abundant study since that time(4). Grant and Root (5) have concluded that hypoxia is the fundamental stimulus for erythropoiesis. However, this response to hypoxia is apparently restricted to homeotherms, and has not been found in poikilothermic animals(6).

The additive (and apparently independent) nature of these two polycythemia-inducing conditions was demonstrated in experiments at high altitude† and at sea level with cockerels, some of which were subjected to repression of sexual development by stilbestrol (estrogen) treatment.

Materials and methods. The experiment was conducted on White Leghorn cockerels of sea level stocks—*i.e.*, not the strain selected for high altitude(7). These were brooded and subsequently pen-reared under ordinary and similar husbandry conditions, and were fed a nutritionally adequate ration. At an age of 2 days, some of the cockerels were taken to an elevation of 10,150 feet, and the remainder retained at sea level. At 45 days age, the high altitude group was taken to an elevation of 12,500 feet, where they remained until termination of the experiment.

At 5 and 45 days of age, some birds of each group (high altitude and sea level) were implanted subcutaneously with one or two 12 mg stilbestrol pellets. This resulted in 5 treatment sub-groups at each elevation, which were designated according to number of pellets and time of their administration:

0-0: no treatment (*i.e.*, treatment controls);
0-1, 0-2: at 45 days of age birds were implanted with either one or two stilbestrol pellets; and,
1-1, 1-2: all these birds received a pellet at 5 days of age and either one or two stilbestrol pellets at 45 days of age.

Records were kept of mortality, and periodically body weights were determined. At approximately 24 weeks of age, the birds were sacrificed and measurements were made of testes weight and hematocrit (by the microcapillary method). Mean data were calculated for each group, and the significance of differences between them was estimated by Student's t-test(8).

Results. 1. *Growth and mortality.* The body weights of the various groups of experimental birds are summarized in Table I.

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† These studies were carried out at the White Mountain Research Station (near Bishop, Calif.), which has laboratories at 4,000, 10,150, 12,500 and 14,250 feet elevations. For further information regarding these facilities contact the Director: Prof. Nello Pace, Dept. of Physiology, Univ. of California, Berkeley.

From these it is evident that the early growth of the high altitude groups (except those receiving 3 stilbestrol pellets) was greater than that of comparable groups at sea level. The differences up to 45 days of age are statistically significant. This greater growth at high altitude has been encountered frequently, but not uniformly, with other groups of chickens. The factors which may be responsible for the apparent growth promotion by this environment have been discussed previously(9). However, by the time of sacrifice (at 24 weeks age) very little difference in body size existed among the stilbestrol-treated groups, but the untreated high altitude controls were about 10% heavier than their sea level counterparts.

Stilbestrol treatment caused a rather marked repression of growth (indicated as ± Δ% from untreated controls in Fig. 1)

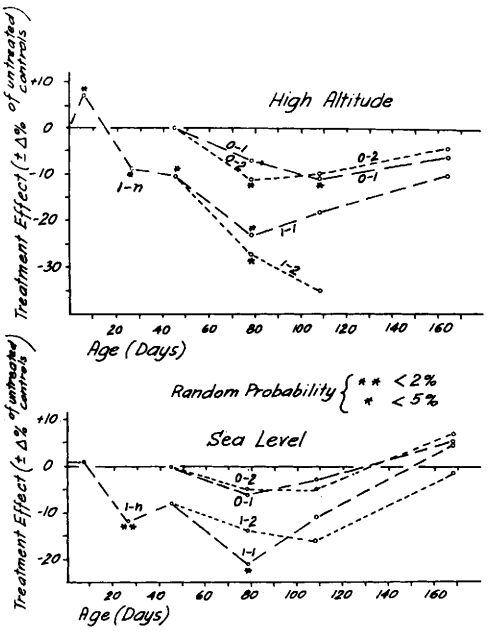

FIG. 1. Effect of stilbestrol treatment on growth of cockerels at high altitude and at sea level.

which was more-or-less proportional to the dosage. Such a response may result from an inhibition of production of androgens, which are known to have growth-promoting properties(10). It also is possible that this growth repression has a sociological basis, rather than physiological, since the estro-

TABLE I. Growth of Cockerels at Sea Level and High Altitude (12,500'): Body Weights, g, Are Summarized as Means and Standard Errors at Various Ages. Treatment groups indicate dosage and time of stilbestrol treatment as explained in the text.

	Age (days)	Treatment group: 0-0	0-1	0-2	Combined 0-n groups	1-1	1-2	Combined 1-n groups
High altitude (12,500 ft):	0	39.8 ± 0.8	36.6 ± 0.7	37.9 ± 0.9	38.0 ± .5	39.3 ± 1.6	37.5 ± 0.9	38.3 ± 0.9
	5	50.6 ± 1.6	48.6 ± 1.4	51.8 ± 1.8	50.2 ± .9	54.9 ± 2.2	52.4 ± 1.3	53.6 ± 1.2
	26	228 ± 10	214 ± 8	225 ± 11	222 ± 6	212 ± 11	190 ± 11	200 ± 8
	45	477 ± 21	483 ± 15	470 ± 22	477 ±11	469 ± 28	395 ± 24	431 ±19
	78	1171 ± 56	1087 ± 46	1045 ± 71		901 ± 73	856 ± 96	
	108	1708 ± 53	1621 ± 62	1539 ± 63		1407 ±146	1107 ±312	
	164	2102 ± 63	1981 ±119	2023 ± 34		1901 ±152	—	
Sea level:	0	39.4 ± 1.3	37.5 ± 1.2	41.0 ± 1.5	39.3 ± 0.8	40.0 ± 1.9	39.5 ± 2.3	39.8 ± 1.4
	7	64.0 ± 2.9	63.8 ± 2.6	68.3 ± 4.6	65.5 ± 2.0	70.8 ± 3.4	60.0 ± 1.9	66.0 ± 2.8
	26	212 ± 7	203 ± 10	205 ± 7	206 ± 5	185 ± 10	179 ± 10	182 ± 7
	45	394 ± 10	373 ± 19	382 ± 15	382 ± 8	326 ± 41	379 ± 41	350 ±25
	71	911 ± 16	878 ± 62	849 ± 49		742 ± 46	830 ±105	
	78	1057 ± 23	977 ± 78	1003 ± 45		837 ± 47	905 ±109	
	108	1523 ± 64	1478 ± 96	1443 ± 96		1353 ± 42	1273 ±120	
	168	1901 ±107	1987 ±129	2027 ±118		1987 ± 47	1897 ±128	

TABLE II. Mortality Summary for Cockerels Raised at High Altitude and at Sea Level, and Treated with Stilbestrol.

	At sea level					At high altitude				
Treatment group	0-0	0-1	0-2	1-1	1-2	0-0	0-1	0-2	1-1	1-2
Initial numbers	5	5	6	5	4	12	16	14	9	12
Mortality (% of initial No.)	0%	0%	17%	40%	0%	17%	44%	70%	56%	100%
Period (days of age)	Mortality rate (% deaths per week)									
0- 45	0	0	0	0	0	0	0	0	0	0
45- 78	0	0	3.4%	8.3%	0	3.5%	5.2%	7.4%	2.3%	12.0%
78-108	0	0	0	0	0	2.3%	0	3.8%	3.2%	9.0%
108-164	0	0	0	0	0	0	1.6%	3.1%	5.0%	12.5%

genized birds became rather submissive and less competitive with other birds in the pen. This growth repression was more pronounced in the high altitude groups.

The stilbestrol-treated cockerels also suffered a severe mortality at high altitude, but not at sea level, and this is summarized in Table II. The mortality at sea level (12%) is not very different from that expected in commercial rearing of chickens, although the 3 birds that died did so shortly after stilbestrol implantation. The mortality encountered in the treatment-controls (group 0-0) at high altitude is less than has usually been encountered with other similar populations newly introduced to high altitude (*e.g.*, frequently this is 25-30% up to 24 weeks of age). The mortality of the stilbestrol-treated birds is greater than would be anticipated, and it was more-or-less evenly distributed through the rearing period, and appeared to be proportional to dosage. The greater mortality of stilbestrol-treated cockerels is somewhat contrary to the usual observation that males suffer a greater mortality under environmental stress than do females(11).

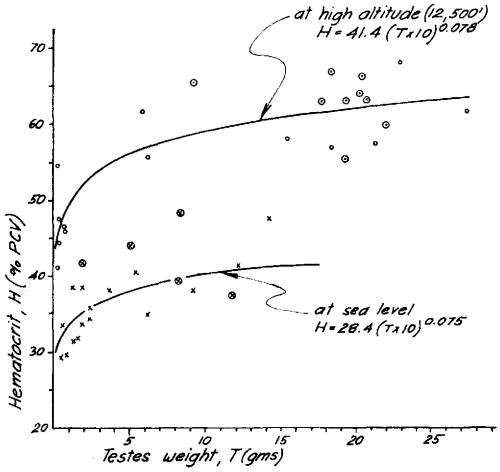

FIG. 2. Relationship between hematocrit and testes size. Data for individual cockerels at high altitude are indicated as O, and at sea level as ×. Circled points denote birds which were not treated with stilbestrol.

2. Testes size and hematocrit. A comparison of testes weight and body weight among all birds revealed no significant correlation ("r" at sea level = 0.185, and "r" at high altitude = 0.160). This lack of correlation applies generally to reproductive organs, since their growth is determined by circulating gonadotrophins, rather than general somatic properties(12). Consequently, testes size was not corrected for variation in body weight for comparison with other parameters.

Significant correlations were found between the logarithms of testes size and hematocrit: "r" at sea level = 0.511, $p<0.05$; "r" at high altitude = 0.725, $p<0.001$. Regressions of hematocrit (H) on testes weight in grams (T) yielded the equations:‡

$$\text{at sea level: } H = 28.4\ (T \times 10)^{0.075}$$

$$\text{at high altitude (12,500'): } H = 41.4\ (T \times 10)^{0.078}$$

The "fit" of the data to these prediction equations is indicated in Fig. 2. It is evident that the high altitude birds had larger gonads, and also, that at each elevation the largest gonads were found in birds which had been implanted with one stilbestrol pellet at 45 days of age. However, there is no reason to believe that these phenomena have any great effect on the derived relationships

‡ Testes weights were increased by an arbitrary factor in this case 10, so that all numbers would be greater than unity. This procedure merely simplifies calculations involving logarithms.

between hematocrit and testes size.

In these equations, the simple numerical coefficients (28.4 at sea level, and 41.4 at high altitude) indicate the influence of chronic hypoxia upon the hematocrit. These are the values predicted for the hematocrit where there is a minimum testicular influence (*i.e.*, where T = 0.1 g; where T = 0, H = 0). The exponential coefficients (0.075 at sea level and 0.078 at high altitude) represent the effect of "maleness" upon the hematocrit. The very close similarity of these coefficients indicates that the effect of factors associated with testes size upon the hematocrit are the same, in both environments.

Discussion. From the results presented, it appears quite possible to separate, at least quantitatively, the effects of "maleness" and hypoxia in determining the level of the hematocrit. In all likelihood, the "maleness" factor, indicated by gonad size, is the level of circulating androgen. At the ages involved herein, which correspond to maximum gonad size(13), comb size and gonad size are highly correlated: r = 0.98(14). In younger animals, this correlation is substantially less: at 50 days, r = 0.25; and 90 days, r = 0.56. Cockerel comb size is quite sensitive to blood androgens to the extent that it has been a standard bio-assay criterion for such materials. Consequently, the gonad sizes reported herein are a suitable index of circulating androgen concentration.

Since only two elevations were involved in this study, the nature and mathematics of the relationships (linear, exponential, etc.) between hypoxia and the hematocrit are not apparent. However, the influence of the ambient oxygen tension on the hematocrit does not appear to be simple. This was examined over a fairly wide range of oxygen concentrations (at normal atmospheric pressure) and in several mammalian species by Campbell, in 1927. From his data(15) it appears that over the range of 100-350 mm O_2 the circulating hemoglobin concentration follows the relationship:

$$(\%\ \text{Hb} - 50) = 95\ e^{-0.43\ O_2}$$

where: % Hb is the hemoglobin concentration relative to that usually present at 150 mm Hg O_2; and O_2 is the oxygen tension in decimeters Hg.

From this it appears that only half of the hemoglobin present at atmospheric sea level oxygen tensions (*i.e.*, "% Hb — 50") is affected by changes in oxygen tension. Thus, a certain part of erythropoiesis is independent of oxygen tension—it either is "spontaneous," or perhaps it is stimulated by some other unidentified environmental factor.

Summary. In cockerels raised at high altitude and sea level, and with the sexual development of some repressed by stilbestrol treatment, it is possible to differentiate the erythropoietic effect of androgens and chronic hypoxia. The actions of these factors appear to be independent of each other, a particular level of androgen (indicated by gonad size) producing equal increments in the hematocrit, in either the presence or absence of hypoxia.

1. Wintrobe, M. M., Clinical Hematology, 5th ed., Lea & Febiger, Philadelphia, 1961, 106.

2. Sturkie, P. D., Avian Physiology, 2nd ed., Comstock, Ithaca, 1965, 5.

3. Nathan, D. G., Gardner, F. H., Blood, 1965, v26, 411.

4. Van Liere, E. J., Stickney, J. C., Hypoxia, U. Chicago Press, 1963.

5. Grant, W. C., Root, W. S., Physiol. Rev., 1952, v32, 449.

6. Altland, P. D., Parker, M., Am. J. Physiol., 1955, v180, 421.

7. Smith, A. H., Abbott, U. K., Poultry Sci., 1961, v40, 1459.

8. Steel, R. G. D., Torrie, J., Principles and Procedures of Statistics, McGraw-Hill, New York, 1960.

9. Smith, A. H., Wilson, W. O., Pace, N., Growth, 1954, v18, 27.

10. Drill, V. A., in Growth in Living Systems, Zarrow, M. X., ed., Basic Books, New York, 1961, 383.

11. Selye, H., Stress, Acta, Inc., Montreal, 1950.

12. Burger, R. E., Lorenz, F. W., Gates, C. E., Poultry Sci., 1962, v41 1762.

13. Bennett, C. H., ibid., 1947, v26, 99.

14. Nalbandov, A. V., Reproductive Physiology, Freeman, San Francisco, 1958, 167.

15. Campbell, J. A., J. Physiol., 1927, v63, 325, Fig. 1.

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